Effect of tooth temperature on the dentin bonding durability of a self-curing adhesives: The discrepancy between the laboratory setting and inside the mouth

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A two-bottle self-curing universal adhesive (Tokuyama Universal Bond; Tokuyama Dental) that does not require a long waiting time or light curing after application of the bonding material has been developed. This study aimed to evaluate the influence of tooth and adhesive temperature during the bonding procedure on the effectiveness of dentin bonding. The results showed that the tooth temperature affected the effectiveness of the dentin bonding; therefore, to determine the precise bonding ability in the laboratory, the temperature of the tooth must be raised until it is the same as that of the oral cavity. In addition, the temperature of the material did not affect bonding effectiveness; this result confirms that it does not matter whether the refrigerated product is used soon after its removal from the refrigerator or after it reaches room temperature in the clinic.

Keywords: Adhesive dentistry, Chemical cure, Dental bonding, Durability, Micro-tensile bond strength

INTRODUCTION

Dental adhesive technology continues to evolve at a rapid pace. The advent of adhesive dentistry was in 1955, following a paper on the benefits of an acid etching by Buonocore¹⁾. Dental adhesives have evolved from no- and total-etch (4th- and 5th-generation) systems to self-etch (6th- through 8th-generation) systems. Self-etch bonding systems are classified as one- and two-step adhesives²⁻⁴⁾. One-step self-etch systems can be further classified into one- or two-component adhesives. In one-component adhesives, also referred to as all-in-one adhesives, all ingredients related to the acidic, priming, and bonding functions are combined into a single bottle, and consist of a complex mix of hydrophilic components. In two-component adhesives, the functional monomers and water are kept separate, which improves the hydrophilic stability and shelf life; however, both components must be adequately mixed before clinical application.

As a dental adhesive that plays an important role in bonding, chemically activated bonding was first developed using two components: one containing a chemical initiator (benzoyl peroxide) and the other a chemical activator (an amine, usually a tertiary aromatic amine). After that, the development of light-activated adhesives and resin composites marked an important advance in dentistry^{5,6)}. However, the drawbacks of light-activated adhesives are that they require a long light

irradiation time and that the irradiation light energy encounters difficulty penetrating sufficiently into deep cavities, resulting in poor polymerization⁷⁻⁹⁾.

A two-bottle self-curing universal adhesive (Tokuyama Universal Bond, Tokuyama Dental, Tokyo, Japan) that does not require a long waiting time or light curing after application of the bonding material has been developed (Table 1). As a result, a reduction in chair time can be expected by shortening the bonding procedure. On the other hand, since it is self-curing, it is considered to be more easily affected by temperature compared with light-cure bonding systems. In clinical practice, adhesives are typically stored refrigerated, and it is recommended to wait to perform the bonding procedure until they reach room temperature, whereas *in vitro*, human and bovine teeth are used to evaluate bonding effectiveness. Therefore, experiments conducted at room temperature, which differs from the temperature in the oral cavity, cannot assess actual bonding effectiveness. The influence of the difference in temperature on light-cure adhesives has been evaluated^{10,11)}, but to our knowledge, the effects of tooth and material temperature on the bonding effectiveness of self-curing adhesives have not been investigated.

Given this background, in the present study, we examined the effect of tooth and material temperatures during bonding operations on the dentin bonding ability of a self-curing adhesive. The null hypotheses were as follows: 1) the temperature of the adhesive would not affect the bond strength, and 2) the tooth temperature

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Table 1 Materials used in the present study

Material	Product	LOT No.	Manufacturer	Composition
Adhesive	Tokuyama Universal Bond ("Bondmer Lightless" in Japan)	5R0079	Tokuyama Dental	Bond A: acetone, phosphoric acid monomer, Bis-GMA, TEGDMA, HEMA, MTU-6
				Bond B: acetone, isopropanol, water, borate catalyst, peroxide, silane coupling agent
Resin cement	Estecem II	6L0099	Tokuyama Dental	Paste A: zirconium silicate colorant, Bis-GMA, TEGDMA, Bis-MPEPP
				Paste B: zirconium silicate colorant, Bis-GMA, TEGDMA, Bis-MPEPP, peroxide, camphorquinone

Fig. 1 Schematic illustration of the temperature measurement.

