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Effect of double-layer application techniques on the dentin micro-tensile bond strength of universal adhesives

Gülşah Tonga[1], Hüseyin Hatırlı[1*] and Kübra Bahar[1]

Abstract

Background This study aimed to evaluate the immediate and 6-month dentin micro-tensile bond strength (μTBS) of three universal adhesives applied using different double-layer application techniques.

Methods Sixty-five human molar teeth were divided into 13 groups ($n=5$) based on adhesive systems and application procedures. Three universal adhesives (G-Premio Bond, GC Corp.; GP, OptiBond Universal, Kerr; OB and Single Bond Universal, 3 M ESPE; SB) and a two-step self-etch adhesive (Clearfil SE Bond, Kuraray Noritake Dental; CSE) were tested. Universal adhesives were applied according to the manufacturer's instructions-(SL-MI) or three different double-layer application procedures: (1) the first adhesive layer was applied without either air thinning or light curing-(DL1), (2) air thinning without light curing the first layer-(DL2), (3) with air thinning and polymerisation each layer-(DL3). The two-step self-etch group was applied according to the manufacturer's instructions. μTBS was measured immediately, after 3 months and 6 months of water storage. Statistical analyses were conducted using Three-Way ANOVA ($p<0.05$).

Results For the immediate and 3-month water storage of SB and GP, the highest μTBS values were observed in Group DL2, while for OB, Group DL3 exhibited the highest μTBS values ($p<0.05$). After 6 months of water storage, differences among the application procedures were not significant for GP and OB ($p>0.05$). The two-step self-etch system demonstrated higher μTBS than all universal adhesive systems at all test periods ($p<0.05$).

Conclusion The application of universal adhesives in two layers, particularly by thinning the first layer with air and polymerising after the second layer, positively affects immediate and 3-month μTBS. However, the benefits of double-layer application on 6-month μTBS appear to be material dependent.

Keywords Universal adhesive, Adhesive application, Double layer, Multi-layer, Air thin, Polymerising

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Background

Universal adhesives have gained widespread use in clinical practice due to their simplified application protocols, reduced technique sensitivity, and enhanced user convenience. However, studies have reported that universal adhesives demonstrate lower bond strength and durability compared to two-step self-etch systems [1–3]. This issue is attributed to the adhesive layer's thin film thickness and the hydrophilic structure [3, 4]. Furthermore, universal adhesives represent a diverse category of materials with distinct variations in pH, chemical composition, and adhesive properties. These differences raise questions regarding the ability to effectively penetrate the smear layer barrier, especially for mild and ultra-mild universal adhesives [1, 2].

The formation of a hydrophilic adhesive layer in universal adhesives is primarily attributed to the substantial effect of oxygen inhibition on polymerisation, inadequate adhesive film thickness, high concentrations of 2-hydroxyethyl methacrylate (HEMA), and incomplete solvent evaporation during the application process [1, 5, 6]. In order to eliminate the adverse effects of thin adhesive layers of universal adhesives, various strategies have been proposed, including applying the adhesive in multiple layers, applying an additional hydrophobic layer, or using flowable composite resin over the adhesive [3, 4, 7–10].

The additional hydrophobic layer application involves using the universal adhesive as a primer and then applying a further layer of hydrophobic adhesive on top. Several studies have investigated this method and demonstrated its positive effects on bond strength [3, 5, 8, 10]. However, the clinical application of this method necessitates using an additional material and prolongs the procedure time. In contrast, applying universal adhesives in multiple layers is a more straightforward technique and, based on our observations, is often performed inadvertently by clinicians with limited experience in dental adhesive systems. Many clinicians believe that applying universal adhesives several times will yield improved bond strength, which may be beneficial for mild and ultra-mild universal adhesives [7]. However, successive application of adhesive layers without air thinning may impede solvent removal, causing solvent accumulation in the adhesive layer, which can negatively affect the polymerisation of the monomers [10, 11].

