Original Article

대한치과재료학회지 48(3) : 135-146, 2021
Korean Journal of Dental Materials (*Korean J Dent Mater*)
ISSN:2384-4434 (Print); 2384-3268 (Online)
Available online at http://www.kadm.org
http://dx.doi.org/10.14815/kjdm.2021.48.3.135

새로 출시된 범용 상아질 접착제의 교반 시간과 건조 시간이 상아질 접착 강도에 미치는 영향

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The effect of agitation and evaporating time of a newly released universal adhesive on dentin bond strength

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접착과정을 단순화하고 다양한 접착모드(산부식 형태)로 사용이 가능한 범용 상아질 접착제가 개발되어왔다. 본 연구에서는 범용 상아질 접착제의 교반 시간 및 건조 시간을 달리 하여, 접착제의 적용 방법이 미세인장접착강도에 미치는 영향을 평가하였다. 18개의 발거 된 인간 대구치를 사용하여 시편을 제작하고, 교반 시간(5, 10, 20초)과 건조 시간(10, 20초)에 따라 6개 그룹으로 나누었다. 시편을 단면적 1 mm^2의 막대 모양으로 절단하여 미세인장접착강도를 측정하였다. 데이터는 ANOVA 및 Tukey's post-hoc test로 분석하였다. 이 후, 주사전자현미경으로 탈락한 표면을 관찰하였다. 용매 증발 시간이 20초인 그룹은 교반 시간이 짧더라도 미세인장접착강도가 유의하게 더 높았다($p<0.05$). 또한, 용매 증발 시간이 짧은 그룹은 교반 시간이 짧아짐에 따라 미세인장접착강도가 유의하게 감소했다($p<0.05$). 본 실험의 결과는 용매를 충분히 건조시킨다면 접착제를 단시간 교반하더라도 충분한 접착강도를 얻을 수 있음을 보여주었다. 이는 주사전자현미경 이미지에 의해 뒷받침되며, 건조 시간이 긴 그룹에서 resin tag가 더 잘 형성되어 있는 접착층이 관찰되었다. 범용 상아질 접착제가 우수한 접착강도를 가지기 위하여 적절한 건조 시간이 확보하는 것이 중요하다.

색인단어 : 교반, 용매 건조, 범용 상아질 접착제, 미세인장접착강도, 주사전자현미경

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Received: Jul. 16, 2021; Revised: Sep. 17, 2021; Accepted Sep. 17, 2021

Introduction

Dental adhesives are used to bond resin composites to enamel or dentin when the restoration is required for the damage of the tooth substrate caused by caries or fracture. These can be classified into 2 types-total etch and self-etch bonding agent-depending on the necessity of the etching procedure. Recently universal adhesives that can be used in both etching modes have been introduced. When these multi-mode universal adhesives are used in self-etch bonding system, the acidic monomers contained in the adhesive remove minerals in the hard tissue without any additional etching process, enabling micro-mechanical bonds (1). When the universal adhesive is applied in practice, the application step and the operator's sensitivity can be reduced to make the bonding procedure more convenient. In the general clinical procedure, a few drops of universal adhesives are rubbed on the exposed dentin surface using a micro-brush. The clinician then uses air from a three-way syringe to evaporate the solvent included in the universal dental adhesives, followed by photo-polymerizing.

Agitation is the process of rubbing the adhesive on the tooth surface while the functional monomers in the universal adhesive dissolve and deform the smear layer. Several studies have suggested that prolonged agitation of the universal dental adhesive during dentin bonding increases the bonding strength (2-4). The reason for this is that agitating the universal adhesive on the dentin surface enables the acidic monomers to be transported deeper into dentin, which leads to an improved micro-mechanical interaction (5-8). This interaction occurs due to the presence of functional monomers, such as 10-MDP, which are acidic molecules that may serve various functions. The functions include etching tooth substrates, enhancing monomer penetration, and imparting the adhesives for chemical interactions with dental substrates (9).

Commercially available universal adhesives contain organic solvents, such as ethanol or acetone, to facilitate the infiltration of monomers into the dentin's wet surface. If the polymerization occurs before the solvent evaporates, the space occupied by the solvent limits the growth of monomers, decreasing the degree of conversion of the resin adhesive. In addition, the dilution effect due to residual moisture and organic solvents reduces the bonding strength, which leads to the reduction of adhesive efficiency (9-13). This can degrade mechanical properties; thus, it is crucial to evaporate the solvent through an appropriate evaporation process. Although solvents are essential components of universal adhesives, when they are applied in practice, they must be wholly removed (evaporated) after sufficient agitation. The process of evaporating the solvent is generally performed using compressed air through a three-way syringe after rubbing the adhesive on the dentin surface (14).

