

Original Article

Effect of accelerated aging and double application on the dentin bond strength of universal adhesive system

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ABSTRACT

Background: Despite the many advantages of simplified adhesive systems, there are concerns about the durability of the adhesive layer over time. The aim was to investigate the effects of various aging methods and double application of an adhesive layer on the bond strength of the universal adhesive system using etch-and-rinse (ER) and self-etch (SE) strategies.

Materials and Methods: In this *in vitro* study, the occlusal enamel of 120 extracted, intact human third molars was removed to expose the dentin. Then, the samples were randomly divided into four groups of thirty according to All-Bond Universal (ABU) adhesive application strategy (ER and SE) and the number of adhesive layers (1 or 2). Then, each group was subdivided into three subgroups of ten according to aging method (control, thermal cycling, and 10% sodium hypochlorite [NaOCl]). The shear bond strength was measured at the strain rate of 0.5 mm/min. Data were analyzed using three-way ANOVA and Tukey's *post hoc* tests ($P < 0.05$).

Results: The effect of adhesive application strategy ($P < 0.001$) and aging method ($P < 0.001$) on the bond strength was statistically significant, but the effect of the double application was not statistically significant ($P > 0.05$). In addition, the interactive effect of adhesive application strategy–aging method was significant ($P = 0.005$).

Conclusion: Using ABU with ER strategy leads to higher dentin bond strength compared to the SE method in the control and thermal cycling groups. However, no significant differences were observed between ER and SE strategies after aging with the NaOCl. Furthermore, the double application might not have any effect on the bond strength and durability.

Key Words: Adhesives, aging, all-bond system, dentin, sodium hypochlorite

Received: 20-Jul-2019
Revised: 14-Dec-2019
Accepted: 03-May-2020
Published: 06-Apr-2021

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INTRODUCTION

Simplified adhesives are very attractive for the clinicians because of saving time, ease of use, and reduced technical sensitivity. Simplification of adhesive systems became possible by introducing

hydrophilic monomers and increasing the solvent content of adhesive formulation to make the adhesive compatible with the wet dentin substrate. However,

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How to cite this article: Bahari M, Oskoei SS, Chaharom ME, Kahnemoui MA, Gholizadeh S, Davoodi F. Effect of accelerated aging and double application on the dentin bond strength of universal adhesive system. Dent Res J 2021;18:25.

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in this way, more residues of solvent remain in the adhesive layer after evaporation, possibly delaying the formation of a highly cross-linked polymer, reducing the degree of conversion, and increasing the permeability of the adhesive layer. As a result, the interface will be highly susceptible to degradation over time.^[1,2]

Reductions in the bond strength, which are usually seen in long-term studies, are due to the hydrolysis of collagen fibrils, which leads to the destruction of hybrid layer. The bond strength and durability are affected by the resin infiltration extension into exposed collagen fibrils. Ideally, adhesive monomers should fully infiltrate the interfibrillar spaces. It has been shown that there is a correlation between the infiltration of dentin by the adhesives and the thickness of the adhesive with rheological and chemical features; however, they might also be affected by the application strategy.^[3,4] Various strategies such as preetching,^[5] increased air-drying time,^[6] warm air-drying,^[7] double application,^[8] active agitation,^[9] or the addition of a hydrophobic layer^[10] have been proposed to reinforce this variable adhesive layer of simplified adhesives.