Previous research indicates that the double-layer application of universal adhesives positively influences bond strength and durability [7]. However, there is no consensus on the optimal clinical application procedure. Essentially, the double-layer application of universal adhesives can be performed using three distinct techniques: (1) Applying the first adhesive layer without either air thinning or light curing as mentioned above, (2) air thinning without light curing the first layer, and (3) air thinning and light curing each layer. While these application methods have been tested separately in different research, no study has evaluated the effect of these three different application methods together in the literature review.

Therefore, this study aimed to determine the effects of different double-layer adhesive application procedures on three universal adhesive systems' immediate, 3-month, and 6-month dentin micro-tensile bond strength (μTBS). The null hypotheses tested were: (1) Double-layer universal adhesive application techniques do not increase the dentin μTBS of adhesive systems, and (2) There is no difference among the different double-layer universal adhesive application techniques.

Methods

This study utilised the G*Power V.3.1.9.6 software to calculate a 95% confidence level (1-α) and 80% test power (1-β). At least 65 teeth were required to obtain an effect size of f = 0.50 between the study groups, and $n = 5$ teeth were used in each group.

Tooth selection and specimen Preparation

Sixty-five human lower third molars were collected following the approval of the study protocol by the Ethics Committee of Tokat Gaziosmanpasa University, Faculty of Medicine (23-KAEK-114). The teeth were stored in 0.5% aqueous chloramine-T solution at 4 °C after extraction and used within one month.

The teeth were embedded in self-curing acrylic resin, and occlusal one-thirds of the crowns were cut using a slow-speed diamond saw (Micracut 152, Metkon, Bursa, Türkiye) under cooling fluid (Metcool II, Metkon) and water mixture, and mid-coronal dentin exposed. The teeth were divided into thirteen groups ($n = 5$) regarding the adhesive systems and application procedure. The teeth were ground for 60 s under water cooling using 240 grit SiC sandpaper and a polishing device (Metkon, Türkiye) to prepare uniform dentin surfaces and smear layers. Ground surfaces were examined for the presence of remnant enamel and pulp exposure.

Adhesive application procedure

Three universal adhesives (G-Premio Bond, GC Corp., Tokyo, Japan; GP, OptiBond Universal, Kerr, Orange, CA, USA; OB, and Single Bond Universal, 3 M ESPE, St. Paul, MN, USA; SB) and a two-step self-etch adhesive (Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan; CSE) were tested in this study. The gold-standard CSE was used in accordance with the manufacturer's instructions (Table 1) and served as a reference. Universal adhesives were used in self-etch mode and subdivided into four groups regarding application procedures.

Table 1 Adhesive systems, composition, and application procedures

Adhesive (Manufacturer)	pH/Classification	Composition	Manufacturer's instructions	Lot No
Clearfil SE Bond (Kuraray Noritake Dental, Tokyo, Japan)	2.0 (primer)/ Two-step mild self-etch	Primer: 10-MDP, HEMA, Hydrophilic dimethacrylate, di-camphorquinone, N, N-diethanol-p-toluine, water Bond: 10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, di-camphorquinone, N, N-diethanol-p-toluine, silanated colloidal silica	- Apply primer 20 S - Dry with mild air flow - Apply bonding - Air flow gently - Light cure for 10 s	000469
Single Bond Universal (3 M Deutschland GmbH, Neuss, Germany)	2.7/Ultra-mild self-etch	10-MDP, HEMA, silane, dimethacrylate resins, Vitrebond™ copolymer, filler, ethanol, water, initiators.	- Apply the adhesive to the entire surface and rub it in for 20 s. - Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. - Light cure for 10 s.	20524B
G-Premio Bond (GC Corporation, Tokyo, Japan)	1.5/Intermediary strong self-etch	10-MDP, 4-META, methacrylate adic ester, distilled water, acetone, photo initiators, silica fine powder.	- Apply using a microbrush - Leave undisturbed for 10 s after application. -Dry thoroughly for 5 s with oil-free air under maximum air pressure. -Light cure for 10 s.	2,304,171
Optibond Universal (Kerr, Orange, CA, USA)	2.5/ Mild/Ultra-mild self-etch	GPDM, HEMA, glycerol dimethacrylate, phosphate dimethacrylate acetone, ethanol, water, filler.	-Apply to the entire surface and agitate for 20 s. -Air dry for 5 s with mild air pressure. -Light-cure for 10 s	9,448,958