(a) Coronal dentin surfaces were obtained. (b) Wet-polishing with #600 grit silicon carbide paper. (c) Storage in distilled water at each temperature for 24 h. (d) Temperature measurement. (e) Storage in distilled water at each temperature for 24 h. (f) Temperature measurement.

would not affect the bond strength.

MATERIALS AND METHODS

Temperature measurement

The present study examined 15 extracted non-carious human molars collected after obtaining patients' informed consent under a protocol reviewed and approved by the institutional review board of Osaka University (protocol No. H30-E51). The samples were randomly divided into three groups (Fig. 1). They were stored in distilled water at 37°C (T_{high}), 23°C (T_{middle}), or 4°C (T_{low}) for 24 h. The adhesive was divided into two subgroups and stored at 23°C (B_{middle}) and 4°C (B_{low}). The temperatures of the teeth and adhesive stored under each condition were measured with a thermal imaging camera (FLIR C2, CHINO, Tokyo, Japan).

Tooth preparation and resin buildup

Another 16 extracted non-carious human molars were used in a bond strength test. All teeth were cut at the height of the contour and exposed dentin and then polished with #600 silicon carbide paper to create a standardized smear layer (Fig. 2). The samples were then

Fig. 2 Schematic illustration of the micro-tensile bond strength test.

(a) Coronal dentin surfaces were obtained. (b) Wet-polishing with #600 grit silicon carbide paper. (c) Storage in distilled water at each temperature for 24 h. (d) Surface preparation and adhesive application. (e), (f) Resin cement was built up and light-cured for 20 s from five directions. (g) All specimens were stored in 37°C distilled water for 24 h. (h) Specimens were cut into 1.0×1.0-mm beams. (i) Beams were stored in distilled water for 24 h, 1 month, or 6 months. (j) μTBS values were measured. Fracture surfaces after μTBS measurements were observed by SEM. μTBS: micro-tensile bond strength, SEM: scanning electron microscope.

randomly divided into four groups and stored in distilled water at 37°C (T_{high}), 23°C (T_{middle}), or 4°C (T_{low}) for 24 h. The adhesive was also divided into two subgroups and stored at 23°C (B_{middle}) or 4°C (B_{low}).

The experimental groups were as follows:

$T_{\text{high}}/B_{\text{middle}}$ group: Clinical situation. The material was used at room temperature.

$T_{\text{high}}/B_{\text{low}}$ group: Clinical situation. The material was used immediately after removal from the refrigerator.

$T_{\text{middle}}/B_{\text{middle}}$ group: Laboratory setting. The tooth and material were used at room temperature.

$T_{\text{low}}/B_{\text{low}}$ group: laboratory setting. The tooth and material were used immediately after removal from the refrigerator.

Since combinations of sample and material temperatures other than those mentioned above cannot occur in clinical and laboratory settings, these were not set as experimental groups.

After the dentin surface treatment, air-drying using a three-way syringe was performed for 10 s. The adhesive was applied to the dentin surface for 10 s and then dried with medium pressure air until the bonding layer did not move. Resin cement (Estecem II, Tokuyama Dental) was built up by 2-mm-thick layers at a time and then light-cured for 20 s from four directions at a maximum light intensity of 2,200 mW/cm² (Satelec Mini LED3, Acteon, Merignac, France) (Table 1). The specimens were stored in water at 37°C for 24 h, and then sectioned into beams with a cross-section area of 1 mm². Micro-tensile bond strength (μ TBS) was measured at 24 h or after storage in water for 1 or 6 months ($n=16$).

