



## Effect of Galardin and its Solvents on the Microtensile Bond Strength of Different Adhesive Systems to Dentin


Mohammad Esmaeel Ebrahimi Chaharom<sup>1</sup>, Mahmoud Bahari<sup>2</sup>, Mahdi Rahbar<sup>3,4</sup>, Sepideh Golbaz<sup>5</sup>


<sup>1</sup>Department of Operative Dentistry, Dental Faculty, Tabriz University of Medical Sciences, Tabriz, Iran.  0000-0002-8163-8510

<sup>2</sup>Dental and Periodontal Research Centre and Department of Operative Dentistry, Dental Faculty, Tabriz University of Medical Sciences, Tabriz, Iran.  0000-0002-1774-7659

<sup>3</sup>Department of Operative Dentistry, Dental School, Tabriz University of Medical Sciences, Tabriz, Iran.  0000-0002-9049-8169

<sup>4</sup>Research Center for Prevention of Oral and Dental Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran.

 0000-0002-9049-8169

<sup>5</sup>Department of Operative Dentistry, Dental Faculty, Tabriz University of Medical Sciences, Tabriz, Iran.  0000-0001-7153-1683

Author to whom correspondence should be addressed: Mahmoud Bahari, Dental and Periodontal Research Centre and Department of Operative Dentistry, Dental Faculty, Tabriz University of Medical Sciences, Gholgasht St., Daneshgah Ave., Tabriz, Iran. Phone: +98 413 335 5965. E-mail: [mahmoubahari@ymail.com](mailto:mahmoubahari@ymail.com).

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### Abstract

**Objective:** To evaluate the effect of a synthetic inhibitor of MMPs (Galardin) and its solvents [ethanol and dimethyl sulfoxide (DMSO)] on the microtensile bond strength ( $\mu$ TBS) of adhesive systems to dentin. **Material and Methods:** Sound human third molars ( $n=180$ ) were randomly assigned into 5 based on solution type: DMSO; ethanol; Galardin + DMSO; Galardin + ethanol; and distilled water as control. Then were further subdivided into 6 based on the adhesive system, i.e. 3-step and 2-step etch-and-rinse (ER), one-step and 2-step self-etch (SE) and universal in ER and SE strategies. The samples underwent a 500-round thermocycling procedure at  $5\pm 5/55\pm 5^\circ\text{C}$  and were sectioned into 1-mm<sup>2</sup> pieces perpendicularly in a cutting machine. The  $\mu$ TBS was measured at a strain rate of 1 mm/min. Data were analyzed with two-way ANOVA and post hoc Games-Howell tests ( $p<0.05$ ). **Results:** The adhesive system and the solution had significant effects on the  $\mu$ TBS ( $p<0.001$ ). The universal adhesive in the SE mode resulted in a significant decrease in  $\mu$ TBS compared to the other adhesives ( $p<0.05$ ). Ethanol, too, resulted in a significant decrease in  $\mu$ TBS compared to other solutions ( $p<0.05$ ). **Conclusion:** Galardin and its solvents, except for ethanol, had no detrimental effects on the immediate  $\mu$ TBS.

**Keywords:** Dental Materials; Dentin-Bonding Agents; Tensile Strength.

## Introduction

Complete penetration of resin into the network of collagen fibers is necessary in order to form a hybrid layer and achieve successful bonding to dentin. Several studies have shown that the integrity of the collagen matrix of dentin in the hybrid layer is a key factor for the longevity of bonded restorations [1,2].

The great resistance of the collagen matrix of dentin against heat and proteolytic agents is attributed to the high rate of intermolecular connections and the strong mechanical structure of this specific connective tissue [3,4]. However, from the time the presence of complex and active forms of matrix metalloproteinases (MMPs) were identified in the human dentin matrix, great attention has been directed toward the proteolytic activity potential of dentin [5]. MMPs are naturally found within the dentin matrix and are zinc- and calcium-dependent enzymes that have the capacity to break down the natural collagen tissue in a neutral pH [6].

Metalloproteinases have an important role in several physiologic and pathologic processes, including the breakdown of dentin collagen fibers [7,8]. It has been shown that the collagen matrix and gelatinolytic activity of human dentin matrix is inhibited by protease inhibitors [8-10]. One of these agents, Galardin (Ilomastate, GM6001), inhibits the protease activity. Galardin is the specific inhibitor of MMPs, introduced by Grobelny, and has an innate inhibitory activity on MMP1, 2, 3, 8 and 9 [11,12].

