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using an in vitro Model of Hypersensitive Dentin**

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# Study on Adhesion of Composite Resin using an *in vitro* Model of Hypersensitive Dentin

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## Abstract

**Purpose:** we prepared an *in vitro* model of hypersensitive dentin, which had the same intrapulpal pressure as in humans and the wet condition inside dentinal tubules closely resembled the condition in the clinical setting, and performed light-cured composite resin filling on it. We then examined the state of adhesion when the dentin was sealed with a light-cured composite resin for the treatment of dentin hypersensitivity.

**Methods:** Human molar teeth were used for this experiment. For the experiment, pieces of coronal dentin were smoothed to a flat surface and their coronal sides were abraded with wet sandpaper until #600, in order to prepare dentin disks of 1 mm thickness. With dentin disks that would be subjected to pressure of 25 mmHg, the same as human intrapulpal pressure, the *in vitro* model of hypersensitive dentin was prepared. Then, adhesives of four types of one-bottle one-step bonding system (BeautiBond Multi (BB), G-BOND PLUS (GB), Scotchbond™ Universal Adhesive (SU) and CLEARFIL® S<sup>3</sup>BOND ND Quick (TB) ) were applied. On the other hand, the control group was prepared through the same procedure except that the dentin disks of 1 mm thickness were not put on the apparatus. After the adhesive was applied, they were preserved in water at 37°C for 24 hours or 6 months and then subjected to the tensile bond strength test, creating four groups: 24h control group, 24h restored dentin group, 6M control group, and 6M restored dentin group (n = 7).

**Results:** Concerning the specimens after 24-hour preservation, TB in the control group and SU and TB in the restored dentin group showed significantly greater bond strength compared with other products. Concerning the specimens after 6-month preservation, in the control group no significant differences were observed among the products, whereas in the restored dentin group SU showed significantly greater bond strength compared with BB. When the 24h control group, the 24h restored dentin group, the 6M control group, and the 6M restored dentin group were compared with each other in each of the products, no significant differences between the 24h control group and the 6M control group were observed except in TB.

**Conclusion:** When dentin hypersensitivity is treated with adhesive resins, bonding systems including an organophosphate monomer (MDP) are more effective. And the moisture from dentinal tubules may permeate and cause adhesion failure with the passage of time.

**Key wards:** hypersensitive dentin, human intrapulpal pressure, tensile bond strength test

## Introduction

When dentin is exposed, transient pain may be felt in response to stimuli including thermal stimuli such as cold air or cold water, mechanical stimuli such as brushing, etc. This pathological condition is clinically called dentin hypersensitivity. Although the mechanism of pain transmission through dentin is not fully understood, there are some theories including the following: 1) nerve endings present in dentinal tubules respond directly to stimuli and cause pain (Direct Innervation Theory),<sup>1,2</sup> 2) odontoblast processes, working as pain receptors, transmit stimuli to pulp and cause pain (Odontoblast Receptor Theory),<sup>3,4</sup> 3) flow of fluid in dentinal tubules stimulates free nerve endings in pulp and causes pain (Hydrodynamic Theory),<sup>5-7</sup> and 4) morphological change of odontoblast processes, not flow of fluid in dentinal tubules, causes excitation of free nerve endings and hence pain (Odontoblast Deformation Theory).<sup>8,9</sup> Currently, the Hydrodynamic Theory is the most widely accepted. According to the theory, which was proposed in 1963 by Brännström et al., stimuli to dentin create some changes in the flow of fluid in dentinal tubules, and the changes stimulate free nerve endings present around odontoblast and thus cause pain.<sup>5,6</sup>