Testing of μ TBS

Next, each beam was attached to a testing apparatus (Ciucchi's jig) using cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Tochigi, Japan). Then, using a desktop testing apparatus (EZ Test, Shimadzu, Kyoto, Japan), each beam was subjected to a tensile force at a crosshead speed of 1 mm/min until failure, after which the fractured specimens were carefully removed from the jig. The μ TBS was calculated by dividing the applied force (N) at the time of fracture by the bonded area (in mm²) and expressed in MPa. The mean bond strength of 25 beams derived from each group represented the μ TBS of that group, with three water storage period values generated per group.

Failure mode analysis

An optical microscope (magnification, 30 $\times$, SZ61, OLYMPUS, Tokyo, Japan) was used to observe the fractured dentin surfaces, and the failure pattern was categorized as cohesive, mixed, or adhesive. Representative specimens were observed using a scanning electron microscope (SEM; JSM-6390, JEOL, Tokyo, Japan) (Fig. 2).

Statistical analyses

The Kruskal–Wallis and Bonferroni tests were used to analyze the μ TBS data, with 5% differences considered statistically significant. EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria), was used to perform all statistical analyses.

RESULTS

Temperature measurement

The temperature of the tooth and the adhesive stored under each condition converged at room temperature (Fig. 3). However, the adhesive temperature stored at 4°C rose remarkably and returned to room temperature the fastest. Next, specimens of μ TBS were prepared within 30 s after removing the teeth from the distilled water at each temperature and immediately after removing the adhesive from the refrigerator.

μ TBS

The bond strength results are summarized in Table 2 and Fig. 4. The results of the Kruskal–Wallis test showed that the “temperature of each experimental group” ($p<0.001$) and the parameters termed “water storage periods” ($p=0.01$) had significant effects. In addition, the μ TBS values were significantly higher in the $T_{\text{high}}/B_{\text{middle}}$ group compared with the $T_{\text{middle}}/B_{\text{middle}}$

Fig. 3 Temperature transition.

Table 2 μ TBS values (MPa) and failure modes

Temperature (tooth/bonding)	Water storage		
	24 h	1 month	6 months
$T_{\text{high}}/B_{\text{middle}}$	27.2 (10.7) [0/7/9]	18.9 (12.9) [1/9/6]	19.5 (14.2) [0/11/5]
$T_{\text{high}}/B_{\text{low}}$	22.5 (8.0) [2/9/5]	19.3 (7.2) [1/11/4]	18.9 (9.0) [0/12/4]
$T_{\text{middle}}/B_{\text{middle}}$	16.5 (13.1) [0/16/0]	11.2 (9.6) [0/16/0]	11.4 (13.5) [0/16/0]
$T_{\text{low}}/B_{\text{low}}$	9.8 (9.8) [0/16/0]	9.1 (7.9) [0/16/0]	6.6 (4.6) [0/16/0]

Numbers in parentheses on the upper line are the standard deviation, and numbers in parentheses in the lower line are the number of beams per failure mode: cohesive in dentin/cohesive in the composite/interface between the adhesive and dentin/interface between the composite and the adhesive/mixed.

Fig. 4 μ TBS data.

Box plot. From above: maximum, 75th percentile, median, 25th percentile, minimum. The same letters on the top means no significant difference between groups. μ TBS: micro-tensile bond strength, 24 h: 24 hours, 1 M: 1 month, 6 M: 6 months.

Fig. 5 Fracture mode.

24 h: 24 hours, 1 M: 1 month, 6 M: 6 months

Fig. 6 SEM images of the fractured surface after μ TBS testing.