This agent has a collagen-like structure and binds to the active segment of MMP and the hydroxamate structure (R-Co-H-OH) and chelates the zinc found in the catalytic end of MMP [13]. The incorporation of some MMP inhibitors (MMPI) such as Galardin into the chemical structure of adhesives results in a significant increase in the immediate bond strength to dentin [14]. However, some authors [13] showed that irrigation of dentin with Galardin resulted in a 27% decrease in bond strength of adhesive systems after one year; nevertheless, it should be pointed out that this decrease was significantly less than that in the control group.

To use Galardin, this chemical substance should be dissolved in a solvent. DMSO is the specific solvent of Galardin. DMSO contains methyl and sulfur groups in its chemical structure and is a strong solvent for a wide variety of materials. It appears the negative ions of DMSO can react with the positively charged ions of calcium in tooth structure to form strong ionic bonds. The relatively high surface energy of this solvent and the strong bond formed between DMSO ions and Ca<sup>2+</sup> ions can affect the bond strength and improve it [15]. The wetting properties of DMSO are very similar to adhesives and result in improvements in the performance of adhesive bonds [16].

One of the other solvents of Galardin is ethanol, with a different effect on the stability of adhesive bonds. Studies have shown that adhesives with ethanol and water as solvents in their chemical structure have greater stability compared to adhesives with only ethanol as a solvent in their composition. An important consideration is the fact that when the adhesive is applied, the maximum amount of the solvent should be eliminated through evaporation because the solvent remnants can prevent polymerization, decreasing resistance against failure [17,18]. Previous

authors showed that alcohols could inhibit the dissolved MMPs and MMPs bound to the matrix of dentin [17]. In this context, the alcohol inhibits the formation of covalent bonds between catalytic zinc ions in the structure of MMP and the oxygen atom of the hydroxyl group of MMPs. Covalent bonds are one of the strongest intermolecular bonds and can exert a favorable effect on the bond strength [19].

A search in valid databases showed that despite the availability of various studies on the effect of Galardin on the bond strength of adhesives, which are sometimes contradictory, too, there is no study available to have evaluated the effect of different solvents of Galardin alone and in combination with Galardin on the immediate bond strength of different adhesive systems. Therefore, the aim was to evaluate the effect of different solvents of Galardin on the microtensile bond strength ( $\mu$ TBS) of different dentin bonding adhesives. The null hypothesis stated that different solvents of Galardin alone or in combination with Galardin have no effect on the immediate  $\mu$ TBS of different adhesives to dentin.

### Material and Methods

A total of 180 sound extracted human third molar teeth were included in the present in vitro study. The teeth were visually inspected and examined with the use of a dental explorer and only sound teeth with no caries, fractures or cracks were selected. The teeth were cleared with a brush and pumice slurry after extraction and stored in 0.5% chloramine T solution (Merck, Germany) until used for the purpose of the study (a maximum of 3 months). The occlusal surface enamel was eliminated by cutting the teeth perpendicular to their long axis with the use of cutting machine under water coolant. The cut surfaces were polished with 600-grit silicon carbide paper under running water for 30 seconds. Then the samples were randomly assigned into 5 groups in terms of the different solvents of Galardin as follows: G1: 100% DMSO (Duksun, Seoul, Korea); G2: Absolute ethanol (Duksun, Seoul, Korea); G3: 5  $\mu$ mol of Galardin (Sigma-Aldrich Company Ltd., Dorset, UK) in DMSO; G4: 5  $\mu$ mol of Galardin in ethanol (EG) and G5: Distilled water (control).

The samples in each group were divided into 6 subgroups in terms of the adhesive system used as follows:

- Subgroup 1: Three-step etch-and-rinse (ER) adhesive system (All-Bond 3, Bisco Inc., Schaumburg, IL, USA) (ALB);
- Subgroup 2: Two-step ER adhesive system (One-Step Plus, Bisco Inc., Schaumburg, IL, USA) (OSP);
- Subgroup 3: Two-step self-etch (SE) adhesive system (Clearfil SE Bond, Kuraray America, New York, USA) (CSE);
- Subgroup 4: One-step SE adhesive system (Clearfil S3 Bond, Kuraray America, New York, USA) (CS3);
- Subgroup 5: Universal adhesive system (All Bond Universal, Bisco Inc., Schaumburg, IL, USA) applied in the SE mode (USE);

- Subgroup 6: Universal adhesive system (All Bond Universal, Bisco Inc., Schaumburg, IL, USA) in the ER mode (UER).

