The Open Dentistry Journal
DOI: 10.2174/0118742106357984241216041551, 2024, 18, e18742106357984

ISSN: 1874-2106

RESEARCH ARTICLE

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Effect of Accelerated Aging on the Microtensile Bond Strength of a Two-Step Adhesive Containing a Universal Adhesive Primer, an *in vitro* study

Mehdi Daneshpooy[1], Mahmoud Bahari[2], Fatemeh Dabaghi Tabriz[1], Parnian Alizadeh-Oskoee[1] and Zhila Fahim[1,*]

[1]*Department of Esthetic and Restorative Dentistry, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

Abstract:

Background: Despite the advantages of one-step universal adhesives, concerns about their bond durability exist. By introducing a two-step adhesive using universal bonding technology, the study aimed to evaluate the effect of accelerated aging on the microtensile bond strength (µTBS) of this adhesive when applied to dentin.

Materials and Methods: In this *in vitro* study, 16 extracted sound human third molar teeth were selected. Specimens were prepared by cutting the occlusal enamel perpendicular to the longitudinal axis and categorized into four groups of 18 each: G2-Bond Universal (GC Corp, Tokyo, Japan) in etch-and-rinse mode (GU-ER), G2-Bond Universal in self-etch mode (GU-SE), G-Premio Bond in etch-and-rinse mode (GP-ER), and G-Premio Bond (GC Corp, Tokyo, Japan) in self-etch mode (GP-SE). The resin composite was bonded to the dental surfaces based on the manufacturer's instructions and light-cured for 10 seconds using an LED curing unit (Demetron A2, Kerr, Scafati, Italy, 1200 mW/cm^2). The bonded specimens were then sectioned into 1 mm^2 sticks and divided into two subgroups (n=36). One subgroup (9 specimens from each adhesive) underwent thermal cycling, while the other was immersed in 10% sodium hypochlorite for three hours to simulate accelerated aging. The µTBS was measured, and failure modes were determined. Data were analysed using two-way ANOVA, the Sidak test, and the Independent Samples T-Test

Results: The highest mean µTBS was associated with GU-SE (29.63 ± 8.59 MPa), while the lowest was observed with GP-ER (18.65 ± 9.33 MPa). The µTBS values decreased following aging ($p < 0.001$). The values for GU-SE and GU-ER were significantly higher than those for GP-ER ($p < 0.001$ and $p = 0.005$, respectively). GU-SE and GU-ER showed a slight, statistically insignificant decrease in bond strength with aging ($p = 0.133$ and $p = 0.060$, respectively). However, GP-SE and GP-ER showed significant reductions in bond strength after aging ($p = 0.004$ and $p = 0.001$, respectively). The interaction between accelerated aging and study groups was not significant ($p = 0.311$), indicating a uniform effect of aging in all groups. Failure modes were similar in groups ($p > 0.05$), with adhesive failure being the most common type. G-Premio had more adhesive failures than G2-Bond, though this difference was not statistically significant.

Conclusion: Two-step universal adhesive performed better than one-step system during aging. In etch-and-rinse mode, the two-step adhesive significantly improved bond strength, while in self-etch, both adhesives showed similar performance.

Keywords: Durability, Tensile strength, Self-etch, Adhesives, Dentin, Substrate.

*Address correspondence to this author at the Department of Esthetic and Restorative Dentistry, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran; Tel: 00989145320704; E-mail: zhilafahim72@gmail.com

Cite as: Daneshpooy M, Bahari M, Dabaghi Tabriz F, Alizadeh-Oskoee P, Fahim Z. Effect of Accelerated Aging on the Microtensile Bond Strength of a Two-Step Adhesive Containing a Universal Adhesive Primer, an *in vitro* study. Open Dent J, 2024; 18: e18742106357984.
http://dx.doi.org/10.2174/0118742106357984241216041551

Received: September 15, 2024
Revised: November 06, 2024
Accepted: November 15, 2024
Published: December 27, 2024

1. INTRODUCTION

Adhering restorative materials to hard dental substrates, such as enamel and dentin, presents challenges due to their structural differences. Enamel's homogeneous structure facilitates the formation of a strong adhesive bond, while dentin's diverse inorganic and organic components make adhesion more complex and sensitive [1]. Over the past 20 years, research shifted from multi-step dental adhesives to simplified versions, which were not thoroughly studied in both laboratory and clinical settings [2]. Universal adhesives, the most recent advancement in dental adhesives, have become increasingly popular due to their adaptability and simplicity of use in adhering to a variety of substrates. These adhesives can be applied using different etching modes [3, 4].

A key difference between conventional one-step self-etch adhesives and universal adhesives is that most universal adhesives contain functional acid monomers [5], which are crucial for forming chemical bonds with the tooth structure [6]. However, some researchers argue that the bond quality of universal adhesives is comparable to that of one-step self-etch adhesives, without showing significant improvement [7]. In contrast, the combination of essential components with varying chemical properties in a single bottle can result in water absorption, phase separation, increased nanoleakage, and reduced bond durability when compared to two-step self-etch adhesives [8]. Furthermore, high concentrations of hydrophilic monomers and incomplete solvent removal during evaporation can delay the formation of a high-crosslink polymer, reduce the degree of conversion, and increase the permeability of the adhesive layer [7]. Overall, *in vitro* studies suggest that the efficacy of hybridization may be reduced when the adhesive application procedure is simplified by combining the primer and adhesive into a single step [2].