Agitation and evaporation are processes performed for different purposes when a universal adhesive is applied to dentin, and many studies have investigated the effects of each process on bonding efficiency. However, these processes cannot always be considered two separate processes. Evaporation of the solvent may occur during the agitation, or agitation may occur during the evaporation process. To date, few studies have investigated the effects of these processes on each other. The purpose of this study was to evaluate the effects of agitation and evaporation by comparing μTBS when the agitating time and solvent evaporating time are altered when a universal adhesive is applied in direct restoration using composite resin. In this study EZ bond (Metabiomed Inc., Chungju, Korea) was used as a universal adhesive since the bonding agent has only few information about applying method as it was released recently. The tested null hypothesis was that when using a universal adhesive, the agitation time and evaporating time do not affect the bonding strength to the dentin-resin interface.

Materials and Methods

1. Specimen preparation and bonding procedure

Eighteen caries-free human molars were collected under the consent of the donors, as per a protocol approved by the Institutional Review Board of Pusan National University Dental Hospital (PNUDH 2020 008). The teeth were stored in 4 ℃, 0.5% thymol solution, and were used within three months after extraction. EZ bond and EZ Fil (Metabiomed Inc., Chungju, Korea) were used as an experimental universal dental adhesive and composite resin, respectively. The compositions of the materials used in this study are described in Table 1.

The root portions of the teeth were embedded in self-cured acrylic resin (Tokuso Curefast, Tokuyama, Tokyo, Japan). The teeth were sectioned horizontally at the midcoronal level to obtain a flat, sound dentin surface using a diamond saw (Accutom-50, Struers, RØdovre, Denmark) with constant water cooling. The exposed dentin surfaces were then wet-polished with 600-grit SiC paper for 60 seconds (s) to standardize the smear layer.

Fig. 1 shows the schematic experimental protocol of this study. The teeth were randomly divided into six experimental groups, three molars in each group, each with different agitation time (5 s, 10 s, 20 s) and evaporation time (10 s, 20 s), as follows:

(1) Group 1: five seconds of agitation of universal dental adhesive onto dentin followed by ten seconds of evaporation of solvent with compressed air
(2) Group 2: ten seconds of agitation of universal dental adhesive onto dentin followed by ten seconds of evaporation of solvent with compressed air
(3) Group 3: twenty seconds of agitation of universal dental adhesive onto dentin followed by ten seconds of evaporation of solvent with compressed air
(4) Group 4: five seconds of agitation of universal dental adhesive onto dentin followed by twenty seconds of evaporation of solvent with compressed air
(5) Group 5: ten seconds of agitation of universal dental adhesive onto dentin followed by twenty seconds of evaporation of solvent with compressed air
(6) Group 6: twenty seconds of agitation of universal dental adhesive onto dentin followed by twenty seconds of evaporation of solvent with compressed air

A single drop of adhesive was applied to each specimen with a new, clean micro-brush. The dentin surface was dried using a three-way air syringe with the air pressure adjusted to 1 bar using a pressure regulator, and the air nozzle was held at 45° to the dentin surface at a distance of 1.5 cm. After applying the adhesive, all specimens were photopolymerized for 20 s using a LED curing unit

Table 1. Compositions of adhesive and composite resin used in this study

Material	Composition
Universal dental adhesive-EZ bond (Metabiomed Inc., Chungju, Korea)	10-MDP, Bis-GMA, UDMA, TEGDMA, HEMA, Ethanol, nano-filler (Silica), Initiator (EDAB), Inhibitor (BHT)
Composite resin-EZ Fil (Metabiomed Inc., Chungju, Korea)	Bis-GMA, UDMA, Bis-EMA, TEGDMA, CQ, Barium Glass

* Abbreviations

10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate Bis-GMA: bisphenol A glycidyl methacrylate, UDMA: urethane dimethacrylate, TEGDMA: triethylene glycol dimethacrylate, HEMA: hydroxyethylmethacrylate, Bis-EMA: ethoxylatedbisphenol A glycidyl methacrylate, EDAB: ethyl 4-dimethylaminobenzoate, BHT: butylated hydroxytoluene CQ: camphorquinone. All materials are provided by the manufacturer.