Abbreviations: 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; **HEMA**: 2-hydroxyethyl methacrylate; **Bis-GMA**: bisphenol A diglycidyl methacrylate; **4-META**: 4-methacryloyloxyethyl trimellitate anhydrate, **GPDM**: Gycerol Phosphate Dimethacrylate

Group SL-MI: (Single layer manufacturers' instructions*)*: Single layer application according to manufacturer's instructions (Table 1).

Group DL1 (Double-layer, the first adhesive layer was applied without either air thinning or light curing) The first adhesive layer was applied without either air thinning or light curing, followed by applying the second layer according to the manufacturer's instructions.

Group DL2 (Double-layer, air thinning without light curing the first layer) The first layer was applied without light curing, followed by the second layer according to the manufacturer's instructions.

Group DL3 (Double-layer, air thinning and light curing each layer) Each layer was applied with air thinning and light curing according to the manufacturer's instructions. Following dentin preparation with sandpaper, adhesive application procedures were performed immediately. Each adhesive system was polymerised for 10 s with an LED light unit (standard mode;1000 mW/cm^2, Valo, Ultradent Products Inc., South Jordan, UT, USA), and light intensity was measured with a radiometer (LM-1, Guilin Woodpecker Medical Instrument Company, Guangxi, China) before each group. 4-mm-thick composite resin build-ups (Filtek™ Z250; 3 M ESPE, USA) were made with 2 mm increments using the Adapt SuperCap matrix (Kerr, Bioggio, Switzerland), and the thickness of each increment was verified using a periodontal probe [12]. Each increment was polymerised for 20 s with an LED light unit (standard mode, Valo, Ultradent Products Inc.). One operator performed all specimen preparation, universal adhesive application, and composite resin build-up procedures.

Aging and microtensile bond strength (μTBS) testing

After the preparation, specimens were kept in distilled water at 37 °C for 24 h. Each tooth specimen was sectioned using a slow-speed diamond saw (Micracut 152, Metkon) under cutting fluid (Metcool II, Metkon) and water mixture cooling to produce 1 ± 0.1 mm^2 rectangular dentin-resin sticks. The precise dimensions of the stick specimens were measured with a digital calliper. Nine central stick specimens were selected from a single tooth for the dentin μTBS test and were randomly subdivided regarding the aging duration: immediate, 3 months, or 6 months of water storage. Sticks of the 3-month and 6-month water storage groups were stored in distilled water at 37 °C for the specified periods in separately compartmentalised containers labelled according to the tooth from which they were obtained. Therefore, 45 sticks were obtained from 5 teeth for each adhesive-application

procedure group, and 15 sticks were selected for each aging period.

For μTBS assessment, the sticks were fixed onto a modified Geraldeli's jig with quick-setting cyanoacrylate glue. The jig was placed in a universal testing machine (Shimadzu Corp., Kyoto, Japan) and subjected to a tensile force (cross speed: 1 mm/min). The force value of the fracture was divided by the bonded area, and μTBS was determined in MPa. The guide of the Academy of Dental Materials on μTBS test protocol was carefully followed during the preparation of the samples and testing [12].

Failure mode analysis

All the failure surfaces were examined with a stereomicroscope (Stemi C-2000; Zeiss, Oberkochen, Germany) at ×10 magnification. Fractured surfaces were classified as interfacial-adhesive failure, cohesive in resin composite, cohesive in dentin, or mixed failure.

Scanning Electron microscopy (SEM) evaluation

The adhesive interfaces were examined using five sticks for each group, one stick from each tooth, after excluding the nine central rods designated for the μTBS test. Specimens were prepared for SEM observation according to the protocol by Ting et al. (25). Specimens were scanned with FE-SEM (Mira 3 XMU, Tescan, Brno, Czech Republic).

