Effect of air-blowing temperature and water storage time on the bond strength of five universal adhesive systems to dentin

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The purpose of this study was to evaluate the air-blowing temperature and water storage time on the micro-tensile bond strength (μTBS) of five universal adhesive systems to dentin. The bond strength with two different air-blowing temperatures (60±2°C and 23±2°C) was measured after water storage at 37°C for 24 h and 100 days respectively. The fracture surface on dentin side was observed by scanning electron microscope (SEM). Three-way ANOVA revealed a significant effect of universal system (p<0.001) and air-blowing temperature (p<0.001) on bond strength to dentin except water-storage time (p=0.145). The interaction within three factors was significantly different (p<0.001). It could be concluded that the μTBS of universal systems to dentin was material-depended. The higher and more stable bonding performance of universal systems on dentin could be achieved by air-blowing at 60±2°C temperature. In addition, the quantity of voids in the adhesive layer of aceton-based universal adhesive was significantly reduced by higher temperature.

Keywords: Universal adhesive systems, Micro-tensile bond strength, Adhesives, Air-blowing temperature, Water storage

INTRODUCTION

The development of adhesive system has achieved great improvements in simplifying the bonding procedure to achieve demineralization and resin penetration simultaneously, meanwhile, researchers have put enormous efforts on 1-step self-etching priming systems (SEs) to improve its bonding performance. Recently, several manufacturers declared an idea that their universal system adhesives could be used as an ideal 1-step SEs. The universal system adhesives, as the latest generation of bonding agent, were designed to minimize operational drawbacks, optimize bond strength, and be capable of bonding to various substances appeared in daily clinical treatment.

Although universal system adhesives could maximize bonding performance to the greater extent, and optimize application procedures, there are many challenges in the clinic operations. Experiments have confirmed that steps clinical applications, such as: adhesive coating, air blowing, photocuring operation will affect the quality of adhesive layer and significantly influence the bonding performance of universal adhesive systems. It was confirmed that higher air-blowing temperature could promote the degree of solvent evaporation to achieve a better adhesive layer, which contribute the durability of bonding agent as well.

In addition, the current hydrophilic trends of the development of dental adhesives, owing to the composite like HEMA, tend to make more water penetration which expedite degradation of resin-dentin bonds. It has also been reported that the composite resin of HEMA-contained 1-step SEs tend to make more water penetration and water saturation in a certain volume after 100 days water-storage, which eventually result in the mechanical degradation of resin-dentin bonds.

With the hydrophilicity of the majority of the universal system adhesives, the impact of both air-blowing temperature (23±2°C/60±2°C) and water storage condition (37°C 24 h/100 days) on the μTBS to dentin have not been intensively studied. Therefore, the aim of this study was to evaluate the effect of air-blowing temperature and water storage time on the μTBS of five universal adhesive systems. The present study was conducted with null hypotheses that: (1) air-blowing temperature has no influence on the bond strength of universal adhesive systems; (2) no significant difference in bond strength exists among universal adhesive systems; and (3) there is no decrease of bonding performance after different storage period.

MATERIALS AND METHODS

Tooth selection and preparation
One hundred extracted, caries-free human third molars were used in the study. The teeth were collected under a protocol reviewed and approved by the institutional review board of School and Hospital of Stomatology, China Medical University, Shenyang, China (G2018026). The teeth were stored in 4°C distilled water after extraction and used within 6 months. Flat dentin surfaces were obtained by using a gypsum model trimmer under...
The universal adhesive systems were applied on the dentin surface strictly following the manufacturer's instructions. The air-blowing procedure was performed for 10 s at a vertical distance of 2 cm to the dentin surface with the air temperatures of 60±2ºC or 23±2ºC, respectively. The air stream was provided by a hair-dryer which has a LED display screen showing the air temperature (KF-5895, CONFU, Guangdong, China). The diameter of the air-blowing port was 5mm. A digital anemometer (GM816, Benetech, Shenzhen, China) was used to monitor the air temperature and measure the flow rate.