(SmartLite PS; Dentsply DeTrey, Konstanz, Germany) at 1000 mW/cm^2. 4 mm thickness of composite resin was built incrementally, which was photopolymerized for 20 s using a LED curing unit. After polymerization, all specimens were stored in distilled water at 37 ℃ for 24 hours. Table 2 shows a summary of the clinical procedures for each group.

2. Microtensile bond strength (μTBS) testing

Using a low-speed diamond saw (Struers Accutom-50, Ballerup, Denmark), the specimens were cut into rod shapes with a cross-section of 1×1 mm. Twenty rod-shaped specimens were randomly selected from each group, and microtensile bond strength was measured using a microtensile tester (Bisco, Schaumburg, IL, USA). The resin-dentin bonded sticks were attached to a jig with cyanoacrylate cement and subjected to a μTBS test in the microtensile tester (Bisco, Schaumburg, IL, USA) at 0.5 mm/min until failure (Fig. 2). The μTBS values were calculated by dividing the load at the failure by the cross-sectional bonding area.

3. Analysis of failure mode

After the μTBS test, all debonded sticks were observed

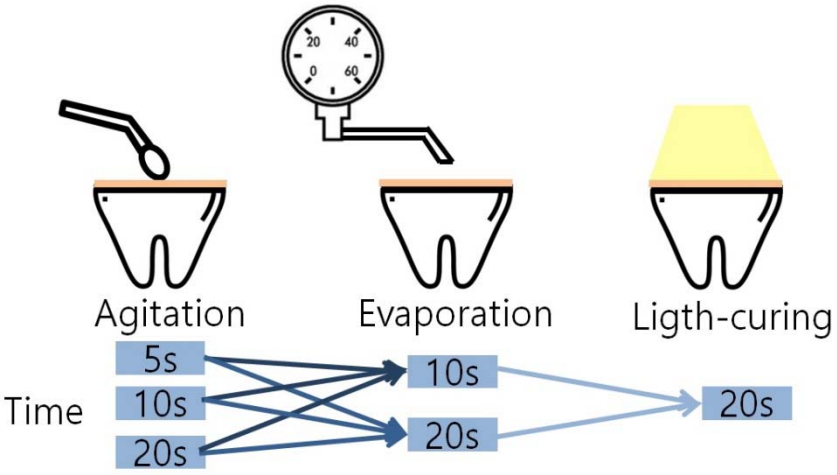

Figure 1. Experimental protocol of this study.

Table 2. Clinical procedure for each group

Group	Agitation Time (Second)	Evaporating Time (Second)	Application Procedure
Group 1	5	10	1. Apply the adhesive with micro-bush to the prepared tooth and rub for the time according to each group. 2. Gently air dry the adhesive to evaporate the solvent at 1.5 cm according to each group. 3. Light activation for 20 s with 1000 mW/cm^2.
Group 2	10	10	
Group 3	20	10	
Group 4	5	20	
Group 5	10	20	
Group 6	20	20	

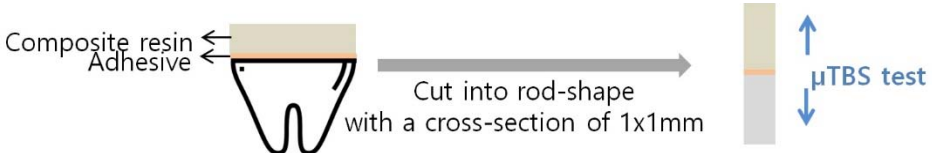

Figure 2. Schematic illustration of microtensile bond strength test specimen.

Table 3. μTBS (MPa) of all experimental groups

	Agitation Time	Evaporating Time	μTBS (Mean (SD))
Group 1	5	10	9.29 (3.24)^{a}
Group 2	10	10	16.57 (3.21)^{b}
Group 3	20	10	18.22 (3.75)^{bc}
Group 4	5	20	19.44 (3.67)^{bc}
Group 5	10	20	20.90 (3.29)^{c}
Group 6	20	20	22.23 (3.24)^{c}

Different superscript lower-case letters indicate significant differences between rows ($p<0.05$).

under a stereomicroscope (Leica, Heidelberg, Germany) at 40× magnification to determine the failure mode. The failure mode was classified as 'cohesive' (exclusively within dentin or resin composite), 'adhesive' (at the resin/dentin interface), or 'mixed' (at the resin/dentin interface that included the cohesive failure of the neighboring substrates).