Statistical analysis

Statistical analyses were performed using SPSS version 19 (IBM SPSS Statistics 19, SPSS Inc., an IBM Company, Somers, NY, USA). The μTBS data were analysed for normality with the Kolmogorov-Smirnov test, and the homogeneity of variance was assessed by the Levene test. The μTBS data were analysed using a Three-Way Analysis of Variance (ANOVA), and post-hoc comparisons were performed with the Bonferroni test ($\alpha = 0.05$).

Results

Micro tensile bond strength

Pre-test failures did not occur in stick specimens. The mean μTBS ± standard deviations of the groups are presented in Table 2. Repeated measure ANOVA results revealed that the adhesive system ($F = 367.797$, $p < 0.001$) and application procedure ($F = 15.198$, $p < 0.001$) had significant effects on μTBS, whereas the effect of water storage ($F = 0.557$, $p = 0.456$) was not significant.

The reference two-step self-etch adhesive, CSE, applied as per the manufacturer's instructions, showed higher μTBS than all tested universal adhesive systems, irrespective of application procedure and water storage ($p < 0.05$), except for the immediate μTBS of the SB subgroup DL2- air thinning without light curing the first layer. For the SL-MI groups, GP showed the lowest μTBS ($p < 0.05$), and the difference between SB and OB was not significant ($p > 0.05$). For the double-layer application groups (DL1, DL2, and DL3), universal adhesive systems (SB, OB, and GP) exhibited μTBS values in decreasing order, respectively ($p < 0.05$). For all universal adhesive systems and CSE, the immediate μTBS values of the groups were similar to those of 3-month and 6-month water storage μTBS values ($p > 0.05$).

For the immediate and 3-month water storage of SB and GP, the highest μTBS values were observed in Group DL2- air thinning without light curing the first layer, and the lowest μTBS values were observed in Group SL-MI ($p < 0.05$). The differences among double-layer application groups were not significant for SB and GP. For the OB, Group DL3- light curing each layer exhibited the highest μTBS values, and the lowest μTBS was exhibited in Group DL1- the first adhesive layer was applied without either air thinning or light curing ($p^{<}0.05$). In addition, the difference among the single-layer manufacturer's instructions, Group DL2- air thinning without light curing the first layer, and Group DL3- air thinning and light curing each layer was not significant ($p > 0.05$).

After 6 months of water storage, differences among the application procedures were not significant for GP and OB ($p > 0.05$). For SB, the highest μTBS was observed in Group DL2- without light curing the first layer, and the lowest μTBS was observed in the SL-MI group ($p < 0.05$). In addition, the difference between Group DL2 and Group DL3- air thinning and light curing each layer was not significant for SB ($p > 0.05$).

Interfacial SEM analysis of different application procedures

Representative interfacial surface SEM images of each adhesive and application group at 2500 × and 5000 × were presented in Fig. 1. Double application procedures increased adhesive layer thickness. The SCE group showed a higher adhesive layer thickness than all other groups. Gap formation between the adhesive resin and the dentin surface was observed in GP DL-2 and OB DL-3.

Fracture mode analysis

The result of fracture mode analysis is presented in Table 3. Adhesive fracture was the most common fracture type for all periods (immediate: 161/185, 3 months: 159/185, 6 months 166/185). Cohesive and mixed fracture types were more common in the specimens with higher bond strength and were proportionally highest in the CSE group.

Discussion

The most effective method for evaluating the bond strength of different adhesive systems and application techniques is μTBS testing, particularly when combined

Table 2 Micro-tensile bond strengths (μTBS) ± standard deviations (SD) in MPa to dentin