Dentin hypersensitivity can be categorized according to the site of occurrence into cervical hypersensitivity, root hypersensitivity, gingival hypersensitivity, postoperative hypersensitivity by an exposed dentinal surface after cavity preparation, etc. Except for postoperative hypersensitivity due to cavity preparation, dentin hypersensitivity often occurs along with gingival recession of not less than 1 mm,<sup>10</sup> and approximately half of all such cases are cervical hypersensitivity, which occurs at the cervical portion on the buccal side,<sup>11</sup> with a wedge-shaped defect—a loss of hard tissue substance—observed in many cases at the cervical portion on the buccal side.<sup>12</sup> Yoshiyama et al.<sup>13,14</sup> reported that an estimated 75% of dentinal tubules are open at the site of a wedge-shaped defect on a hypersensitive tooth, allowing dentin hypersensitivity to occur through the hydrodynamic pain transmission mechanism. In other words, the surface of a tooth with dentin hypersensitivity is considered to be in the wet condition clinically to a certain extent. Treatments used for dentin hypersensitivity include application of liquid medicine, iontophoresis, laser irradiation, and sealing the site of occurrence with adhesive materials.<sup>15-17</sup> For a hypersensitive site with a wedge-shaped defect, sealing with adhesive materials is often chosen in the clinical setting.

In this study, we prepared an *in vitro* model of hypersensitive dentin, which had the same intrapulpal pressure as in humans and the wet condition inside dentinal tubules closely resembled the condition in the clinical setting, and performed light-cured composite resin filling on it. We then examined the state of adhesion when the dentin was sealed with a light-cured composite resin for the treatment of dentin hypersensitivity.

## Materials and methods

### 1. Preparation of *in vitro* model of hypersensitive dentin

This study was conducted with the approval of the Medical Ethics Committee of Osaka Dental University (No. 110767).

Human molar teeth extracted at the Department of Oral and Maxillofacial Surgery in Osaka Dental University Hospital were used for this experiment, with the consents of the patients from whom the teeth were extracted. After extraction, the teeth were preserved in physiological saline solution frozen at  $-40^{\circ}\text{C}$  and thawed immediately before use. For the experiment, pieces of coronal dentin were smoothed to a flat surface with a model trimmer and their coronal sides were abraded with wet sandpaper until #600, in order to prepare dentin disks of 1 mm thickness. After the abrasion, the disks were cleansed for 10 minutes with an ultrasonic cleaner.

The method developed by Zenny et al.<sup>18</sup> for preparing an *in vitro* model of hypersensitive dentin was used to reproduce the model (Fig. 1). Although Zenny et al. treated the coronal side with 10% phosphoric acid solution and the root side with 10% sodium hypochlorite in order to open dentinal tubules, we observed the surfaces of dentin disks and chose those with open dentinal tubules because chemical treatments could not be performed due to the fact that adhesives would be applied on the dentinal surfaces. With dentin disks that would be subjected to pressure of 25 mmHg, the same as human intrapulpal pressure, the *in vitro* model of hypersensitive dentin was prepared.<sup>19-21</sup>

### 2. Bond strength test

The restored dentin group was prepared through the following procedure: a piece of double-sided adhesive tape with a hole of 3 mm inside diameter was taped on the adherend surface of the *in vitro* model of hypersensitive dentin, and a brass mold of 3.5 mm inside diameter and 2.0 mm height, which acted also as a tensile jig, was placed on it. Then, adhesives of four types of one-bottle one-step bonding system (Table 1) were applied according to the manufacturers' instructions (Table 2). On the other hand, the control group was prepared through the same procedure except that the dentin disks of 1 mm thickness were not put on the apparatus. After the adhesive was applied, they were preserved in water at  $37^{\circ}\text{C}$  for 24 hours or 6 months and then subjected to the tensile bond strength test, creating four groups: 24h control group, 24h restored dentin group, 6M control group, and 6M restored dentin group. For the bond strength test, each of the specimens was lined with an acrylic board because it would fracture with only a dentin disk of 1 mm thickness. The universal testing machine IM-20 (INTESCO) was used for the bond strength test, and the tensile strength was measured at a cross head speed of 0.3 mm/min to calculate the mean and standard deviation ( $n = 7$ ).

### 3. Observation of fracture surface with SEM

After the bond strength test, a thin layer of gold was deposited on the fracture surface with an ion coater according to the standard practice, and the fracture surface was observed with a scanning electron microscope. Fracture surfaces were categorized according to the state of fracture into the following: interfacial failure (not less than 70% area of the fracture surface is interface), dentin cohesive failure or

bonding layer cohesive failure (not less than 70% area of the fracture surface is dentin or bonding layer, respectively), and mixed failure (neither of the above).