### Experimental groups and application

The teeth were randomly divided into 5 groups corresponding to five universal systems (n=20). Each group was further assigned to 2 subgroups (n=10) corresponding to air-blowing temperatures of 60±2ºC and 23±2ºC, respectively. Each subgroup was split into two sets (n=5) for different water-storage time (24 h and 100 days) under 37ºC. Five universal adhesive systems selected in this study were: ① Adhesive Universal Vivapen (AUV; Ivoclar, Schaan Liechtenstein); ② GLUMA Bond Universal (GBU; Heraeus, Hanau Germany); ③ All Bond Universal (ABU; Bisco, Schaumburg, IL, USA); ④ Single Bond Universal Adhesive (SBU; 3M, Neuss, Germany); and ⑤ Clearfil Universal Bond (CUB; Kuraray, Tokyo Japan).

### Table 1 The chemical composition of five universal systems in the study and manufacturer's instruction for application of the universal systems

| Materials & lot no. | Code | Main composition and pH | Manufacturer’s instruction |
|---------------------|------|-------------------------|---------------------------|
| Adhesive Universal Vivapen Lot no. U49773 (Ivoclar) | AUV | MDP, HEMA, Ethanol, Water, Bis-GMA, Dimethacrylate resins, MCAP, Fillers, Initiators pH=2.5 | 1. Adhesive applied to air-dried tooth surface with rubbing action for 20 s. 2. Medium air pressure applied to surface for 5 s. 3. Adhesive photo-polymerized for 10 s. |
| GLUMA Bond Universal Lot no. 010027 (Heraeus) | GBU | 10-MDP, 4-META, acetone, water, light-activated methacrylate monomers pH=1.6–1.8 | Use the brush to apply GLUMA Bond Universal to the entire surface of prepared teeth without delay. Gently rub in the adhesive for 20 s. Air dry carefully with a gentle oil-free air flow until the adhesive film no longer moves. Light-cure for 10 s. |
| All Bond Universal Lot no. 1700005046 (Bisco) | ABU | MDP, Bis-GMA, HEMA, ethanol, water, initiators pH=3.2 | Apply two separate coats of adhesive, scrubbing the preparation with a microbrush for 10–15 s per coat. Do not light cure between coats. Evaporate excess solvents by thoroughly air-drying with an air syringe for at least 10 s, there should be no visible movement of the material. The surface should have a uniform glossy appearance. 3. Light cure for 10 s. |
| Single Bond Universal Adhesive Lot no. 624090 (3M ESPE) | SBU | 10-MDP, HEMA, ethanol, water, dimethacrylate resins, phosphate monomer, methacrylate-modified polyalkenoic acid copolymer, filler, initiators, silane pH=2.7 | Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. Direct a gentle stream of air over the liquid for about 10 s until the solvent is evaporated completely. Light-cure for 10 s. |
| Clearfil Universal Bond Lot no. REF #3290-KA (Kuraray) | CUB | 10-MDP, Bis-GMA, HEMA, ethanol, water, hydrophilic aliphatic dimethacrylate, colloidal silica, silane coupling agent, CQ pH=2.3 | 1. Apply bond to the dentin surface and rub it in for 10 s. 2. Dry the dentin surface sufficiently by blowing mild air for more than 5 s until bond does not move. 3. Light cure for 10 s. |

10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloxy-propoxy)-phenyl] propane; HEMA: 2-hydroxyethyl-methacrylate; CQ: camphoroquinone; MCAP: methacrylated carboxylic acid polymer; 4-META: 4-methacryloyloxyethyl trimellitate anhydride
air speed of air flow at the adhesive surface. The speed of the air was 20 m/s and the air flow were 0.1 m$^3$/min$^{15}$. After air-blowing the adhesive layers were light-cured for 20 s (2,000 mW/cm$^2$, DemiPlus, Kerr, USA). All bonded surfaces were built up with resin composite (Filtek Z350 XT, Shade A2, 3M ESPE, St. Paul, MN, USA, Lot no: N95673) in three increments of 2 mm for each time. Each incremental layer was light-cured for 20 s at a vertical distance of 1 mm to the surface. The resin-dentin samples were stored in distilled water at 37ºC using a thermostat water bath (Cole-Parmer, USA) for 24 h and 100 days, respectively, before $\mu$TBS test.