After application of the adhesives according to manufacturers' instructions in each subgroup, all the dentin surface was covered with Valux Plus composite resin (3M Espe, St Paul, MN, USA) up to a height of 6 mm in three layers at an approximate thickness of 2 mm. Each layer was separately light-cured with Demetron A2 light curing unit (Kerr Dental, Scafatti, Italia) for 30 seconds. Then the samples underwent a 500-round thermocycling procedure at  $5\pm 5/55\pm 5^{\circ}\text{C}$  and were sectioned into 1-mm<sup>2</sup> pieces perpendicularly in a cutting machine. The  $\mu\text{TBS}$  was determined in each group in a  $\mu\text{TBS}$  test machine (Bisco Inc., Schaumburg, IL, USA) at a strain rate of 0.5 mm/min. After the  $\mu\text{TBS}$  tests, the failure modes were determined under a stereomicroscope (Nikon SMZ800, Nikon Corp., Tokyo, Japan) at a magnification of  $\times 10$  and categorized as follows: Type I: Cohesive failure in dentin; Type II: Cohesive failure in the composite resin block; Type III: Adhesive failure and Type IV: Mixed failure.

#### Statistical Analysis

Data were analyzed with IBM SPSS Statistics for Windows Software, version 16 (IBM Corp., Armonk, NY, USA) using two-way ANOVA. Statistical significance was set at  $p < 0.05$ . Post hoc Games-Howell test was used for two-by-two comparisons of the adhesives in case of significant difference.

#### Ethical Aspects

This research was approved by the Ethics Research Committee of the Tabriz University of Medical Sciences.

#### Results

Table 1 presents the means and standard deviations of  $\mu\text{TBS}$  values in the study groups. Kolmogorov-Simonov and Shapiro-Wilk tests were used to evaluate normal distribution of data, which showed that data were distributed normally ( $p > 0.05$ ). Levene's test was used to evaluate the homogeneity of the variance, which showed that this presupposition did not hold ( $p < 0.05$ ).

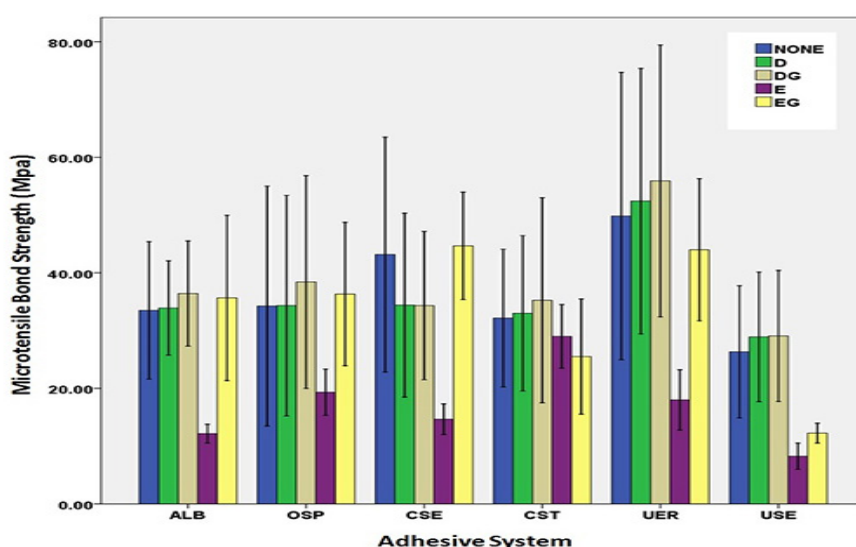
Two-way ANOVA showed that the type of irrigation solution and the type of the adhesive system significantly affected the  $\mu\text{TBS}$  ( $p < 0.001$ ). However, the cumulative effect of these two variables was not significant ( $p > 0.05$ ).

Base on the results of post hoc Games-Howell tests for two-by-two comparisons of the adhesives, the differences in the mean  $\mu\text{TBS}$  values between the USE group and other adhesive groups were significant except for the ALB groups ( $p < 0.005$ ) and conservatively significant in the case of the ALB group ( $p = 0.053$ ). However, the differences between the other adhesives were not significant ( $p > 0.05$ ) (Figure 1).