4. Scanning electron microscope (SEM) image analysis

Two specimens per group were additionally fabricated for SEM image analysis. Using a low-speed diamond saw (Struers Accutom-50, Ballerup, Denmark), the specimens were cut in a longitudinal direction at a thickness of 1 mm. The cross-sectional surface was cleaned with distilled water using an ultrasonic cleaner. The treated cross-sectional surface was observed using SEM.

5. Statistical analysis

The μTBS data were analyzed using an ANOVA and Tukey's post hoc test at $p = 0.05$ (SPSS 21.0; SPSS Inc., Chicago, IL, USA).

Results

1. μTBS

Table 3 presents the results of μTBS in which the difference is shown in superscripted letters. The μTBS in Group 1 was significantly lower than those of other groups ($p<0.05$). The difference of the μTBS of Group 2 was not statistically significant from those of Groups 3 and 4 ($p>0.05$), but was significantly lower than those of Group of 5 and 6 ($p<0.05$). The average bonding strength increased in the order of Group 3, 4, 5, and

6, but the difference was not statistically significant ($p>0.05$).

2. Analysis of failure mode

Table 4 presents the number and percentage of specimens according to fracture pattern mode. In failure mode analysis, adhesive failure was predominant among all groups. But the percentage of each failure mode was similar in all groups.

3. Scanning electron microscopy

SEM image analysis of the cross-sectional view showed that groups with 10 seconds of evaporation time (Group 1, 2, and 3) had relatively irregular adhesive layers compared with the groups with 20 seconds of evaporation time (Group 4, 5, and 6). The groups with 20 seconds of evaporation time showed a relatively uniform adhesive layer, which also infiltrated well into the tubules, regardless of the adhesive agitation time (Fig. 3).

Discussion

Considering that agitation time and evaporating time influenced bond strength when using the universal adhesive, the null hypothesis was rejected. In this study, the effects of agitating the universal adhesive and evaporating the solvent on microtensile bond strength were evaluated by varying agitation and evaporation time. In modern dentistry, dental adhesives have been developed to simplify the application process, resulting in reduced technical sensitivity and chair time (15-17).

Universal adhesives contain both hydrophilic and hydrophobic monomers, diluents, a photoinitiator system, and a solvent such as ethanol or acetone. Universal dental adhesives are multi-mode adhesives, and when they are used in self-etch mode, the acidic monomers contained in the adhesives dissolve and deform the smear layer to form a micro-mechanical bond (18).

Due to the weak acids of universal adhesives and their low etching ability, they are likely to create a relatively weak hybrid layer. The bonding quality of the self-etching system can vary depending on the thickness and density of the smear layer. Therefore, the universal adhesive should have the ability to infiltrate into the smear layer and impregnate the underlying dentin (19). The agitation motion -rubbing the adhesive on the dentin surface- leads to impregnation of monomers inside the smear layer at a higher rate, which eventually improves the adhesive-interface quality (9). The solvent of universal adhesive plays a role in infiltrating the adhesive into the wet dentin's

Table 4. Number and percentage of specimens (%) according to fracture pattern mode

	Cohesive Failure	Adhesive Failure	Mixed Failure
Group 1	6 (30)	9 (45)	5 (25)
Group 2	5 (25)	9 (45)	6 (30)
Group 3	4 (20)	12 (60)	4 (20)
Group 4	5 (25)	10 (50)	5 (25)
Group 5	4 (20)	10 (50)	6 (30)
Group 6	7 (35)	8 (40)	5 (25)