Recently, a new group of two-step universal adhesives called G2-Bond Universal (GC Corp, Tokyo, Japan) has been introduced to enhance the durability of the substrate-adhesive interface [8]. While universal adhesives typically function as hydrophilic polymers with permeable constituents [2], G2-Bond Universal provides a more hydrophobic bond layer owing to its two-step application method [8]. Most studies have primarily focused on single-step universal adhesives or compared them to traditional self-etch systems [9,10]. Evidence from a recent systematic review suggests that most commercially available universal adhesives are single-bottle systems, containing less resin and more solvent than those with separate primer and adhesive components. This composition may lead to thinner adhesive layers after curing, resulting in suboptimal polymerization. Unlike traditional adhesives, G2-Bond Universal is a two-bottle, HEMA-free universal bonding agent [11]. The absence of HEMA in this adhesive structure enhances the hybrid layer's resistance to hydrolytic degradation [12]. Similarly, Tokuyama Universal Bond (Tokuyama Dental Corp, Tokyo, Japan) and LuxaBond Universal (DMG America LLC, Englewood, NJ, USA) are two-bottle systems, but their components are blended before application [11]. In contrast, G2-Bond Universal functions as a true two-step adhesive, highlighting the importance of comparing it to other single-step, HEMA-free universal adhesives.

There is limited information available on the degradation of bonds in two-step universal adhesives under aging conditions. Moreover, G2-Bond Universal is designed for use in both etching modes, making it essential to evaluate the impact of prior acid conditioning of dentin on its adhesive strength. Therefore, the present study aimed to investigate the effect of accelerated aging on the microtensile bond strength (μTBS) of this two-step universal adhesive when applied to dentin.

2. MATERIALS AND METHODS

The present *in vitro* experimental study approved by the Ethical Research Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.492),

2.1. Specimens Preparation

The sample size was determined based on the design of a pilot study, which resulted in a total of 72 samples from 16 teeth, and therefore, 16 extracted sound human third molar teeth were used. Teeth from patients aged 20-35 years, free of caries, fractures, or cracks, were selected to minimize dentin sclerosis and accurately simulate individual conditions. The teeth were assessed visually and with an explorer to confirm compliance with these standards. After cleaning, they were stored in a 0.5% T chloramine solution (Merck, Darmstadt, Germany) for disinfection and immersed in distilled water for 24 hours before the experiment [13]. To prepare the specimens, a precise cut was made *via* the occlusal enamel of teeth, perpendicular to their longitudinal axis. The cut surfaces were polished with 600-grit silicon carbide sandpaper (Phoenix Beta, Buehler, Germany) under running water for 60 seconds to standardize the smear layer [14, 15].

2.2. Adhesive Application Procedure

Table **1** shows the materials used in this study. Specimens were divided into four groups of 18 each based on adhesive type and etching mode:

[1] G2-Bond Universal in etch-and-rinse mode (GU-ER): A two-step universal adhesive system designed to enhance bonding effectiveness using the previous acid conditioning of dental substrate.

[2] G2-Bond Universal in self-etch mode (GU-SE): A two-step universal adhesive system that simplifies the bonding process by eliminating the need for separate etching.

[3] G-Premio Bond in etch-and-rinse mode (GP-ER): A one-step universal adhesive system offering enhanced adhesion by promoting optimal enamel and dentin etching.

[4] G-Premio Bond in self-etch mode (GP-SE): A one-step universal adhesive system that provides an alternative for bonding procedures by eliminating the need for acid etching.

Table 1. Materials used in this study.

Code	Adhesive Systems	Adhesive System Type	Components	Application Mode
GP-SE GP-ER	G-Premio Bond (GC corp, Tokyo, Japan)	One-step Universal Adhesive	10-MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, silica, initiators. PH=1.5	Self-etch strategy: 1. Apply using a micro brush and wait for 10 s 2. Dry for 5 s at maximum air pressure 3. Light cure for 10 s. Etch-and-rinse strategy: 1. Apply etchant for 15 sec. 2. Rinse thoroughly for at least 15 sec. 3. Remove rinsing water completely by blowing gently with an air syringe or by blot-drying with a cotton pellet. 4. Apply adhesive as for the self-etch mode
GU-SE GU-ER	G2-Bond Universal (GC corp, Tokyo, Japan)	Two-step Universal Adhesive	Primer: 4-MET, MDP, MDTP, dimethacrylate monomer, acetone, water, photoinitiator, filler. PH=1.5 Adhesive: dimethacrylate monomer, bis-GMA, filler, photoinitiator	Self-etch strategy: 1. Apply the primer and wait for 10 s 2. Dry with moderate air blow for 5 s 3. Apply the bond 4. Gentle air blowing to make the film uniform 5. Light cure for 5 s. Etch-and-rinse strategy: 1. Apply etchant for 15 sec. 2. Rinse thoroughly for at least 15 sec. 3. Remove rinsing water completely by blowing gently with an air syringe or by blot-drying with a cotton pellet. 4. Apply adhesive as for the self-etch mode.
Composite resin	**Type**		**Composition**	**Filler Content% (w/w)**
Gradia Direct (GC corp, Tokyo, Japon)	Microhybrid Composite		UDMA, dymethacrylate, camphorquinone, FAS, silica, prepolimerized filler, powder	73