The double application increases the resin saturation inside the collagen network, thereby increasing the quality of the resin–dentin interface. In addition, it can easily be done at the chairside. However, several studies have shown that the influence of double application on the bond strength and bond durability of self-etch (SE) adhesive systems depends on adhesive type that cannot be generalized.^[11,12] It has also been reported that the double application of one-step SE adhesives could probably lead to a uniform infiltration of the adhesive into smear layer-covered dentin if a one-step SE adhesive is applied in two layers.^[13,14] However, Fujiwara *et al.* reported that the double application might be ineffective for two-step SE adhesives for clinical applications.^[12]

Multimode or universal adhesive systems (UASs) have newly been introduced, claiming that one monomer solution can be used with both adhesive strategies without affecting the bonding performance. The main advantage of these systems is that they can be used in both etch-and-rinse (ER) and SE strategies and the so-called select-etch strategy. Therefore, this feature has attracted the attention of clinicians.^[13,15,16] Yet, some researchers believe that the bond quality of UASs is like the conventional single-step SE adhesives without showing progress. Furthermore, it has been reported that the adhesive layer of UASs

might susceptible like other SE adhesives.^[17,18] Furthermore, despite the ever-increasing popularity of UASs, there are concerns about bond durability. Therefore, the purpose of this study was to investigate the effects of various aging methods and double application on the bond durability of the ultra-mild UAS applied with ER and SE strategies.

MATERIALS AND METHODS

In this *in vitro* study, 120 extracted, carious-free, and intact human impacted third molars were stored at the temperature of 4°C for 1 month in a 0.5% chloramine-T solution. To determine the sample size, the results of the pilot study and the formula

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

were used. Considering $\alpha = 0.05$, $Z_{1-\beta} = 1.28$, $Z_{1-\alpha} = 1.96$, $\mu_1 = 40.76$, $\mu_2 = 27.47$,

$S_1 = 20.17$, $S_2 = 7.22$, the sample size calculated 10 for each subgroup and 120 in total.

The occlusal enamel was removed using a low-speed diamond disk (Isomet Low-Speed Saw, Buehler, Lake Bluff, IL, USA) under abundant water spray to expose the dentin of occlusal surface and was finished by 600-grit silicon carbide papers (Struers, Cleveland, OH, USA) for 15 s to standardize the smear layer.

In this study, All-Bond Universal (ABU) adhesive system (Bisco, Schaumburg, IL, USA) was used. After preparing smooth dentin surfaces, the samples were randomly divided into four groups of thirty according to UAS application strategy (ER and SE) and the number of adhesive layers (one layer according to the manufacturer's instruction and two layers according to double-application strategy):

Group 1: Etch-and-rinse – one layer

Etchant gel (37% phosphoric acid, Ultra-Etch, Ultradent, South Jordan, USA) was applied for 15 s and rinsed for 10 s. After drying for 10 s, the ABU was applied according to the manufacturer's instructions in one layer and light cured by light-emitting diode (LED) light-curing unit (Demetron A.2, Kerr, Scafati, Italia) with a capacity of 1000 mw/cm² for 10 s.

Group 2: Etch-and-rinse – two layers

All stages were similar to the first group, except that an extra layer was applied before the photopolymerization of the first layer of adhesive.

Group 3: Self-etch – one layer

ABU was applied according to manufacturer's instructions for SE method in one layer and then light cured by LED light-curing unit (Demetron A.2, Kerr, Scafati, Italia) with a capacity of 1000 mw/cm² for 10 s.

Group 4: Self-etch – two layers

All stages were similar to Group 3, except that an extra layer was applied before the photopolymerization of the first layer of adhesive.

After bonding procedures, Z250 composite resin (3M ESPE, St. Paul, MN, USA) was applied at a height of 5 mm and a diameter of 3 mm using transparent plastic cylinders and light cured for 40 s on each side. One person performed all bonding steps. It should be noted that the intensity of light was evaluated periodically by a radiometer (Demetron, Model 100, Kerr, Danbury, CT).

Then, each group was subdivided into three subgroups of ten according to aging method. In the first subgroup as control, the samples were stored in distilled water for 24 h at 37°C. In the second, thermal cycling was performed by applying 3000 cycles at 5°C–55°C with dwell time of 30 s for each and the transfer time of 5 s. In the third, samples were stored in 10% sodium hypochlorite (NaOCl) (Ogna Laboratory Farmaceutici, Muggio, Italy) for 3 h. The shear bond strength (SBS) was measured using a universal testing machine (Hounsfield Test Equipment, Model H5KS, Surrey, UK) at a crosshead speed of 0.5 mm/min. The SBS was calculated by dividing the peak failure force (N) into the bonded surface area (mm²) in MPa. Data were analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using three-way ANOVA and Tukey's *post hoc* test with a significance level of $P < 0.05$.