**Table 1. Mean and standard deviations (SD) of microtensile bond strength values and distribution of fracture patterns.**

Adhesive System	Solution	Microtensile Bond Strength		Fracture Pattern		
		Mean	SD	Adhesive	Mixed	Cohesive
ALB	Control	33.50	11.33	50.0	50.0	-
	DMSO	33.91	7.77	33.3	66.6	-
	DMSO+Galardin	36.41	8.67	-	33.3	66.6
	Ethanol	12.16	1.53	75.0	25.0	-
	Ethanol+Galardin	35.66	13.62	-	50.0	50.0
OSP	Control	34.25	19.78	16.6	50.0	33.3
	DMSO	34.33	18.20	-	66.6	33.3
	DMSO+Galardin	38.41	17.54	16.6	16.6	66.6
	Ethanol	19.33	3.81	50.0	50.0	-
	Ethanol+Galardin	36.33	11.82	33.3	33.3	33.3
CSE	Control	43.16	19.36	-	50.0	50.0
	DMSO	34.41	15.16	-	100.0	-
	DMSO+Galardin	34.33	12.22	-	66.6	33.3
	Ethanol	14.66	2.52	83.3	16.6	-
	Ethanol+Galardin	44.66	8.86	-	50.0	50.0
CS <sup>s</sup>	Control	32.16	11.36	16.6	50.0	33.3
	DMSO	33.00	12.81	33.3	66.6	-
	DMSO+Galardin	35.25	16.91	-	50.0	50.0
	Ethanol	29.00	5.24	66.6	33.3	-
	Ethanol+Galardin	25.50	9.49	50.0	50.0	-
UER	Control	49.83	23.69	-	66.6	33.3
	DMSO	52.41	21.89	-	50.0	50.0
	DMSO+Galardin	55.91	22.41	-	33.3	66.6
	Ethanol	18.00	4.96	66.6	33.3	-
	Ethanol+Galardin	44.00	11.72	16.6	33.3	50.0
USE	Control	26.33	10.89	66.6	33.3	-
	DMSO	28.91	10.69	50.0	50.0	-
	DMSO+Galardin	29.08	10.80	-	66.6	33.3
	Ethanol	8.25	2.16	100.0	-	-
	Ethanol+Galardin	12.25	1.63	50.0	-	50.0

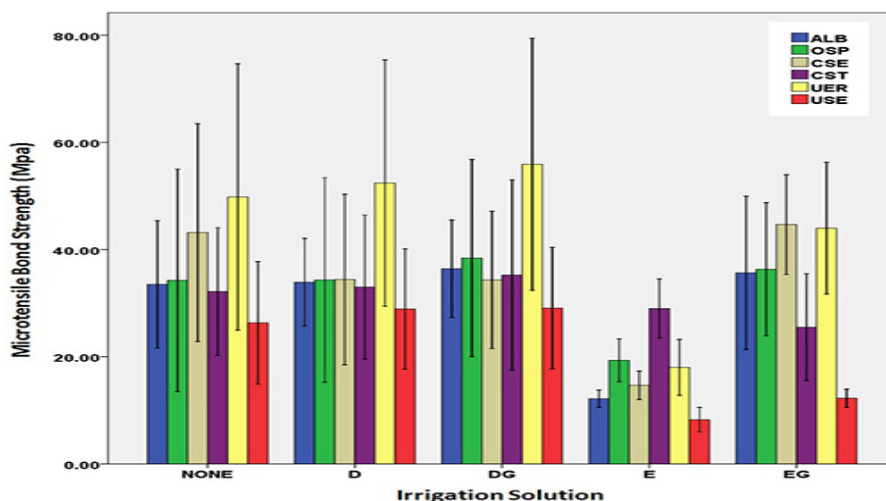
ALB (All Bond Universal); OSP (One Step Plus); CSE (Clearfil SE Bond); CS<sub>3</sub> (Clearfil S<sub>3</sub> Bond); UER (Universal Adhesive in Etch-and-Rinse Mode); USE (Universal Adhesive in Self-Etch Mode).



ALB (All Bond Universal), OSP (One Step Plus), CSE (Clearfil SE Bond), CS<sub>3</sub> (Clearfil S<sub>3</sub> Bond), UER (Universal Adhesive in Etch-and-Rinse Mode), USE (Universal Adhesive in Self-Etch Mode), D (DMSO), E (Ethanol), DG (DMSO + Galardin), DE (DMSO + Ethanol).