	Single Bond Universal			G-Premio Universal			Optibond Universal			Clearfil SE Bond		
	24-h	3-m	6-m	24-h	3-m	6-m	24-h	3-m	6-m	24-h	3-m	6-m
Group SL-MI (Single layer manufacturers' instructions)	47,44 ± 10,91 B, b,x	46,88 ± 9,60 B, b,x	46,34 ± 6,22 C, b,x	24.88 ± 5.58 B, c,x	24.66 ± 3.66 B, c,x	28.49 ± 4.15 A, c,x	43,58 ± 10,11 AB, b,x	41,59 ± 7,46 AB, b,x	42,90 ± 5,86 A, b,x	64,04 ± 13,64 a, x	66,10 ± 9,29 a, x	68,10 ± 8,38 a, x
Group DL1 (Double-Layer, the first adhesive layer was applied without either air thinning or light curing)	51,81 ± 8,59 AB, b,x	51,57 ± 8,08 AB, b,x	50,01 ± 4,04 BC, b,x	29.53 ± 6.89 AB, d,x	33.83 ± 7.47 A, c,x	32.82 ± 12.94 A, d,x	40,82 ± 4,48 B, c,x	34,88 ± 5,56 B, c,y	40,78 ± 6,58 A, c,x			**
Group DL2 (Double-layer, air thinning without light curing the first layer)	58,14 ± 7,70 A, a,x	56,87 ± 7,60 A, b,x	57,42 ± 5,51 A, b,x	34.67 ± 10.49 A, d,x	32.98 ± 6.26 A, d,x	34.34 ± 5.35 A, d,x	43,04 ± 6,75 AB, c,x	41,75 ± 4,44 AB, c,x	42,91 ± 4,18 A, c,x			**
Group DL3 (Double layer, air thinning and light curing each layer)	52,26 ± 8,64 AB, b,x	53,16 ± 10,22 AB, b,x	52,94 ± 5,80 AB, b,x	29.16 ± 6.67 AB, d,x	30.11 ± 4.61 AB, d,x	34.36 ± 5.05 A, d,x	43,46 ± 9,24 A, c,x	42,54 ± 10,04 A, c,x	40,29 ± 4,38 A, c,x			**

*The superscript uppercases indicate significant difference among application protocols (in vertical columns), the superscript lowercases indicate significant differences among adhesive systems (in horizontal rows) in a certain period of time ($p < 0.05$). 'x and y' indicate significant difference among aging periods (in horizontal rows) of a certain adhesive system ($p < 0.05$)