Abbreviations: bis-GMA: bis-phenol A diglycidylmethacrylate; 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; 4-MET: 4-Methacryloyloxyethyl trimellitate, MEPS: methacryloyloxyalkyl thiophosphate methylmethacrylate, MDTP: 10-methacryloxydecyl dihydrogen thiophosphate, UDMA: Urethanedimethacrylate, FAS: fluoro-alumino-silicate.

G2-Bond Universal and G-Premio Bond are universal adhesives developed by GC Corp, headquartered in Tokyo, Japan. G2-Bond is designed as a two-step system, while G-Premio features a one-step application process. The adhesives were applied based on the manufacturer's instructions. Single operator who is an expert in restorative dentistry carried out the procedure. In the self-etch (SE) mode, no acid etchant was used. After applying the G-Premio bonding agent for ten seconds, the surface was completely dried for five seconds using the maximum air pressure. To optimize control over the adhesive layer's thickness on dentin, several precise application methods were implemented. Specifically, the bonding agent was applied as a single layer with a disposable applicator brush, ensuring even coverage across the dentin surface with a 20 seconds scrubbing action [16].A controlled air stream was then directed over the adhesive for five seconds until the layer stabilized, allowing the solvent to evaporate and forming a uniform, slightly shiny film [17]. The adhesive was then light-cured for ten seconds using an LED curing unit (Demetron A2, Kerr, Scafati, Italy) at 1200 mW/cm^2. For the G2-Bond application, the primer was applied in a single layer using a precise micro brush over the entire surface of the dentin. It was allowed to dwell for ten seconds, after which the surface was dried with moderate air blowing for five seconds. The bonding agent was then applied in the same manner as G-Premio, lightly air-dried to create a uniform film, and subsequently light-cured. In etch-and-rinse (ER) mode, etching was performed with a 37% phosphoric acid etching agent (Ultra-etch, Ultradent, South Jordan, UT, USA). After applying the etchant and rinsing for at least 15 seconds, residual water was removed gently using an air syringe. The adhesives were then applied following the same procedure as in SE mode. The details of the adhesive application and composition are provided in Table **1**. After adhesive application, the substrate was restored with a resin composite (Gradia Direct, GC, Tokyo, Japan) in 2 mm increments, achieving a total height of 6 mm. The thickness of each increment was carefully controlled using a probe, and each layer was individually light-cured for 20 seconds with curing unit [13].

2.3. Aging Procedure

The restored teeth were stored in distilled water at 37°C for 24 hours. They were then sectioned vertically into sticks with a cross-sectional area of 1 mm^2 using a cutting machine (Thin Sectioning Machine Inc., Rochester, NY, USA) [18]. After measuring the thickness and width of each stick with a digital caliper (Mitutoyo, Tokyo, Japan), the intact sticks (n = 72) were randomly divided into two subgroups (n = 36 each) [19]. One subgroup underwent thermocycling with 500 cycles at 5°C–55°C and a dwell time of ten seconds [20], while the other was immersed in a 10% sodium hypochlorite (NaOCl) solution at 37°C for three hours to simulate accelerated aging [18].

2.4. Microtensile Bond Strength (μTBS) Test & Failure Mode Analysis

Bond strength was measured using a universal testing machine (Bisco, Schaumburg, IL, USA) with a loading speed of 0.5 mm/min [18]. Failure modes were then observed under a stereo microscope (Nikon SMZ800, Tokyo, Japan) at 10× magnification and classified as follows [21]: Type I: cohesive failure within the substrate; Type II: cohesive failure within the composite; Type III: adhesive failure; Type IV: mixed failure. Fig. (1) illustrates the study design.

2.5. Statistical Analysis

To investigate the effect of accelerated aging and the type of adhesive system on microtensile bond strength (µTBS), the normality of data was first assessed. The Kolmogorov-Smirnov test indicated that the tensile bond strength values followed a normal distribution ($p = 0.832$). Consequently, a two-way analysis of variance (ANOVA) was employed to compare bond strength among the groups. Levene's test confirmed that the bond strength variable exhibited equal variances ($p = 0.120$), making the use of Sidak post hoc test appropriate. The effect of aging on µTBS for each adhesive type was analyzed using an Independent Samples T-Test. Additionally, a Chi-square test assessed the relationship between failure modes and study groups. The significant level was in p-value <0.05.