#### 4. Statistical analysis

The results of the bond strength test were statistically analyzed with the one-way analysis of variance and the Newman-Keuls method ( $P < 0.05$ ). The results of morphological categorization of fracture surfaces were statistically analyzed with the Mann-Whitney U-test ( $P < 0.05$ ).

## Results

### 1. Bond strength test

The results of the bond strength test after 24-hour preservation are shown in Fig. 2 and the results after 6-month preservation are shown in Fig. 3. In addition, the results of each of the products are shown in Fig. 4. Concerning the specimens after 24-hour preservation, TB in the control group and SU and TB in the restored dentin group showed significantly greater bond strength compared with other products. Concerning the specimens after 6-month preservation, in the control group no significant differences were observed among the products, whereas in the restored dentin group SU showed significantly greater bond strength compared with BB but no significant differences were observed among other products. When the 24h control group, the 24h restored dentin group, the 6M control group, and the 6M restored dentin group were compared with each other in each of the products, no significant differences between the 24h control group and the 6M control group were observed except in TB. In addition, no significant differences were observed in each of the products between the 24h restored dentin group and the 6M restored dentin group and between the 6M control group and the 6M restored dentin group. Between the 24h control group and the 24h restored dentin group, significant differences were observed in all the products except for SU.

### 2. Forms of failure at interface

Forms of failure at the interface by the bond strength test after 24-hour and 6-month preservation are shown in Table 3.

Although interfacial failure was often observed in BB and SU and bonding layer cohesive failure and mixed failure were often observed in TB and GB, no significant differences were observed among the groups. In TB, significant differences were observed in the 24h restored dentin group and the 6M restored dentin group compared with the 24h control group. In GB, significant differences were observed in the 24h restored dentin group and the 6M restored dentin group compared with the 24h control group and in the 24h restored dentin group and the 6M restored dentin group compared with the 6M control group. However, no significant differences were observed in each of the products between the 24h control group and the 6M control group and between the 24h restored dentin group and the 6M restored dentin group.

## Discussion

The effect of moisture at bonding interfaces has been regarded as important since Sano et al.<sup>22</sup> reported nanoleakage on bonding interfaces observed under a scanning electron microscope (SEM) in 1995 and Tay et al.<sup>23</sup> reported water treeing observed under a transmission electron microscope (TEM) in 2003. The aging mechanism of resin-dentin bonding structures can be explained in terms of hydrolysis of an exposed collagen fibril layer, hydrolysis of a bonding resin, and disappearance of silane coupling agents between

matrix and filler of a composite resin. The presence of moisture on bonding interfaces is regarded as essential for these aging mechanisms. Many studies have been conducted on the effect of humidity at adherend surfaces on bond strength and the effect of wet conditions at adherend surfaces of teeth on dentinal adhesiveness in reproduced oral environments.<sup>24-27</sup> Some studies have reported that moist adherend surfaces of teeth increased the bond strength of dentin in the priming adhesive system but decreased it in the self-etching system<sup>28</sup> and that when a dentinal adherend surface, treated with blot-drying after rinsing in water, was subjected to a bond strength test in the one-step adhesive system, the effect of the treatment varied depending on the products used<sup>29</sup>. In the clinical setting, the dentinal surface is considered to be in the wet condition due to the leakage of pulpal fluid. According to the standard adhesion theory,<sup>30</sup> the presence of moisture on dentinal surfaces is a factor that may inhibit adhesion, and so the control of moisture on adherend surfaces has been regarded as an important factor that may affect dentinal adhesiveness. Thus, hydrophilic HEMA has often been used in dentin bonding systems, but it is considered that HEMA indeed increases bond strength at an early stage but after an extended period of immersion in water the bond strength declines as the absorbency increases depending on the proportion of contained HEMA.<sup>31</sup> Consequently, acetone or a hydrophilic monomer has been used in recent products in order to be less sensitive to moisture. In BeautiBond Multi and G-BOND PLUS, both of which were used in this experiment and are one-bottle one-step bonding system, acetone is used to deal with the effect of moisture. In Scotchbond™ Universal Adhesive and CLEARFIL® S<sup>3</sup>BOND ND Quick, organophosphate MDP, which is reported to be stable and durable in the presence of moisture,<sup>32</sup> is used as an adhesive monomer to deal with the effect of moisture.