**Micro-tensile bond strength ($\mu$TBS) test**

After different water-storage duration period (24 h and 100 days), specimens were sectioned into resin-dentin slabs with a cross-sectional area about 1.0 mm$^2$ by using a low speed diamond saw (SYJ-150, Shenyang Kejing Auto-instrument, Shenyang China). Each slab was stuck to the grip using glue (Aron Alpha Gel-10, Toagosei, Tokyo, Japan) and subjected to a tensile test by using a universal testing machine (WDD-200, Weidu Wenzhou China) at a crosshead speed of 1 mm/min. The $\mu$TBS was calculated by dividing the applied force in newton (N) at the time of fracture by the bonded area (mm$^2$) and recorded in MPa.

**Scanning electron microscope (SEM) observation and failure mode analysis**

After the tensile bond strength tests, the fractured specimens were mounted on an aluminium stub and sputter-coated with gold for 150 s. The fractured surface on the dentin side was observed by using a SEM (JSM-7001F, JEOL, Tokyo, Japan) under high magnification ($\times$2,000). The failure modes observation was accomplished by a digital stereomicroscope ($\times$600). The failure modes were classified as follows: A: mixed failure (cohesive failure within dentin and composite); B: adhesive failure; C: failure at dentin or composite resin.

**Statistical analysis**

The $\mu$TBS data of the present study were statistically analyzed with SPSS version 19.0 (CA, USA) by a three-way ANOVA (the universal adhesive, the air-blowing temperature and the water storage time) and Games-Howell. The statistical significance level was set at $\alpha$=0.05. The schema of this research method was shown in Fig. 1.

**RESULTS**

$\mu$TBS and statistical analysis

The $\mu$TBSs of five universal adhesive systems under different conditions were listed in Table 2. No significant difference was detected in CUB under different experimental condition. In the 24 h storage group, the bond strength of AUV and GBU was significantly improved when the air-blowing temperature increased from 23±2ºC to 60±2ºC, inversely, the bond strength of ABU and SBU was not changed. On the other hand, the bond strengths of GBU, ABU, and SBU in the 60±2ºC group are much larger than those in the 23±2ºC group after 100 days storage.

Three-way ANOVA analysis revealed that the $\mu$TBS was significantly affected by both air-blowing temperature ($p<0.001$, $F=35.284$) and universal adhesive ($p<0.001$, $F=14.809$), while the water storage time does not affect $\mu$TBS in the present study does not affect $\mu$TBS ($p=0.145$, $F=2.138$). Moreover, the interaction among air-blowing temperature, universal adhesive and water storage time was significantly different ($p<0.001$, $F=8.725$). The significant interaction of each two of the three variables were: universal adhesive and air-blowing temperature ($p<0.001$, $F=10.115$), air-blowing temperature and water storage time ($p=0.003$, $F=1.076$) respectively. It is worthy to note that no interaction between water storage time and universal adhesive was detected in the present study ($p=0.145$, $F=2.138$).

Table 3 exhibits the mean $\mu$TBS values (MPa) of all five adhesives under different experimental conditions. The Game-Howell test indicated that the mean $\mu$TBS of 60±2ºC and 100 days group was significantly higher.

![Fig. 1 Brief Experimental Design.](image-url)
Table 2  Micro-tensile bond strength values (MPa) for five universal adhesives at different air-blowing temperatures in two different times (mean±SD)

|         | AUV      | GBU      | ABU      | SBU      | CUB      |
|---------|----------|----------|----------|----------|----------|
| 24 h    |          |          |          |          |          |
| 23±2ºC  | 23.55±7.75¹,AC | 18.72±5.68¹,A | 23.66±7.88¹,AC | 30.59±9.61¹,BC | 31.26±9.86¹,BC |
| 60±2ºC  | p<0.05   | NS       | NS       | NS       | NS       |
| 100 days|          |          |          |          |          |
| 23±2ºC  | 27.67±6.63¹,P | 15.99±8.66¹,YL | 21.64±4.76¹,PQ | 28.85±8.72¹,P | 29.03±9.01¹,P |
| 60±2ºC  | NS       | 31.59±6.74²,XY | 36.02±7.08³,X | 26.70±5.44³,Y | 38.03±9.47³,X | 28.22±4.19²,Y |