**Figure 1. Error-bar graph of microtensile bond strength values based on different adhesive systems.**

In addition, based on the results of this post hoc test in relation to two-by-two comparisons of different irrigation solutions, the differences in the mean  $\mu$ TBS values between the ethanol (E) and other solvents were significant ( $p < 0.05$ ). However, there were no significant differences between the other solutions ( $p > 0.05$ ) (Figure 2).



ALB (All Bond Universal), OSP (One Step Plus), CSE (Clearfil SE Bond), CS3 (Clearfil S3 Bond), UER (Universal Adhesive in Etch-and-Rinse Mode), USE (Universal Adhesive in Self-Etch Mode), D (DMSO), E (Ethanol), DG (DMSO + Galardin), DE (DMSO + Ethanol).

**Figure 2. Error-bar graph of microtensile bond strength values based on different solutions.**

## Discussion

MMPs and cysteine cathepsin found in dentin and dentinal fluid can decrease the bond strength over time due to their contribution to the enzymatic breakdown of the hybrid layer. Changes in pH due to acid etching, acidic monomers and adhesive resin monomers are factors involved in the regulation or changing the level of MMPs and cysteine cathepsin activity, which result in an increase in the breakdown of the hybrid layer collagen [20]. Use of MMPIs as a pretreatment before denting bonding has been suggested to prevent the breakdown of collagen fibers or to decrease the extent of breakdown and increase the bond strength [14].

The present study was carried out to evaluate the effect of a synthetic MMPI (Galardin) and its solvents (ethanol and DMSO) on the immediate  $\mu$ TBS of various adhesive systems to dentin. The results showed that ethanol could result in a significant decrease in the immediate  $\mu$ TBS compared to the control group, refuting the null hypothesis of the study. In fact, the results of the present study showed that a significant relationship between the  $\mu$ TBS and the type of the irrigation solution, with ethanol decreasing the bond strength significantly, compared to other solvents.

Consistent with the results of the present study, irrigation with 100% ethanol resulted in a significant decrease in the  $\mu$ TBS of ER adhesive compared to the control group (water) after 24 hours [21]. In addition, it was showed that use of absolute alcohol resulted in a non-significant decrease in  $\mu$ TBS compared to the control group [22]. Some authors evaluated the effect of ethanol and chlorhexidine (CHX) on the bond strength and reported that both solvents resulted in a non-

significant decrease in bond strength after 24 hours, with greater decrease in bond strength with the use of ethanol compared to CHX [23].

When simplified protocols are used, evaporation of water from the water-saturated collagen matrix before irrigation with absolute ethanol can result in the collapse and shrinkage of collagen fibrils. Collapse of the matrix of demineralized dentin is an active process, which results in rapid formation of hydrogen bonds between adjacent collagen peptides, resulting in hardening of the matrix under collapse conditions. By considering the theory of solubility parameters ( $\delta h$ ), different solvents such as ethanol ( $\delta h=20$ ), methanol ( $\delta h=24$ ) and water ( $\delta h=40.4$ ) have the capacity to break down inter-peptide hydrogen bonds and therefore can soften the matrix to some extent. In this context, the matrix expands rapidly when water and methanol are used but the rate of expansion is much slower when ethanol is used. Therefore, when the water present in the adhesive cannot re-expand the collapsed matrix after chemical dehydration of dentin with 100% ethanol, it can result in poor infiltration of monomer and formation of an inadequate hybrid layer of infiltrated resin [21].

It was previously reported that simplified dehydration protocols that use one to three applications of 100% ethanol should not be used in the ethanol wet bonding (EWB) technique because they result in a decrease in bond strength at both 24 hours and 6 months after bonding [24]. The use of the simplest EWB technique (30 seconds of irrigation with 100% ethanol) could completely replace water at the interface. Ethanol has a vapor pressure of 52.5 mmHg at 23°C, while the vapor pressure of water is 21.05 mmHg, i.e. ethanol more rapidly evaporates compared to water. Therefore, 30 seconds of irrigation is not sufficient to replace water with ethanol within the acid-etched intertubular dentin and dentinal tubules completely. Most possibly residual water in the acid-etched dentin results in poor wetting by the hydrophobic adhesive.