(a) $T_{\text{high}}/B_{\text{middle}}$ Mixed failure. (b) $T_{\text{high}}/B_{\text{low}}$ Cohesive failure in resin cement. (c), (d) $T_{\text{middle}}/B_{\text{middle}}$ and $T_{\text{low}}/B_{\text{low}}$, Adhesive failure, Innumerable bubbles are recognized in the bonding material. Ad: adhesive, Rc: resin cement, De: dentin, SEM: scanning electron microscope, μ TBS: micro-tensile bond strength.

group ($p=0.001$), and in the $T_{\text{high}}/B_{\text{low}}$ group compared with the $T_{\text{low}}/B_{\text{low}}$ group ($p<0.001$). By contrast, no significant difference in bond strength was seen in the $T_{\text{high}}/B_{\text{middle}}$ and $T_{\text{high}}/B_{\text{low}}$ groups ($p=0.87$). After 6 months of storage in water, the bond strength was significantly lower than that after 24 h ($p=0.01$).

Failure modes

Interfacial fractures between the dentin and resin cement were observed in all groups (Table 2 and Fig. 5). Cohesive failure in resin cement and mixed failure were confirmed in some $T_{\text{high}}/B_{\text{middle}}$ and $T_{\text{high}}/B_{\text{low}}$ group samples. SEM observation revealed a fractured surface on the resin composite side after the μ TBS test (Fig. 6). In the $T_{\text{high}}/B_{\text{middle}}$ group, dentin with a trace of resin cement and grinding on the dentin side of the fracture surface was confirmed, as was mixed fracture (Fig. 6a). Cohesive failure in resin cement was observed in the $T_{\text{high}}/B_{\text{low}}$ group (Fig. 6b). Many bubbles were confirmed in the adhesive on the resin cement side (Fig. 6c, d).

DISCUSSION

To date, only a limited number of studies have been carried out to investigate the effect of temperature on dentin bonding. Therefore, in the present study, the influence of tooth and adhesive temperature during the bonding procedure on the effectiveness of dentin bonding was evaluated. The results indicate that it took a longer time for teeth stored in distilled water at each temperature to rise to room temperature, whereas it only took a short time for the adhesive stored in the refrigerator to rise to room temperature. The differences in teeth and materials were influenced by their volume and properties (*i.e.*, solid or liquid). Based on these results, the teeth were used within 30 s after removal of distilled water at each temperature and the adhesive was used immediately after removal from the refrigerator for the bond strength test. When used after 30 s, the temperature of T_{high} was 30–35°C and that of T_{low} was 10–15°C. Also, when B_{low} was used immediately,

the temperature was about 18–20°C.

Significantly higher bond strength was observed in the $T_{\text{high}}/B_{\text{middle}}$ than in the $T_{\text{middle}}/B_{\text{middle}}$ group ($p=0.001$) and in the $T_{\text{high}}/B_{\text{low}}$ group compared with the $T_{\text{low}}/B_{\text{low}}$ group ($p<0.001$). Therefore, the null hypothesis that tooth temperature would not affect bond strength was rejected. The fact that a self-curing adhesive is affected by temperature is in agreement with a previous report that investigated the effect of temperature on self-curing composite resin; that study reported finding temperature dependence, in that the higher the temperature, the faster the acceleration of the polymerization¹². Interfacial fractures were observed in both the $T_{\text{middle}}/B_{\text{middle}}$ and $T_{\text{high}}/B_{\text{low}}$ groups, and many bubbles were confirmed in the adhesive on the resin side in the SEM observation (Fig. 6c, d). This was considered due to the residual solvent left over from the insufficient polymerization of the adhesive¹³⁻¹⁵ or the absorption of water from the dentin side due to the delayed polymerization⁶. These results suggest that polymerization is not promoted when the tooth temperature is low, and the polymerization of the self-curing adhesive does not proceed well. These findings are in agreement with the report of Kamemizu *et al.*¹². Another previous study explained why the bond strength of the photopolymerization type adhesive decreases at low temperatures by saying that under low temperature conditions, the viscosity of adhesive systems increases considerably¹⁶. It has been shown that the higher the viscosity of an adhesive, the more difficult the substrate wetting because the spreading velocity of the material is substantially reduced¹⁷. These results indicate that caution is needed when measuring bond strength in the laboratory because the actual adhesive capacity in the clinical setting cannot be judged unless the tooth temperature is raised to the same temperature as the body temperature. However, we could find no mention of the tooth temperature in ISO standards or in the adhesive dentistry literature.