RESULTS

The mean and standard deviation of bond strength values are summarized in Table 1. The Kolmogorov–Smirnov test showed that the data have a normal distribution ($P = 0.18 > 0.05$). Three-way ANOVA showed that the effect of adhesive application strategy ($P < 0.001$) and aging method ($P < 0.001$) on bond strength was significant, but the effect of the double application was not statistically significant ($P > 0.05$). In addition, the interactive effect of adhesive application strategy–aging method was significant ($P = 0.005$), but in other cases,

the interactive effect of the two variables was not significant ($P > 0.05$).

Two-by-two comparison of various aging methods with Tukey's *post hoc* test showed that there are significant differences between the aging methods with each other as well ($P < 0.001$). Furthermore, SBS values in the control and thermal cycling groups in the ER application method were significantly higher than that in SE method ($P < 0.001$), but there was no significant difference between the adhesive application strategies in the NaOCl group ($P > 0.05$). The error bar diagram associated with comparing SBS values in groups and subgroups is shown in Figure 1.

DISCUSSION

Since clinical trials are expensive and take a long time to do, artificial aging is implemented for laboratory simulations. Among the several methods, thermal cycling and water storage have been favored. Thermal stresses are capable of acting in two ways: first, crack propagation could be directly induced by cyclic thermal fluctuations through adhesive interfaces, and second, through changing gap dimensions could aggravate the percolation of oral fluids.^[19,20] The ISO TR 11405 (1994) suggested that protocol for thermal cycling is 500 cycles at 5°C–55°C with dwell time of >20 s.^[20] However, controversies exist regarding

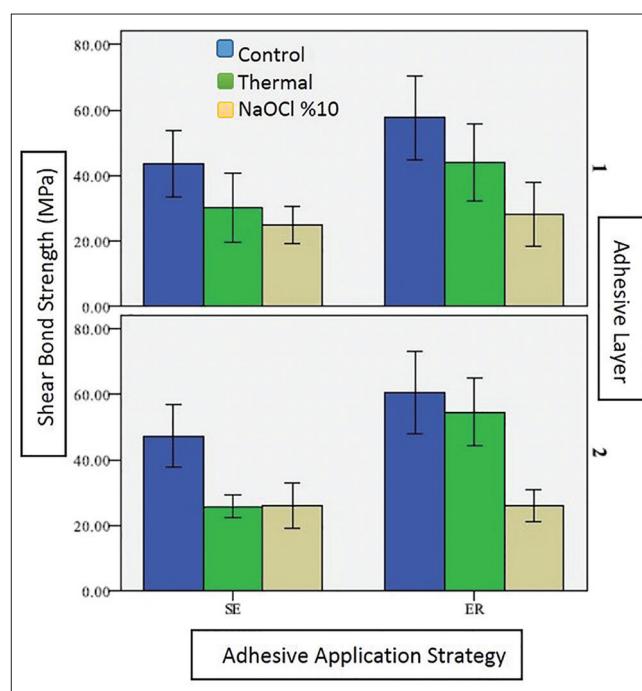


Figure 1: The error bar diagram showing a 95% confidence interval of mean bond strength values.