3. RESULTS

Comparison of bond strengths among the groups using two-way ANOVA revealed significant effects of the aging process ($p < 0.001$) and the type of adhesive used ($p < 0.001$) on µTBS. There was a significant reduction in mean µTBS values after accelerated aging compared to the values before aging ($p < 0.001$).

Table 2 and Fig. (2) indicate the mean µTBS values for all study groups. The highest mean µTBS was associated with GU-SE, with an average of 29.63 ± 8.59 MPa, while the lowest was observed with GP-ER, averaging 18.65 ± 9.33 MPa.

Table 3 presents the Sidak post hoc test results for pairwise comparisons of the adhesives. The results indicate that the values for GU-SE and GU-ER were significantly higher than those for GP-ER ($p < 0.05$), with no significant difference between GU-SE and GU-ER ($p = 0.775$). No significant differences were observed in the mean µTBS values among the other pairs of study groups ($p > 0.05$).

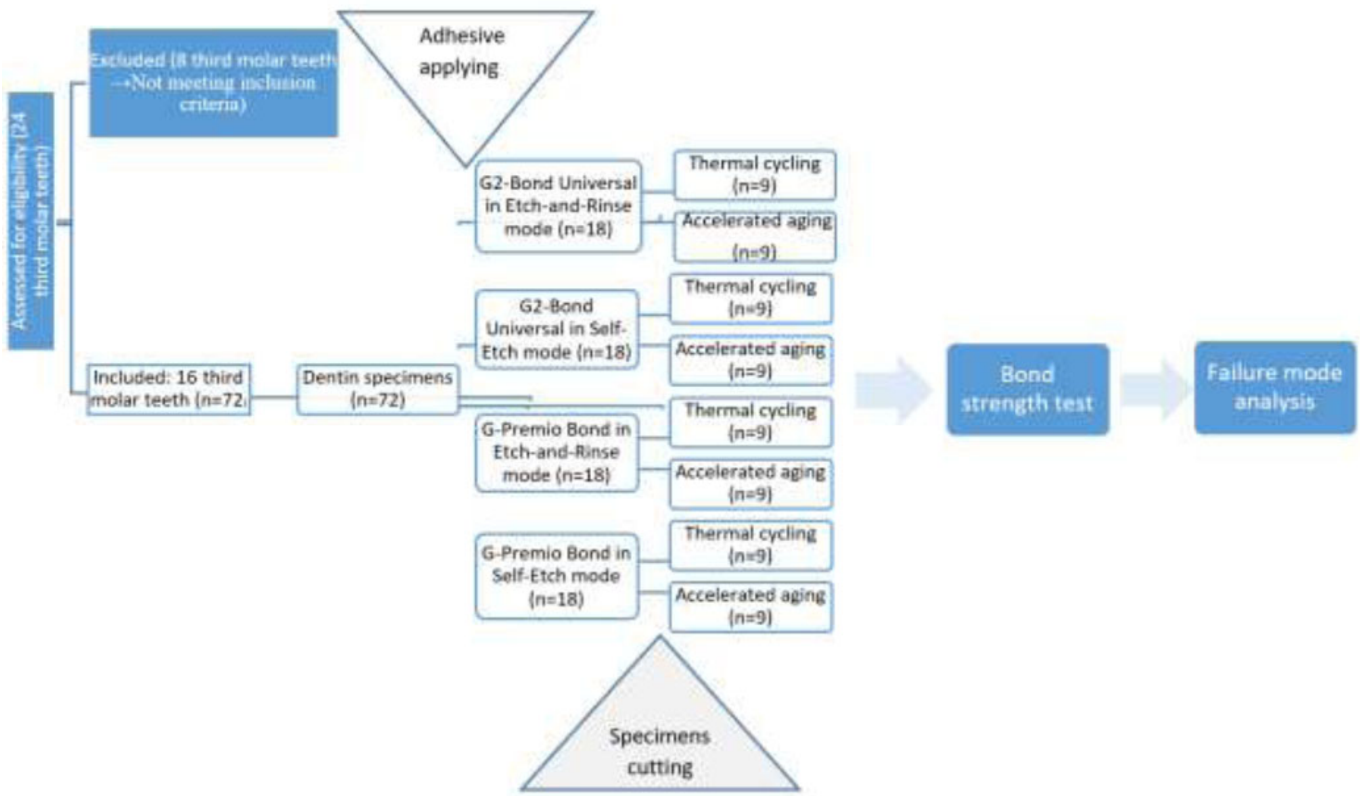

Fig. (1). Study Design Overview. 16 third molar teeth were divided into four groups: G2-Bond Universal in etch-and-rinse mode, G2-Bond Universal in self-etch mode, G-Premio Bond in etch-and-rinse mode, and G-Premio Bond in self-etch mode. Following adhesive application, specimens were sectioned using a cutting machine and divided into two subgroups: one underwent thermal cycling, and the other was immersed in 10% sodium hypochlorite. Bond strength (MPa) was measured using a microtensile strength tester, and failure modes were analyzed with a stereomicroscope. There were no detected exclusion specimens during the analysis process.