**NA

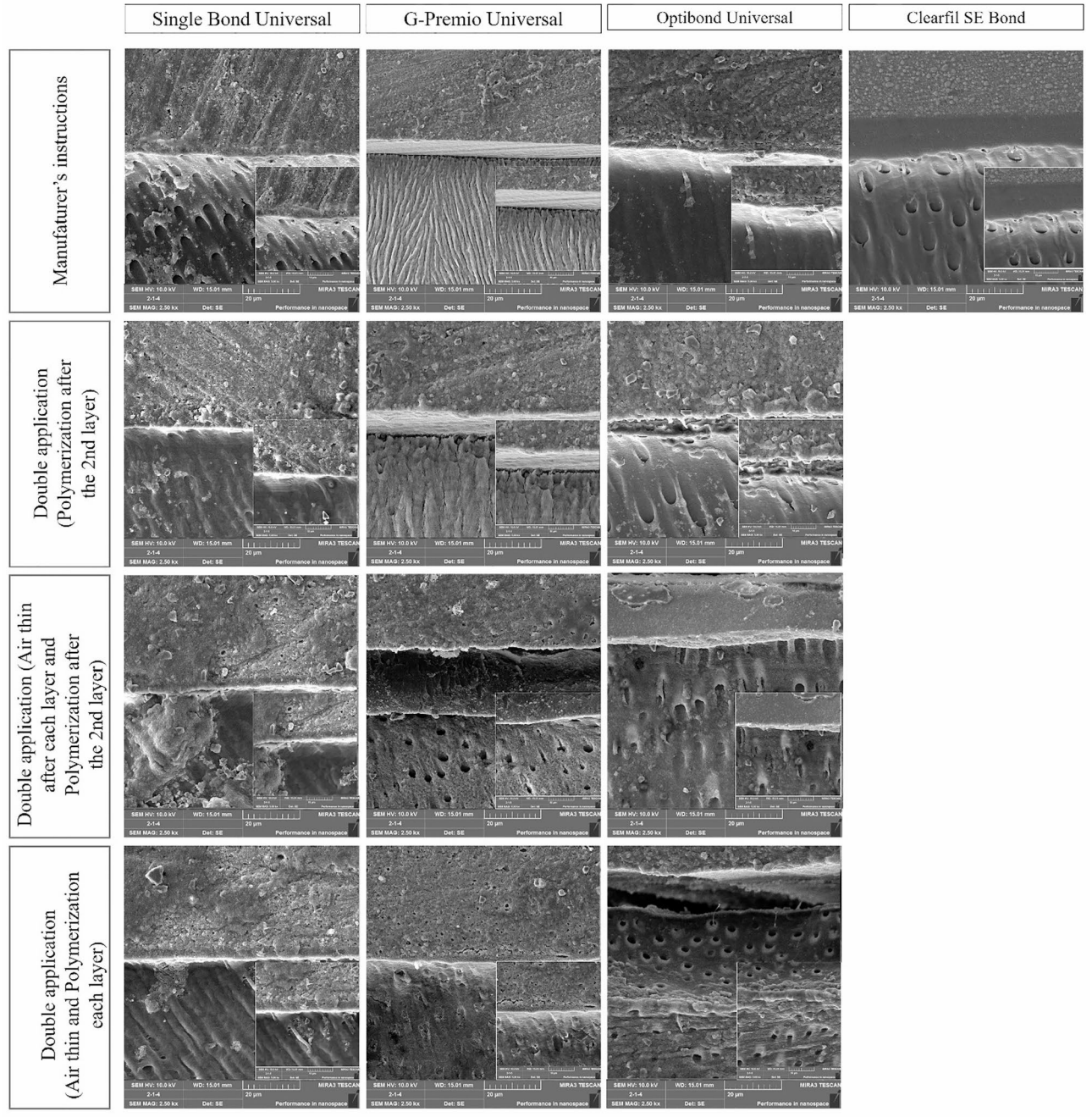


Fig. 1 Representative interfacial surface SEM images of each adhesive and application groups at 2500 × (inserts 5000x)

with aging periods [13]. This approach provides a more detailed and reliable assessment by simulating the long-term performance of adhesive interfaces, allowing for the evaluation of bond durability under clinically relevant conditions [12]. This study evaluated immediate, 3-month, and 6-month dentin μTBS of different universal adhesives applied with various double-layer application procedures.

The present study's findings indicate that DL2- air thinning without light curing the first layer improved the immediate and after 3-month water storage μTBS of GP and SB. In addition, for OB, DL3- double-layer application with air thinning and light curing each layer exhibited higher immediate and after 3-month water storage μTBS than the DL1- double-layer application, the first adhesive layer was applied without either air thinning or light curing. Therefore, the null hypothesis that (1) Double-layer application techniques do not increase the dentin μTBS of universal adhesive systems and (2) There is

Table 3 Distribution of fracture modes (A/CC/CD/M)*

	Single Bond Universal			G-Premio Universal			Optibond Universal			Clearfil SE Bond		
	24-h	3-m	6-m	24-h	3-m	6-m	24-h	3-m	6-m	24-h	3-m	6-m
Group SL-MI (Single layer manufacturers' instructions)	12/2/0/1	13/2/0/0	13/1/1/0	15/0/0/0	14/0/0/1	11/0/3/1	13/0/0/2	14/0/1/0	15/0/0/0	12/0/2/1	12/0/2/1	11/2/1/1
Group DL1 (Double-Layer, the first adhesive layer was applied without either air thinning or light curing)	8/3/3/1	10/0/4/1	14/1/0/0	13/1/1/0	15/0/0/0	12/0/0/3	13/1/1/0	15/0/0/0	13/1/0/1		**	
Group DL2 (Double-layer, air thinning without light curing the first layer)	13/2/0/0	11/2/1/1	12/1/0/2	12/2/0/1	12/0/3/0	12/2/1/0	13/1/0/1	13/0/2/0	15/0/0/0		**	
Group DL3 (Double layer, air thinning and light curing each layer)	13/1/1/0	13/1/1/0	11/1/1/2	10/3/0/2	14/1/0/0	12/2/0/1	14/0/0/1	15/0/0/0	15/0/0/0		**	

*A: Adhesive failure; CC: cohesive failure in resin composite; CD: cohesive failure in dentin; M: mixed failure

**NA

no difference among the different double-layer universal adhesive application techniques was partially rejected.