Table 1: The mean (MPa) and standard deviation of shear bond strength values

Adhesive application strategy	Number of adhesive layers	Aging methods	Mean SBS (MPa)	SD	SEM	Maximum	Minimum
ER	1	Control	57.65 ^{Aa}	18.04	5.70	80.86	36.57
	1	Thermal cycling	43.85 ^{Aa}	16.50	5.21	74.29	22.86
	1	NaOCl 10%	28.34 ^{Ba}	13.59	4.30	52.86	13.48
	2	Control	60.43 ^{Aa}	17.60	5.56	84.76	33.48
	2	Thermal cycling	54.61 ^{Aa}	14.37	4.54	83.81	39.62
	2	NaOCl 10%	26.03 ^{Ba}	6.62	2.09	40.71	18.81
SE	1	Control	43.53 ^{Aa}	13.99	4.42	68.09	27.14
	1	Thermal cycling	30.03 ^{Aa}	14.75	4.66	65.71	17.62
	1	NaOCl 10%	24.97 ^{Ba}	7.89	2.49	38.95	16.19
	2	Control	47.15 ^{Aa}	13.29	4.20	70.00	30.05
	2	Thermal cycling	25.68 ^{Aa}	4.86	1.53	34.14	17.48
	2	NaOCl 10%	26.00 ^{Ba}	9.69	3.06	44.14	15.09

Similar lowercase demonstrates no statistically significant differences between one and two layers of adhesive ($P>0.05$). Different uppercase shows statistically significant differences according to adhesive application strategy and aging method ($P<0.05$). SD: Standard deviation, SEM: Standard error of mean, NaOCl: Sodium hypochlorite, ER: Etch-and-rinse, SBS: Shear bond strength

the number of cycles corresponding to 6 months and 1 year of physiologic aging in the oral cavity. Some authors suggested that 10,000 cycles correspond to 1-year *in vivo* aging. However, most of the authors applied a number of cycles $<10,000$, showing that 10,000 cycles do not correspond to 1 year of *in vivo* aging. We used 3000 cycles in this study, which was applied by many authors.^[20,21]

Recently, the adhesive interface aging with a 10% NaOCl solution has been proposed as well. NaOCl is a nonspecific deproteinase that forms superoxide radicals in aqueous solution, which can cause peptide ring oxidation in proteins such as collagen.^[22] This degradation potential is responsible for removing organic components from the interface dentin, which is due to its ability to dissolve collagen fibrils enclosed by adhesive resins.^[22,23] It has been shown that aging in 10% NaOCl for 1 h and 3 h indicates very similar degradation patterns and microtensile bond strength to those in aging for 6 months and 12 months of water storage.^[22] Therefore, the storage of samples in a 10% NaOCl solution is a fast and reliable method for testing the adhesive interface durability.

The findings of this study showed that both aging procedures significantly reduced the bond strength. In addition, the bond strength in the NaOCl aging was also significantly lower than the thermal cycling group. Unprotected collagen fibrils in the base of hybrid layer may cause higher water absorption and inflation of polymer materials, which may generate the host-derived protease reaction, degrade collagen fibrils, and deteriorate the adhesive bond.^[24] Furthermore, differences in hydrophilicity

due to different concentrations of 2-hydroxyethyl methacrylate (HEMA) in the adhesive composition can also affect the hydrolytic stability of adhesives. HEMA can keep water, and water absorption is strengthened in the presence of HEMA. Given that ABU is an adhesive containing HEMA, aging can cause hydrolytic degradation and reduce the strength of this adhesive interface, as time goes on.^[25-27]

Similarly, Taschner *et al.* reported a significant decrease in bond strength of another HEMA-containing UAS, Scotchbond universal, after 6 months of water storage and NaOCl aging. Furthermore, they showed that NaOCl aging decreases bond strength more than that by water storage.^[11] Another recent study revealed a significant reduction in bond strength of several UASs including ABU after 1 year of water storage as well.^[18] Infiltration of adhesive monomers into interfibrillar collagen spaces depends not only on hydrophilicity but also on the molecular size of monomers. Demineralized collagen network acts as molecular sieves, in a manner in which molecules smaller than 1000Da could easily be diffused into interfibrillar collagen spaces, whereas larger molecules could not.^[28] It seems that the effect of HEMA in water sorption and bond deterioration is so much so that it can neutralize the positive effect of 10-MDP. Considering that 10-MDP has a relatively stable hydrophobic bond with collagen, while HEMA does not interact with collagen, interaction between these molecules in adhesive composition may produce aggregates that reduce the hydrophobicity of 10-MDP and compromise its interaction with collagen.^[29,30] While, as suggested in the literature,