Table 2. Microtensile bond strength (MPa) in study groups (Mean ± Std. deviation).

Adhesive System	Without Aging (MPa)	With Aging (MPa)	Total (MPa)	*P* -value
G-premio Bond in Self-Etch mode (GP-SE)	28.50 ± 6.84	18.17 ± 7.12	29.63 ± 8.59	0.004
G-premio Bond in Etch-and-Rinse mode (GP-ER)	25.61 ± 7.18	12.95 ± 6.71	26.76 ± 6.88	0.001
G-bond Universal in Self-Etch mode (GU-SE)	32.69 ± 2.49	27.58 ± 10.59	24.70 ± 9.21	0.133
G-bond Universal in Etch-and-Rinse mode (GU-ER)	29.95 ± 7.35	23.67 ± 5.03	20.73 ± 9.56	0.060
Total (All Adhesives)	28.97 ± 6.63	20.73 ± 9.56	-	<0.001

Table 3. Between-group comparison of the adhesive systems.

(*I*)	(*J*)	Mean Difference(*I-J*)	*p*-value*
-	GP-ER	5.20	0.145
GP-SE	GU-SE	-5.77	0.079
-	GU-ER	-2.91	0.760
-	GU-SE	-10.97	<0.001
GP-ER	GU-ER	-8.11	0.005
GU-SE	GU-ER	2.86	0.775

Note: * Sidak test.

The interaction between accelerated aging and study groups was not significant ($p = 0.311$), indicating a uniform effect of aging in all groups.

Table **4** presents the Independent Samples T-Test results evaluating the effect of aging on µTBS for each adhesive type. It shows that G2-Bond demonstrated more stable dentin bond strength during aging, irrespective of the application mode ($p > 0.05$). In both the GP-SE and GP-ER adhesive systems, the mean µTBS values after accelerated aging were significantly lower than those before aging ($p < 0.05$).

The Chi-square test was used to assess the association between failure modes and study groups. Table **5** shows the frequency of specimens based on failure mode in the study groups, as well as the results of Chi-square test. Analysis revealed no statistically significant association between the study groups and the failure modes ($p > 0.05$). Relatively similar patterns were observed in all groups, with adhesive failure being the most common type. Fig. (**3**) illustrates the various patterns of failure observed at specimen interfaces under a stereo-microscope.

Table 4. Effect of aging on µTBS for each adhesive type.

No. of Specimens	Adhesive System	Accelerated Aging	Mean (SD)	t	df	*p*-value*
18	GP-SE	No	28.50 (6.84)	3.30	18	0.004
		Yes	18.17 (7.12)			
18	GP-ER	No	25.61 (7.17)	4.07	18	0.001
		Yes	12.95 (6.71)			
18	GU-SE	No	32.69 (2.49)	1.605	12.766	0.133
		Yes	27.58 (10.59)			
18	GU-ER	No	29.55 (7.35)	2.012	17	0.060
		Yes	23.67 (5.03)			

Note: * Independent Samples T-Test.

Table 5. Frequency of specimens based on failure mode in study groups (n = 9).

-	Without Aging				With Aging			
-	GP-SE	GP-ER	GU-SE	GU-ER	GP-SE	GP-ER	GU-SE	GU-ER
Cohesive(substrate)	D (1)	D (0)	C (2)	C (2)	D (0)	D (0)	D (1)	B (4)
Cohesive(composite)	D (0)	D (1)	D (1)	C (2)	D (1)	D (1)	C (2)	D (1)
adhesive	A (6)	A (6)	B (4)	C (3)	A (6)	A (7)	B (5)	C (3)
mixed	C (2)	C (2)	C (2)	C (2)	C (2)	C (2)	D (1)	D (1)
p-value*	0.777				0.245			

Note: *Chi-square test. Grading key for failure modes: A: 6–7 specimens, B: 4–5 specimens, C: 2_3 specimens, D: 0_1 specimens..