NS means no significant differences (p>0.05).
The same capital letters indicates no significant differences in μTBS for each row (p>0.05).
The same number indicates no significant differences within each column at cold and warm air temperatures (p>0.05).

Table 3  The mean micro-tensile bond strength values (MPa) of all five adhesives in the different experimental groups (mean±SD)

|         | 24 h         | 100 days     |
|---------|--------------|--------------|
| 23±2ºC  | 25.56±9.37ab | 24.24±8.95a  |
| 60±2ºC  | 28.20±9.57b  | 32.11±7.95c  |

The same lower case letters indicate no significant differences (p>0.05).

Fig. 2  Failure modes of the five universal adhesives bonded to dentin.

Fracture modes
The result of the failure analysis is exhibited in Fig. 2. The percentage of Type C (failure at dentin or composite resin) significantly increases in the 60±2ºC group comparing with that of the 23±2ºC group after 24 h water-storage, except ABU. In contrast, the summation of Type A and B (A: mixed failure; B: adhesive failure) failure modes was increased after 100 days water-storage.

SEM observation of the fractured dentin surface after μTBS testing
Figure 3 presents the SEM images of the dentin surfaces after the μTBS test. It can be observed that multiple voids exist in the adhesive layer of GBU under all conditions (Figs. 3E–H).

DISCUSSION
Proper chemical bond between acidic functional monomer in adhesives and hydroxyapatite (HA) in dental substance is essential for a successful restoration. For
the composition of universal systems, the proportion of water and organic solvents can reach up to 80% approximately\(^17\). Owing to the volatility of solvents, adhesive can remove the residual water sufficiently and control the solvent mixed in an adhesive layer by air-blowing application\(^15\) to achieve the ideal bonding performance. However, it is challenging to achieve complete evaporation in an oral environment\(^14\). Besides, the residual solvent will lead to the obstruction of polymerization and the deterioration of hybrid layers, resulting in damages to their clinical manifestations\(^18\), either for the bond strength or for the durability of the adhesives\(^8\).

Among most of organic solvents, acetone and ethanol have been widely incorporated in commercial adhesives. The volatility of solvent can be related to the boiling temperature and the vapor pressure. Lower boiling temperature and higher vapor pressure make acetone-based adhesives more difficult to store than ethanol-based adhesives, and easier to volatilize at the same air-blowing temperature\(^19\). Therefore, this experiment wants to study the different reactions among acetone-based universal adhesive (GBU) and other four ethanol-based universal adhesives.

Accordingly, it is obviously for 1-step SEs that the air-blowing application is a highly important variable for the bonding performance comparing with the other application steps\(^20\). In our previous study\(^8\), it was confirmed that the bond strength of both 1-step SEs\(^21\) and universal systems\(^16\) could be significantly influenced by prolonging air-blowing time appropriately. As one of the most important variables of the air-blowing application, the air temperature ranged from 37ºC to 80ºC was investigated in previous studies\(^10,20,22-27\). Among these studies, the effect of evaporation temperature around 37ºC still seems controversial with the uncertain detrimental impact\(^20,27\). The temperature of 80ºC applied in the root canal dentin has the limited scope of application\(^22\). Therefore, 60ºC was selected as the middle level air temperature in current study, because the solvent evaporation of 60ºC has increased the bonding strength by nearly 20% comparing with that of the room temperature \textit{in vitro} study\(^10,15\).