10-MDP-containing adhesives have better bond durability.^[31,32]

As another remarkable finding, the present study showed that the double application of the UAS has no significant effect on the dentin bond strength. On the other hand, the double application did not increase the immediate bond strength and durability. This is in agreement with Taschner *et al.* who reported that UAS showed no difference between single- and double-application strategies irrespective of storage condition. Contrarily, Fujiwara *et al.* showed that the double application of UAS increases the SBS and shear fatigue strength.^[12] Although it is not possible to justify such a difference in the results of studies with certainty, the bond strength of the UAS and its durability seems to be largely influenced by its application strategy to the substrate and to some extent its acidity. Contrary to the present study, Fujiwara *et al.* applied UAS with active agitation method with twice time as long as manufacturer's instructions. Furthermore, they used UAS which has more acidity than the ABU. Several studies have shown that the active agitation and the longer duration of application cause functional monomers to penetrate more into the dentin structure, which results in more uniform adhesive layer. Furthermore, In the case of ABU, there are two different solvents in the composition (ethanol and water), which have different evaporation power. In a thinner layer, these two substances are easily evaporated, but in a thicker layer, ethanol evaporates faster and the volume of ethanol decreases before reaching the azeotrope, allows the resin monomers to be fall out in the solution, and causes phase separation inside the adhesive layer. This causes incomplete evaporation of the remainder of the adhesive water, reduces the degree of conversion, and reduces its mechanical properties.^[9,33]

Regarding the efficiency of the adhesive application strategy, the results showed that bond strength in ER was greater than SE in the control and thermal cycling groups. However, there was no significant difference between SE and ER in the NaOCl group. Similarly, several studies demonstrated that the ABU adhesive in SE mode shows significantly lower bond strength compared to the ER. In other words, the ABU is the only UAS with lower bond strength in the SE mode compared to ER. ABU is an ultra-mild adhesive (PH = 3.1). The low acidity of ABU is inadequate for effective dentin etching and infiltration of monomers. Etching with phosphoric

acid removes the smear layer, accelerates the surface dentin demineralization, and results in the formation of a thick hybrid layer, which is fully integrated with dentin.^[32,34] However, Wagner *et al.* showed that the bond strength of ABU in SE mode is not significantly different compared to ER. The reason for such differences in the literature may be related to the adhesive application strategy.^[35] It has been shown that the use of ultra-mild UAS with active agitation strategy can significantly improve bond strength in comparison with their use according to the manufacturer's instructions.^[9]

It should be pointed out that the present study was *in vitro* and has been designed based on SBS test where the force is applied to the interface monotonically and gradually increasing until the bond is broken. This is very different from clinical conditions and cannot simulate the frequent and cyclic loading factor, which is very important in interface fatigue failure. Therefore, care should be taken in generalizing the results to clinical conditions.

CONCLUSION

Considering the limitations of this experimental study, it can be concluded that:

- Using ultra-mild UAS with ER strategy creates higher dentin bond strength compared to the SE method in the control and thermal cycling groups. However, no significant differences were observed between the two application strategies in the NaOCl-aged group
- The double application might not have any effect on the bond strength and durability of the ultra-mild UAS in any of the ER and SE strategies.

Acknowledgments

The authors express their grateful thanks to Dental and Periodontal Research Centre and the Vice-Chancellor of Research and Technology of Tabriz University of Medical Sciences for their support.

Financial support and sponsorship

This study was financially supported by Dental and Periodontal Research Centre at Vice-Chancellor for Research and Technology, Tabriz University of Medical Sciences, Tabriz, Iran.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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