The effect of EWB and CHX on the bond strength of a hydrophobic 3-step ER adhesive showed that the samples bonded in the EWB group (with or without CHX) exhibited significantly higher  $\mu$ TBS immediately after bonding and after one year compared to the water wet bonding (WWB) group (with or without CHX) [25]. An improvement in the  $\mu$ TBS of bis-GMA/IEG-DMA hydrophobic adhesive resin to dentin under EWB condition might be justified by hydro solubility parameters. Replacing water within the acid-etched dentin with ethanol makes the dentin matrix more hydrophobic. The hydro solubility parameter for total cohesive forces ( $\delta t$ ) changes from 30.5  $(\text{J}/\text{cm}^3)^{1/2}$  to 25.1  $(\text{J}/\text{cm}^3)^{1/2}$ , allowing the bis-GMA/IEG-DMA ( $\delta t=21.2$   $[\text{J}/\text{cm}^3]^{1/2}$ ) adhesive resin to better be mixed with saturated dentin.

Therefore, EWB results in more effective infiltration of the bis-GMA/IEG-DMA hydrophobic resin into demineralized dentin compared to WWB, which is not consistent with the results of the present study because in the study above, a completely hydrophobic experimental adhesive was used [25] but in the present study adhesives with hydrophilic elements were used. In addition, in the present study there was a significant difference in the  $\mu$ TBS between solution E and solution EG, with a higher  $\mu$ TBS of solution EG compared to solution E; however, there was no significant difference in the  $\mu$ TBS between solution EG and the control group. It might be

hypothesized that incorporation of Galardin into ethanol compensated its effect on decreasing the  $\mu$ TBS, which might be attributed to its effect on solubility parameter.

Dimethyl sulfoxide was used as a criterion for measuring the surface energy due to its proper wetting properties and low viscosity and revealed that the wetting properties of DMSO are very similar to those of adhesives and result in improvements in the performance of adhesive bonds [16]. Therefore, it is possible that the high surface energy and proper wetting properties of DMSO result in proper wetting of the surface, allowing the adhesive to bond strongly to the tooth surface, increasing the bond strength. However, the present study showed that use of DMSO solution alone had no significant effect on the  $\mu$ TBS of the tested adhesives. Similarly, a previous study demonstrated that use of DMSO had no effect on the immediate bond strength [15]. However, after storage for 6 and 12 months, the bond strength in the control group significantly decreased in the control group and significantly increased in the DMSO group. In addition, after 6 and 12 months the control group exhibited significantly lower  $\mu$ TBS compared to the DMSO group.

However, it was showed that the bond strength of the three-step ER adhesive exhibited a significant increase after 24 hours when DMSO was used but it had no effect on the bond strength of SE adhesive [26], which is different from the results of the present study in relation to ER adhesive. Such discrepancy might be attributed to the differences in the type of the adhesive systems evaluated. There is polyalkenoic acid copolymer in the chemical structure of the adhesive used in the study by Stape. It has been showed that the demineralization process can begin by polyalkenoic acid with a high molecular weight, resulting in the exposure of the porous collagen network by selective dissolution of hydroxyapatite crystals [27]. In addition, the ionic bonds are formed between the carboxyl groups of polyalkenoic acid and calcium in the residual hydroxyapatite crystals, which might increase the bond strength. In another research, DMSO was used at a concentration of 50% [26]; however, in the present study its concentration was 100%. Furthermore, DMSO up to a concentration of 4% had no effect on the immediate bond strength [26].

In the present study, groups in which Galardin solution in DMSO was used did not exhibit a significant difference in the immediate  $\mu$ TBS of different adhesives compared to the control group. Similarly, it was showed that use of Galardin had no effect on the immediate bond strength of two-step ER adhesive; however, it resulted in significant breakdown of the bond after one year [13]. However, it was showed that incorporation of some MMPI, such as Galardin and batimastat (BB94), into the chemical structure of adhesives resulted in a significant increase in immediate bond strength to dentin [14], which is different from the results of the present study. Such a discrepancy might be attributed to the fact that in the study above Galardin was used in the chemical structure of the adhesive primer, while in the present study it was used in the form of a solution in ethanol and DMSO solvent and in a separate one-step procedure. In addition, the adhesive systems used in the two studies were different from each other. A previous study evaluated the effect of different concentrations of Galardin on the shear bond strength of self-adhesive resin cements to dentin and reported that Galardin dissolved in DMSO resulted in an increase in the shear bond strength of resin



cement at both high and low concentrations and the increase was directly correlated with the concentration of Galardin [28]. The discrepancy between the results of the study above and the present study might be attributed to differences in the materials used in the two studies; in the study above self-adhesive resin cement was used, while in the present study different adhesive systems were used. In addition, in the study above the shear bond strength was evaluated but in the present study,  $\mu$ TBS was evaluated. Similarly, researchers evaluated the effects of other MMPIs (benzalkonium chloride, CHX and EDTA) on the  $\mu$ TBS of universal adhesives and concluded that use of MMPIs had no effect on the short-term  $\mu$ TBS in the All Bond Universal adhesive system with ER strategy [29].