HEMA as a hydrophilic monomer can significantly improve bond strength to dentin by enhancing the wetting of dentin and preventing the phase separation\(^11,19,28\). In the present study, no voids within adhesive layer were observed in four HEMA-contained adhesives (Figs. 3A–D, H–T). This morphological feature is attributed to the water within the adhesive layer that could be transferred to the surface and be removed by air-blowing application\(^8,15\). On the other hand, the relatively higher temperature up to 60ºC is still limited on removing water within the adhesive layer of HEMA-free universal
system, because the voids were observed under different experimental conditions of GBU in present study (Figs. 3F–H).

In present study, the adverse effect of HEMA-free attributes to the phase separation resulted from the residual water within the adhesive layer and results in the undesirable bonding performance of GBU in 23±2°C groups. Furthermore, acetone contained in GBU as a solvent, which has a very high vapor pressure with corresponding high volatility result in the joint effect on the residual water29,30. The typical phase separation of GBU has been confirmed by panorama SEM observation in our previous study8. Contrarily, significant higher bond strength of GBU was achieved in the 60±2°C groups, which corresponds well to the decrease of voids quantity and the reduction of voids size (Figs. 3G, H) within the adhesive layer. Hence, the warm air should be considered as an effective way to increase the bond strength of HEMA-free acetone-based adhesive by removing the residual moisture in the adhesive layers25,19,21-26.

Besides, high air temperature could active molecules to move drastically, resulting better polymerization and monomer reactions to improve the quality of the adhesive layer and minimize the degradation of the dentin collagen fibers19. In present study, seven of ten experimental groups indicated that bonding performance was improved under 60±2°C conditions. Furthermore, three-way ANOVA has confirmed that bond strength of universal systems was statistically influenced by the air-blowing temperature (p<0.001, F=35.284). Therefore, the first null hypothesis that the air-blowing temperature could be improved by higher temperature in air-blowing step, which was material-dependent in clinical situation.

Five universal adhesives selected in this study contain 10-MDP. The functional monomer 10-MDP was considered as one of the most promising monomers for chemical bonding to dental substrates, as it establishes an ionic bond with HA most readily and intensively18,31. In this study, varied bonding efficacy was found and the stable bond strength of CUB was detected under different air-blowing temperatures. The stability of CUB corresponded well to the decrease of voids quantity and the reduction of voids size (Figs. 3G, H) within the adhesive layer. Hence, the warm air should be considered as an effective way to increase the bond strength of universal adhesives was rejected.

In the present study, the bond strengths of SBU, GBU and ABU in 60±2°C group increase significantly after 100 days water-storage (Table 2). These performances are contrary to the previous result of one-bottle resin adhesives after 100 days of water storage, which found that the typical degradation pattern of filler reduction and gap formation within adhesive layers19. The formation of compact adhesive layers was achieved since warm air-blowing can evaporate more the solvent contents of the universal adhesives10. The stable nanolayer was also composed by 10-MDP-Ca salt25. They work together to make sure the durability of adhesives and have a positive effect in the conversion of monomers25. Additionally, more 10-MDP-Ca bond7,35 and post-cured polymerization might have been created as the water storage period prolonged11,36. Under the circumstance of 37°C water bath, the heat challenge may accelerate post-cured polymerization of the resin by increasing the degree of conversion of the adhesive and lead to improve the bond strength11.

In this study, the statistical analysis revealed that the bond strength of different universal systems was not influenced (p=0.145) by the water storage time of 100 days. Although some changes of bond strength happened in parts of adhesives selected in this study, no significant decrease was detected between 24 h and 100 days water-storage period. Furthermore, the bond strength of all five adhesives in 60°C group statistically increased after 100 days water-storage comparing with that of the other groups (p<0.001) (Table 3). Therefore, the third null hypothesis that there is no decrease in bonding performance after different storage period was accepted. Consequently, the durability of the universal system under different air-blowing temperatures should be further investigated.

CONCLUSIONS

Within the limitations of the current study, the following conclusions can be drawn:

1. The bond strength of universal adhesive systems could be improved by higher temperature in air-blowing step, which was material-dependent in clinical situation.

2. The bond strength of universal adhesive systems selected in this study did not statistically decrease after 100 days water storage.

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