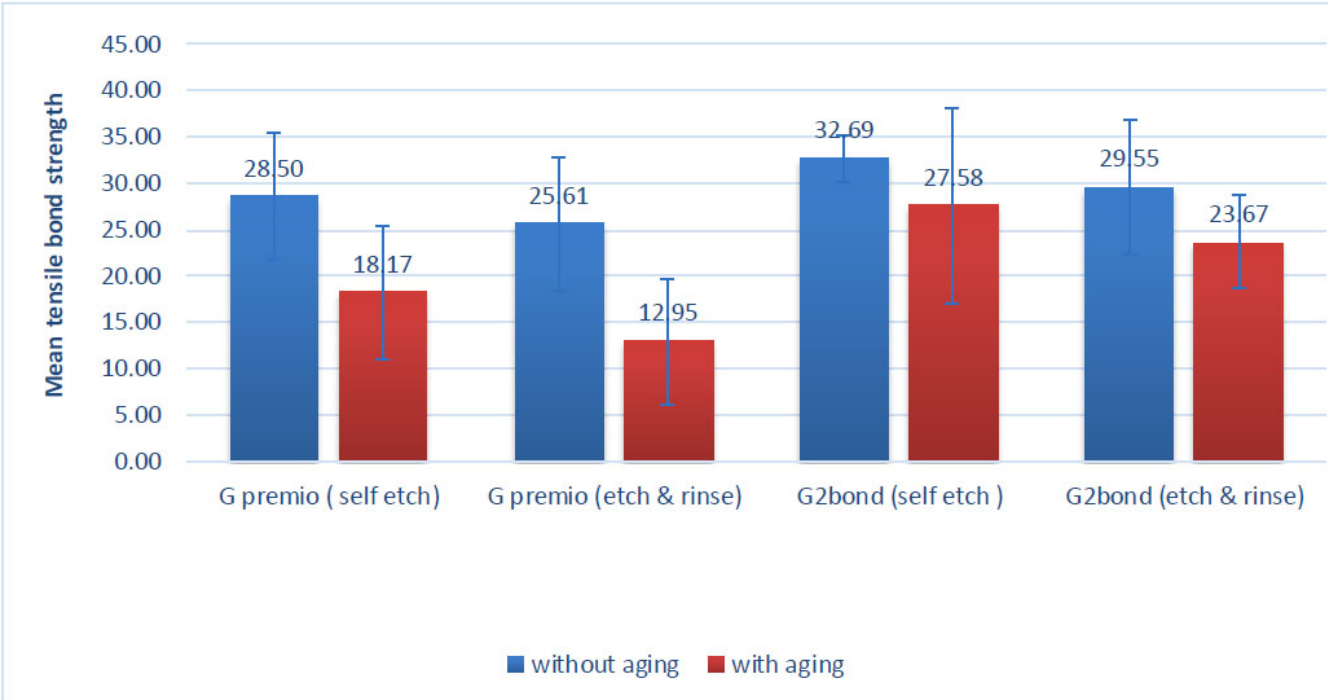

Fig. (2). Mean tensile bond strength (MPa) in study groups.

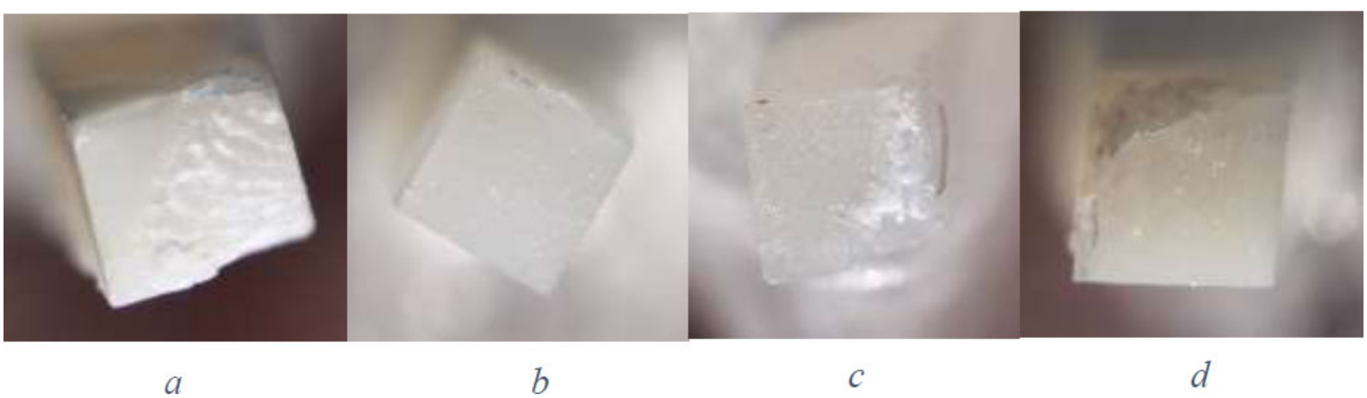

Fig. (3). Various patterns of failure observed at specimen interfaces under a stereomicroscope. (**a**) Cohesive failure within the substrate; (**b**) Cohesive failure within the composite; (**c**) Adhesive failure; (**d**) Mixed failure.

4. DISCUSSION

The primary objective of adhesive restorative materials is to establish a tight and durable bond to dental substrates. Regarding the rapid development and frequent updates of new dental adhesives, assessing their bond degradation and restoration durability is crucial [2]. Clinical trials are often costly and time-consuming, making accelerated aging a practical alternative for laboratory simulations [7]. A recently introduced method involves exposing the adhesive interface to a 10% NaOCl aqueous solution, which is designed to simulate *in vivo* degradation by removing non-resin-infiltrated organic components at the tooth-adhesive interface [22, 23]. Studies showed that aging in 10% NaOCl for 1 and 3 hours produces degradation patterns comparable to water storage for 6 and 12 months. Consequently, immersing specimens in a 10% NaOCl solution is regarded as a rapid and reliable method for evaluating adhesive interface durability [7].