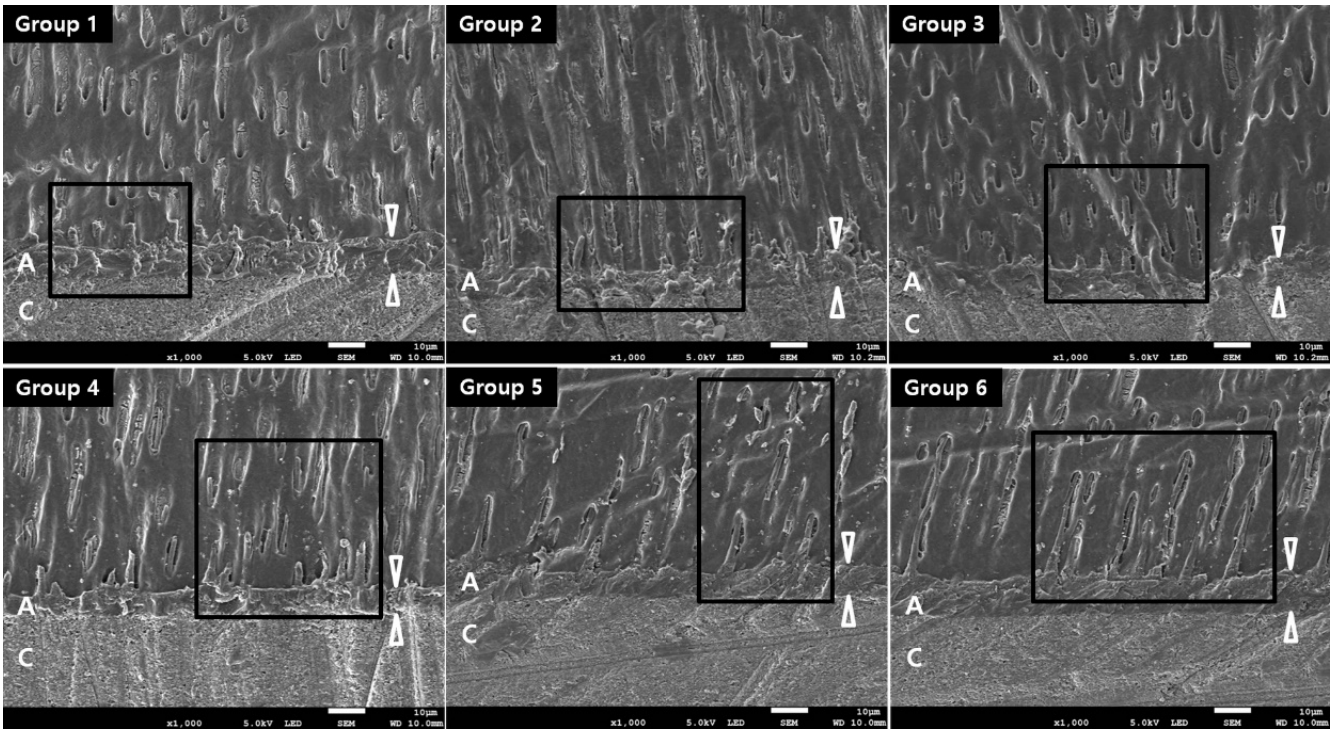

Figure 3. Micrographs of resin-dentin interface of experimental group observed under a SEM with a backscattered detector. Original magnification - × 1,000. A : Adhesive layer, C : composite resin, The black lined box : resin tag structure. The resin tags in Group 1 appear shorter and less infiltrated than those in other groups. The resin tags in Groups 4, 5 and 6 appear to infiltrate similarly well. The adhesive layers of the groups with more evaporation showed more uniformity in the images.

substrate, but it must be removed to prevent adverse effects on the bonding strength. The process of evaporating the solvent is clinically performed by blowing compressed air. During this process, water and the organic solvent contained in the universal adhesives are removed, preventing polymerization inhibition due to residual monomers (20).

Group 1, which had the shortest adhesive agitation time and solvent evaporation time, exhibited the lowest μTBS value of the groups. When the cross-sectional view was analyzed under SEM, Group 1 showed an adhesive layer with a relatively irregular thickness and shorter tags than the other groups. This indicates that shortening the agitation time and evaporation time during the adhesive applying process leads to not only shallow etching depth and monomer infiltration depth but also the insufficient formation of the resin tag.

In the groups with 10 s of solvent evaporating time, Groups 2 and 3 had significantly higher μTBS than group 1 ($p<0.05$), respectively. Group 3 exhibited higher μTBS than Group 2, but the difference was not significant ($p>0.05$). Therefore, when the evaporating time was equal to 10 s, μTBS tended to increase with agitation time. This result is consistent with those of previous studies, which showed the active application of the universal adhesive used in self-etch mode improved the adhesive's degree of conversion and bonding strength compared with the passive application (17, 19, 21). Agitating the universal adhesive on the dentin surface enables the acidic monomer of the adhesive to infiltrate deeper into the dental matrix, which leads to an improved micro-mechanical interaction (5-8). This is because the action

of actively rubbing the adhesive on the tooth surface increases the reaction time of acidic monomers, which improves their dentin infiltration rate and provides better conditioning. These factors can increase the number of open dentin tubules and consequently create greater bonding strength. In addition, as the universal adhesive is agitated, the smear plug may be partially or entirely removed, creating patent dentinal tubules to enable a more stable bonding. On the other hand, the groups with 20 s of solvent evaporation time, Groups 4, 5, and 6, did not show a significant difference in μTBS even with different adhesive agitation times ($p > 0.05$). As long as the solvent is adequately evaporated, sufficient bonding strength can be obtained even when the adhesive is agitated for a short period. The reason may be that compressed air used for evaporation has the effect of rubbing the adhesive on the dentin surface.