Despite advantages such as user-friendliness, reduced technique sensitivity, and acceptable bonding effectiveness, universal adhesive systems have a more hydrophilic structure with a thinner film thickness than two-step self-etch adhesives (Fig. 1.) [5, 14]. Three different clinical procedures were introduced in the literature to overcome the problems related to the thinner film thickness: (1) Application of an additional hydrophobic layer separately [3, 4, 6, 8]. (2) Application of a thin layer of flowable composite after adhesive [15]. (3) Double-layer application of the adhesive system [7]. The double-layer application of the adhesive system is easier to implement clinically as it does not require extra material. However, there is no consensus in the literature on the optimal method for double-layer adhesive application. In some of the studies, each adhesive layer was applied and light cured according to the manufacturer's instructions [16–18], while in other studies, the second layer was applied without polymerising the first layer [7, 19]. However, these studies do not specify whether the adhesive was thinned with air before the application of the second layer or not.

When immediate and after 6-month water storage dentin μTBSs were evaluated, Group DL2: air thinning without light curing the first layer, significantly increased the μTBS compared to single-layer application of GP and SB. Moreover, for GP and SB, DL1- the first adhesive layer was applied without either air thinning or light curing, and DL3- air thinning and light curing each layer, also enhanced μTBS; however, the increase was not statistically significant compared to the single-layer application. Double-layer application may benefit universal adhesives by enhancing etching ability through additional acidic monomers introduced with the second application [7, 10] and by mitigating the adverse effects of the oxygen inhibition layer and polymerisation shrinkage stress of the composite resin restoration by increasing the adhesive layer thickness [11, 20]. However, accumulating solvents and water in the adhesive could limit sufficient polymerisation, cause gap formation, and potentially compromise mechanical properties and bond strength [21]. The relatively lower μTBS values in Group DL1- without air thinning and light curing the first layer may be related to this issue. In addition, lower μTBS values in the Group DL3- air thinning and light curing each layer, may be due to the inadequate polymerisation caused by the oxygen inhibition effect when both layers were separately air-thinned and polymerised [20].

As an important finding of this study, the effect of double-layer adhesive applications has interesting results for the OB. Group DL1- the first adhesive layer was applied without either air thinning or light curing showed the lowest immediate and 3-month water storage μTBS,

while no significant difference was observed between the other double-layer and single-layer application procedures. Zheng et al. have stated that evaporation of the solvent before the light curing could be more difficult for a thicker adhesive layer [22], and filled adhesives may form a thicker adhesive layer in single-layer applications [23, 24]. Accordingly, OB has a 7% filler load and ternary solvent system (water, ethanol, and acetone), and the application of double layers without air thinning and light curing could adversely affect the removal of solvents from the adhesive layer.

After the 6-month water storage, Group DL2- air thinning without light curing the first layer, showed the highest μTBS for SB consistent with the short-term results. Furthermore, all double-layer application groups exhibited significantly higher μTBS than the single-layer group for SB. This finding suggests that SB benefits from double-layer application and thinning the first layer with air. SB is one of the most commonly used adhesive systems in previous studies, and similar to the results of this study, prior research has reported that a double-layer application increases the bond strength of SB [25, 26]. SB has a pH value of 2.7 and is classified as an ultra-mild self-etch adhesive system [2]. Double-layer application procedures may contribute to the μTBS by prolonging the active application and refreshing the acidic monomers [7].