As another important finding of the present study, the  $\mu$ TBS exhibited a significant relationship with the type of the adhesive system, with significantly lower bond strength with the USE adhesive system compared to other adhesive systems, except for ALB; however, the difference in the  $\mu$ TBS of USE and ALB adhesive can be considered significant in a conservative manner. Similarly, it has been reported that All Bond Universal adhesive in the SE mode (AlSe) exhibited a significantly lower bond strength compared to the ER mode and its bond strength was lower than those of the other mild SE adhesives were [30]. Recent meta-analysis did not report a significant difference in the bond strength between ER and SE strategies for mild universal adhesives; however, in relation to the ultra-mild All Bond Universal adhesive, the bond strength with the ER strategy was significantly higher than that with the SE strategy [31]. In other words, All Bond Universal adhesive was the only universal adhesive that exhibited a decrease in bond strength with the SE strategy compared to the ER technique. SE adhesives are different from many aspects, especially in the composition of resin monomer, water contact and acidity. Adhesives with lower acidity are more sensitive to variations in the underlying layers [32]. The thickness of the smear layer, biofilm and any surface change or contamination can prevent a direct contact between the adhesive and the sound tooth structure, preventing the formation of an effective bond [33,34]. In the present study, AlSe had a pH of 3.1 and is considered an ultra-mild adhesive, while CS3 had a pH of 2.6 and CSE had a pH of 2, with greater acidity.

It is believed that the smear layer forms a real physical barrier, making it very difficult to form a bond and a hybrid layer and its complete integration with dentin. After initial etching with phosphoric acid, the smear layer is removed and the surface layer of dentin is demineralized, resulting in greater penetration of the adhesive and formation of a well-impregnated hybrid layer [35,36]. However, it has been showed that All Bond Universal adhesive in the SE mode did not result in a significant difference in the  $\mu$ TBS of the adhesive to dentin compared to the ER mode; however, in the ER mode longer resin tags and a thicker hybrid layer were observed [37]. In other words, use of an etching step before applying the adhesive resulted in greater penetration of the adhesive into dentin; however, it had no effect on the adhesive bond strength to dentin after 24 hours. They attributed the differences between the results of studies, in which bond strength of AlSe adhesive was lower than that in the ER mode, to the use of the active adhesive. Several studies have

shown that use of active self-etch adhesives compared to their use based on manufacturer's instructions results in significant improvements in the bond strength [38,39].

In relation to failure modes, based on the data of the study, in the USE adhesive subgroup with ethanol solution, all the failures (100%) were adhesive and in the CSE adhesive with solvent D, all the failures (100%) were mixed. Irrespective of the type of the adhesive, in solution E the most frequent failure mode was adhesive and in the solution EG there was mixed failures, indicating an increase in bond strength by solution EG compared to solution E in the present study. In addition, in none of the groups irrigated with ethanol solution, cohesive failure was observed, while with other irrigation solutions a mixture of different failure modes was observed.

In different subgroups of USE adhesive, adhesive failure was predominant failure mode compared to other failure modes; this indicates that compared to other adhesive groups in which the mixed failure mode was dominant, the results of the present study confirmed the lower bond strength of USE adhesive compared to other adhesives.

The discrepancy between the results of the present study and some previous studies, regarding the fact that use of DMSO and Galardin solutions might improve the immediate bond strength, shows that further studies are necessary on the subject. Such studies should evaluate different time intervals in vivo to assess the longevity of the bond to dentin over time.

## Conclusion

The results of the present study showed that  $\mu$ TBS was affected by the type of the adhesive system and the type of the irrigation solution. In this context, USE adhesive resulted in a significant decrease in the  $\mu$ TBS compared to other adhesive types. In addition, ethanol solution resulted in a decrease in  $\mu$ TBS compared to other solutions and the control group.

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**Conflict of Interest:** The authors declare no conflicts of interest.

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