The properties of monomer solutions, including viscosity and the degree of conversion, are important parameters in bond effectiveness^{18,19}, and can be altered by the temperature of adhesive systems. At present, most manufacturers recommend storing adhesive materials at room temperature; however, many dentists continue to utilize the traditional practice of refrigerating materials to extend their shelf life¹⁶. In the present study, no significant difference in bond strength was seen in the $T_{\text{high}}/B_{\text{middle}}$ or $T_{\text{high}}/B_{\text{low}}$ group ($p=0.87$). Similar to the present study, Loguercio *et al.* evaluated the effects of different adhesive temperatures (5, 20, 37, and 50°C) on resin–dentin bonding effectiveness and found no significant difference in terms of μTBS , degree of conversion, or adhesive layer thickness between the refrigerated temperature (5°C) and the room temperature (20°C)¹⁰. In addition, several studies have investigated temperature in adhesive operations, but most have reported finding positive effects (e.g., heat treatment for bonding surfaces)²⁰⁻²². Surprisingly, few studies have confirmed the negative effects of

temperature on adhesives. To the best of our knowledge, no report has been published on the temperature effect of this self-curing adhesive. Since the adhesive is greatly affected by the tooth temperature, a lower temperature of the adhesive was not expected to affect the adhesive properties. However, the results suggest that dentin bonding ability is not significantly affected, even just after the adhesive is removed from the refrigerator and the temperature is low in the clinic. In other words, it does not matter if the refrigerated product is used soon after its removal from the refrigerator or after it reaches room temperature.

The tooth and adhesive temperature effects on dentin bonding were confirmed; it was not affected by the temperature of the adhesive itself, but was affected by the temperature of the tooth. Unlike other adhesives, there are surprisingly few reports of this two-bottle self-curing universal adhesive. This is probably because the bond strength is low unless the tooth temperature is raised in the laboratory (*i.e.*, publication bias). Similar to the temperature of the tooth, that of the resin cement likely affects the durability of the dentin bond because the resin cement mount is much larger than that of the adhesive. The present study was carried out using resin cement at room temperature only. Additionally, as an indirect effect of the tooth and material temperatures, the effects of condensation and humidity also need to be investigated. Another limitation of the present study is that it is unclear whether the results apply to other self-curing adhesives because only one self-curing adhesive was evaluated. Therefore, these issues will need to be investigated in future studies.

In the present study, flat dentin was used to evaluate the effectiveness of dentin bonding; however, self-curing adhesive is more useful in deep cavities, and even in root canals, this self-curing adhesive can cure without light. Its bonding effectiveness to root canal dentin should therefore be evaluated. Moreover, it has been reported that the bond strength of the light-activated adhesive is improved by blowing warm air on the dentin²⁰⁻²²; thus, even in the case of self-curing adhesives, there is a possibility that the bond strength may be further improved by a rise in the temperature of the teeth induced by warm air when compared with light-cure bonding systems. These issues should also be examined in further studies considering the adhesion mechanism of self-curing adhesives. In any case, the results of this study indicate that to accurately ascertain the performance of adhesives accurately in clinical practice, the temperature of the teeth needs to be close to that of the oral cavity.

CONCLUSIONS

When using a self-curing adhesive:

1. The tooth temperature affects the effectiveness of the dentin bonding. To determine the precise bonding ability in the laboratory, the temperature of the tooth must be raised until it is the same as that of the oral cavity.

2. The temperature of the material does not affect bonding effectiveness. This result confirms that it does not matter if the refrigerated product is used soon after its taking from the refrigerator or after it reaches room temperature in the clinic.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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