Groups with an adhesive agitation time of either 5 or 10 s showed significantly greater μTBS as the evaporation time increased. However, when the adhesive was agitated for 20 s, no correlation between evaporation time and μTBS was observed. In other words, the groups with the adhesive agitated on dentin for 20 s exhibited sufficient bonding strength even with a short evaporation time. This could be due to the solvent being continuously evaporated, without the use of compressed air, during the agitation of the adhesive on dentin. The solvent is an essential component of universal adhesives. When the adhesive remains in the bottle, the solvent prevents the phase separation of monomers. When the adhesive is applied onto dentin, the solvent enables infiltration and diffusion into the collagen network. However, the solvent must be removed after completing its roles to prevent adverse effects on bonding strength (22-24). The remaining solvent may result in an increased formation of voids due to the dilution of monomers, which can hinder their polymerization. This can also lead to reduced resin-dentin bonding strength (25). Therefore, clinicians must remove the solvent to achieve an appropriate degree of monomer conversion.

Universal adhesives contain functional monomers that can be chemically bonded to the dental matrix. Depending on the application method of the adhesives, the functional monomers can be transported to a deeper inter-prismatic area. The groups in which the solvent was evaporated for 20 s displayed similar bonding strength, regardless of the agitation time. In addition, the SEM cross-sectional image analysis that exhibited a thin adhesive layer with well-formed tags in the same groups supported this result. Therefore, in order to increase the bonding strength of the universal adhesive, it is crucial to evaporate the solvent sufficiently. If adequate evaporation cannot be performed, a clinician is recommended to at least lengthen agitation time. In this study, one adhesive was used to evaluate how the adhesive strength varies depending on the application method of the universal bonding agent. However, further studies may be needed to generalize the result to various commercially available universal adhesives.

The universal adhesive used in this study was an ethanol-based adhesive, and it is advantageous to evaporate the solvent prior to polymerization completely. In a clinical situation, complete solvent evaporation is difficult to achieve, but it is essential to have sufficient time to allow the solvent to evaporate to the greatest extent possible. In this experiment, when 20 seconds of evaporation time was secured, a decrease in bonding strength was prevented.

Conclusion

When a universal adhesive is used for dentin bonding, similar bonding strength could be obtained when the adhesive had adequate evaporation time, regardless of the duration of agitation. Therefore, it can be concluded

that securing adequate evaporation time is vital to obtain optimal bonding strength.

Acknowledgement

The authors have no conflicts of interest relevant to this article.

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Universal adhesives that simplify bonding procedures and be used in multi-etch mode have been developed. In this study, the effects of agitation and solvent evaporation time of a universal adhesive on microtensile bond strength (μTBS) were evaluated by varying the times of these two procedures. Eighteen human molars were used to fabricate specimens, and the teeth were randomly divided into six experimental groups. Each group had different agitation time (5 s, 10 s, 20 s) and evaporation time (10 s, 20 s). The specimens were cut into a rod-shape with a cross-sectional area of 1 mm^2, and their μTBS was measured. The data were analyzed using an ANOVA and Tukey's post hoc test. After this, the debonded surface was observed using scanning electron microscopy (SEM). In the groups with a solvent evaporation time of 20 s, μTBS was statistically higher, even with a short agitation time ($p<0.05$). Furthermore, for the groups with the shorter evaporation time, bonding strength decreased statistically as the agitation time shortened ($p<0.05$). The results of this experiment suggest that if the solvent was adequately evaporated, sufficient bonding strength could be obtained even when the adhesive was agitated for a short time. This is supported by the results of SEM image analysis, which revealed a uniform adhesive layer with well-infiltrated tags in the groups with the prolonged evaporation time. It may be crucial to secure an adequate evaporation time in order to obtain optimal bonding strength.

KeyWords : Agitation, Solvent evaporation, Universal adhesive, Microtensile bond strength, Scanning electron microscopy