This study's results indicated that "accelerated aging" and "adhesive type" significantly affected µTBS. Specifically, µTBS values to dentin were markedly lower after accelerated aging compared to the groups weren't expose to ageing process. This finding was consistent with the study by Yuan *et al*., which evaluated the effect of NaOCl solution on the dentin bond strength of universal adhesive systems [24]. They observed that immersion in NaOCl solution led to a decrease in microtensile bond strength among study groups [24]. NaOCl is a nonspecific deproteinase that oxidizes peptide bonds in proteins, such as collagen, by forming superoxide radicals in an aqueous solution. This process dissolves the collagen fibrils encapsulated by resins, resulting in the removal of organic components from the dentin interface [7]. Similarly, based on the failure mode results, adhesive layer degradation increased after storing the specimens in NaOCl solution.

The present study found that aging had a similar effect on μTBS values in all groups. However, G2-Bond showed a modest, statistically insignificant decrease in dentin bond strength during aging with SE and ER modes, whereas G-Premio showed a more pronounced and statistically significant reduction in bond strength. The stable dentin bond strength of G2-Bond may be attributed to the efficacy of its universal adhesive-derived primer and adhesive layer properties. The adhesion steps of a one-step universal adhesive system are combined into a single procedure, requiring the use of a single bottle that contains a mixture of solvent, water, and hydrophilic and hydrophobic monomers [8]. Conversely, a two-step universal adhesive system requires the sequential application of its components. Initially, a primer composed of acidic monomers dissolved in an aqueous solution is applied, followed by a hydrophobic resin layer without any solvent in the subsequent step [8, 25, 26]. In the one-step universal adhesive system, the complexity of the composition and the absence of a separate hydrophobic resin layer can lead to incomplete polymerization and reduced bond durability [25, 27, 28]. However, in a two-step protocol, the hydrophobic bonding layer increases the concentration of hydrophobic monomers, thereby reducing the relative concentration of solvents and hydrophilic monomers within the adhesive interface, which explains the slight reduction in bond strength during storage [29]. This observation is reflected in the failure mode results. The data collected through the analysis of stereo-microscope images showed that the number of adhesive failures for G-Premio was greater than that of G2-Bond, although this difference was not statistically significant. Examples of cohesive failure (substrate/composite) were observed in G2-Bond, indicating a high bond strength of the adhesive layer that the substrate or composite could not compete with. Notably, similar method are used in orthodontics to assess the orthodontic adhesive failure by examining the adhesive remnant left on teeth or bracket base [30,31].

A recent systematic review by Hardan *et al*. demonstrated that adding a hydrophobic layer improves the sealing of universal adhesives and enhances both short-term and long-term bonding performance [3]. They noted that the MDP monomer requires an appropriate duration of 20 seconds to achieve a stable nanolayer structure with calcium salts. Applying a second coat of the monomer without curing the first allows the initial layer to interact sufficiently with hydroxyapatite, promoting additional bonding [3]. Their study focused on single-step adhesives [3]. The presence of the hydrophobic layer suggests that a two-step universal adhesive can benefit from this configuration as well.

Regarding the efficiency of adhesive types, the results showed that mean μTBS values for GU-SE and GU-ER were significantly higher than those for GP-ER. However, no significant difference was observed between the other study groups. In the two-by-two comparison of adhesives, the following factors should be considered: the degree of hydrophilicity similarity between the surfaces, the acidity level (pH), the implementation of a two-step approach, and the chemical bonding of functional monomers. An important factor for improving adhesion is the level of hydrophilicity similarity between two surfaces [32]. Phosphoric acid-etched dentin has lower wettability, a reduced degree of polarization, and is less hydrophilic compared to ground dentin (SE group) [6]. The second bottle of G2-Bond does not contain water or solvent; therefore, it is designed for a more hydrophobic surface [32]. As the results showed, the bond strength values for GU-ER were higher than those for GP-ER.

The chemical composition of an adhesive system directly influences its bonding ability. The adhesive systems tested include 10-methacryloyloxydecyl dihydrogen phosphate monomer (10-MDP). One of the most significant attributes of 10-MDP is its capacity to chemically bind with hydroxyapatite in dental substrates, improving adhesion to dental tissue. In SE mode, the residual hydroxyapatite surrounding collagen fibrils functions as a receptor for chemical interaction with 10-MDP, thereby enhancing both hydrolytic stability and adhesive performance [33]. Furthermore, the smaller size of hydroxyapatite crystals in dentin, along with their cross-orientation, makes dentin chemically more reactive [34, 35]. Considering studied adhesives are similar in composition and contain functional monomers, using G-Premio in SE mode produces an acceptable bond strength to dentin, without significant differences compared to the G2-Bond. Additionally, using a two-step strategy of the GU-SE system further improved bond strength compared to GP-ER.