Conversely, there was no difference in μTBS between different application procedures after 6 months of water storage for GP and OB. The deterioration in the adhesive structure with long-term water storage occurs by hydrolysis of hydrophilic monomers and collagen degradation [27]. The bond strength of universal adhesives after 6 months of water storage is considered to be closely related to the content of the adhesive system [28, 29]. GP has a lower pH value (pH: 1.5), and this may cause dentin over-acidification in the especially double layer application groups by extending the application time and formation of unprotected-collagen fibres in the hybrid layer, which may lead to enzymatic degradation over time [7]. These factors may explain the reduced bond strength in the double-layer application groups at 6 months.

Most universal adhesives, such as GP and SB, contain 10-methacryloxydecyl dihydrogen phosphate (10 MDP) as a functional monomer [3, 30]. The fact that SB shows higher μTBS than GP in all application procedures and evaluation periods may be due to additional components, such as Vitrebond in its structure, ethanol-based solvent chemistry, and its longer and active application procedure compared to GP. OB contains a ternary solvent system (water, ethanol, and acetone) and glycerol phosphate dimethacrylate (GPDM) as a functional monomer, and its application procedure is similar to SB. There is a limited number of studies in the literature evaluating the bond strength of OB [9], and similar to the others, the results of this study showed μTBS values between the SB and GP for OB.

Clearfil SE Bond (CSE), a two-step self-etch adhesive system, is widely recognized as the gold standard for dentin bonding [2]. Current literature suggests that applying universal adhesives in a double layer can positively influence bond strength [5, 8]. This study aimed to compare the increase in bond strength expected from a double-layer application with that of both a single-layer application of universal adhesives, following the manufacturer's recommendations, and the bond strength of the gold standard self-etch adhesive. As a result of separate primer and adhesive application steps, the reference two-step self-etch adhesive, CSE, has a more hydrophobic adhesive layer with higher mechanical properties and a 4 to 10 times thicker adhesive layer than universal adhesives [28, 31]. Accordingly, the results of this study showed that the CSE group demonstrated higher μTBS than all universal adhesive groups applied in single-layer and/or double-layer after 3 months and 6 months of water storage.

This study has some limitations that should be considered. Although aging was conducted through water storage for different periods, this method does not fully replicate the complexity of the oral environment. Future research should incorporate additional aging factors to simulate intraoral conditions better.

Conclusion

Within the limitations of this in vitro study, the application of universal adhesives in double layers by thinning the first layer with air and polymerisation after the second layer positively affects immediate and short-term μTBS. Applying universal adhesives in double layers without separately polymerising each layer did not provide additional benefits. Using universal adhesives with different double-layer applications did not improve the bond strength over the two-step self-etch adhesive system.

Abbreviations

HEMA	2-hydroxyethyl methacrylate
μTBS	micro-tensile bond strength
GP	G-Premio Bond, GC Corp., Tokyo Japan
OB	OptiBond Universal, Kerr, Orange, CA, USA
SB	Single Bond Universal, 3 M ESPE, St. Paul, MN, USA
CSE	Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan
SL-MI	Single layer manufacturer's instructions
DL1	Double-Layer, the first adhesive layer was applied without either air thinning or light curing
DL2	Double-layer, air thinning without light curing the first layer
DL3	Double layer, air thinning, and light-curing each layer
SEM	Scanning Electron Microscopy
ANOVA	analysis of variance
10 MDP	10-methacryloxydecyl dihydrogen phosphate

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Author contributions
GT.: Conceptualization; methodology; supervision; data curation; visualization; writing-original draft; writing-review and editing. HH.: Conceptualization; methodology; supervision; data curation; visualization; writing-original draft; writing-review and editing. KB.: Conceptualization; methodology; visualization; writing-review and editing.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study received ethical approval from the Ethics Committee of Tokat Gaziosmanpasa University, Faculty of Medicine (23-KAEK-114) and in accordance with the Declaration of Helsinki. Written informed consent with an easy Turkish language as mother language of participants was signed by the recruited participants to permit the use of the investigation data.

Consent for publication
Not applicable.

Informed consent
Informed consents were obtained from the patients.

Competing interests
The authors declare no competing interests.

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