Tsujimoto *et al*. compared the fatigue bond strength of different adhesive systems, finding that G2-Bond Universal, which uses a primer, showed a higher dentin fatigue bond strength than other adhesive systems in SE mode. This contrasts with the findings of present study [32]. Variations in study results can be attributed to several factors, including methodology, the types of adhesive systems used, the type of bond strength test, and the aging method. Unlike the adhesives in Tsujimoto's study, which varied in composition and pH [32], those in the current study were compositionally similar, differing mainly in application. This study used a tensile bond strength test with sodium hypochlorite aging, whereas Tsujimoto's study used a shear fatigue test. The increased bond strength for G2-Bond in SE mode in Tsujimoto’s study, compared to Clearfil SE Bond 2 and Scotchbond Universal Plus Adhesive, is attributed to G2-Bond's more hydrophobic nature, as well as the absence of HEMA, which further enhances the hydrophobicity of both the primer and adhesive [32]. Notably, both adhesives investigated in the present study were HEMA-free.

In the current study, the bond strength of both adhesives in ER mode was comparable to that in SE mode, suggesting that etching had only a minor effect with the same adhesive. This finding is in line with a systematic review by Negarkar *et al.,* which demonstrated that there is no significant difference between ER and SE modes for the bond strength of G-Premio, according to its pH level (pH = 1.5, Intermediate strong) [36]. Given the similarity in pH between G-Premio and G2-Bond, this result may also apply to G2-Bond. Universal adhesives with high acidity do not create a significant difference in bond strength, as their etch patterns are nearly identical in SE and ER modes [37]. Although it has been suggested that chemical bonding in SE mode helps improve bond strength, in ER mode, numerous resin tags develop due to deeper adhesive penetration, resulting from smear layer removal and tubule opening. These deeper resin tags strengthen the adhesive interface by aligning perpendicularly to the applied stress [6]. However, some studies report varying outcomes on the bond strength superiority of universal adhesives in SE or ER modes. Wegner *et al*. demonstrated that the effect of pH could be minimized using different application techniques. They observed that ultra-mild adhesives applied with active agitation showed comparable bond strength in both SE and ER modes [38]. Also in the study done by Bahari *et al*., bond strength varied between ER and SE modes in both the control and thermal cycling groups, in contrast to the results obtained with NaOCl aging [7]. These findings emphasize that both application and aging methods influence outcomes, highlighting the need for further research employing alternative techniques.

This study was conducted as a laboratory experiment using the µTBS test. Van Noort *et al*. emphasized that the method selected for assessing bond strength can significantly influence results and their clinical relevance [39]. The µTBS test provides improved stress distribution across the adhesive interface compared to traditional shear bond strength (SBS) methods, resulting in more reliable evaluations of adhesive performance [18,39]. Conversely, SBS is not a reliable indicator of adhesive bond quality for resin composites since it is impacted by the cohesive strength of the base material rather than the adhesive interface [39].

5. LIMITATIONS

Bond strength evaluations are predominantly used to rank adhesive systems and may not be a complete predictor of clinical performance. In the oral environment, bond strength can be influenced by factors such as masticatory forces, pH changes, and thermal fluctuations [18]. Thus, caution is needed when applying these results to clinical situations and further clinical studies are warranted.

CONCLUSION

Aging led to a reduction in bond strength in all adhesive systems evaluated. Two-step universal adhesive showed better performance than one-step system during aging. In etch-and-rinse mode, the two-step universal adhesive significantly enhanced bond strength, while in self-etch mode, both two-step and one-step systems exhibited comparable performance. When using the same type of adhesive, bond strength was similar in both etch-and-rinse and self-etch modes.

CLINICAL SIGNIFICANCE

Currently, one-bottle universal adhesives are widely used in dental practices due to their reduced clinical steps. However, concerns remain because all components are contained in a single bottle and used in a one-step process. For restorations requiring enhanced longevity, the two-step universal adhesive may offer a more durable clinical option.

RECOMMENDATIONS FOR FUTURE RESEARCH

Incorporating scanning electron microscopy (SEM) analysis in future studies could offer a more detailed understanding of the differences and similarities in the performance of adhesives. Additionally, exploring the effects of various application methods for universal adhesives may provide valuable insights for optimizing bond strength. Further studies are also needed to evaluate the impact of alternative aging methods on adhesive durability.

AUTHOR'S CONTRIBUTION

M.D., M.B.: Study conception and design; Z.F.: Data collection; M.B., F.D.T., P.A.O.: Analysis and interpretation of results; Z.F., M.D.: Draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

GU-ER	=	G2-Bond Universal in etch-and-rinse mode
GU-SE	=	G2-Bond Universal in self-etch mode
GP-ER	=	G-Premio Bond in etch-and-rinse mode
GP-SE	=	G-Premio Bond in self-etch mode
SE	=	Self-etch
ER	=	Etch-and-rinse
NaOCl	=	Sodium hypochlorite
µTBS	=	Microtensile Bond Strength
SBS	=	Shear Bond Strength
SEM	=	Scanning Electron Microscopy

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Research Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.492).

HUMAN AND ANIMAL RIGHTS

All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from patients for participation in the study.

AVAILABILITY OF DATA AND MATERIAL

All the data and supporting information is provided within the article.

FUNDING

The study was financially supported, reviewed, and supervised by Tabriz University of Medical Sciences.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

The authors extend their gratitude to the Vice Chancellor for Research at Tabriz University of Medical Sciences for their support.

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