Although it is difficult to exactly reproduce the outer layer of teeth with dentin hypersensitivity, it is possible to reproduce the environment where fluid in dentinal tubules can flow. In this experiment, we made a comparison between specimens in which fluid in dentinal tubules can flow (the restored dentin group) and specimens with no flow of fluid in dentinal tubules (the control group), the latter of which have been used for traditional experiments.

The results of the bond strength test after 24-hour preservation showed that the bond strength of BB, TB and GB was significantly lowered in the 24h restored dentin group compared with the 24h control group, but that the bond strength of SU, which uses the same MDP as TB for an adhesive monomer, was not significantly lowered. Although the bond strength of TB in the 24h control group was significantly greater than that of other products and its bond strength in the 24h restored dentin group was also the greatest (10.4 MPa), significant differences were observed. These results indicated the effectiveness of MDP in wet conditions. In addition, in comparison within the 24h restored dentin group, SU and TB showed significantly greater values than BB and GB, and no significant differences were observed between SU and TB. In contrast, the bond strength of BB and GB, both of which use acetone to deal with the presence of moisture, was lowered. In this experiment, the restored dentin group was subjected to pressure of 25 mmHg, the same as intrapulpal pressure. As a result, the moisture continued to be provided even in 10 seconds, which is the necessary period for the treatments with BB and GB, and this may be the reason why the moisture did not evaporate sufficiently even with the use of acetone. Furthermore, among the products used for this study, TB and GB contain filler but SU and BB do not. Although some studies have reported that the presence of filler may increase mechanical strength or bond strength but also facilitate brittleness,<sup>33,34</sup>

the bond strength of both GB and BB, the former of which contains filler and the latter does not, was lowered, so it was not confirmed in this experiment whether the presence or absence of filler can change the bond strength.

On the other hand, concerning the bond strength after 6-month preservation, no significant differences were observed between the 6M control group and the 6M restored dentin group in each of the products. It is speculated that hydrolytic degradation on bonding interfaces or in bonding resins led to the decline of bond strength in the 6M control group. Thus, when compared with each other, TB showed a significantly greater value in the 24h control group, but in the 6M control group no significant differences among the products were observed. Only TB does contain HEMA among the products used for this study. It is speculated that greater bond strength was shown in the specimens after 24-hour preservation because HEMA is hydrophilic but that in the specimens after 6-month preservation the bond strength was lowered due to the hydrolytic degradation. In addition, while SU and TB showed significant differences compared with other products in the 24h restored dentin group, in the 6M restored dentin group a significant difference was observed only between BB and SU and no significant differences were observed among

other products. This indicated that the differences of bond strength among the products disappeared due to the hydrolytic degradation.

These results indicated that the presence or flow of fluid in dentinal tubules affects bond strength and that it is necessary to pay attention to the decline of bond strength when a dentinal surface is sealed with a light-cured composite resin filling for the treatment of dentin hypersensitivity in the clinical setting.

In this experiment, the intrapulpal pressure was set for dentin hypersensitivity only at the time of the bond strength test, which means that the pressure was not kept continuously and that the specimens were only preserved in water at 37°C during the periods of 24 hours and 6 months. In this experiment, no product showed significant differences between the 24h restored dentin group and the 6M restored dentin group. However, if chronological changes under the internal pressure had kept being observed, the hydrolytic degradation in bonding layers might have advanced, causing a continuous decline of bond strength in the restored dentin group. Furthermore, while distilled water was used as fluid in dentinal tubules for this experiment, proteins and enzymes are present in actual fluid. It is considered necessary in the future to establish an experimental system into which these elements can be incorporated.

## Conclusions

By using an *in vitro* model of hypersensitive dentin, which had the same intrapulpal pressure of 25 mmHg as in humans and the conditions of dentinal tubules closely resembled the conditions in the clinical setting, the state of adhesion when the dentin was sealed with a light-cured composite resin for the treatment of dentin hypersensitivity was examined, and the following results were obtained.

1. When dentin hypersensitivity is treated with adhesive resins, bonding systems including an organophosphate monomer (MDP) are more effective.
2. The moisture from dentinal tubules may permeate and cause adhesion failure with the passage of time.

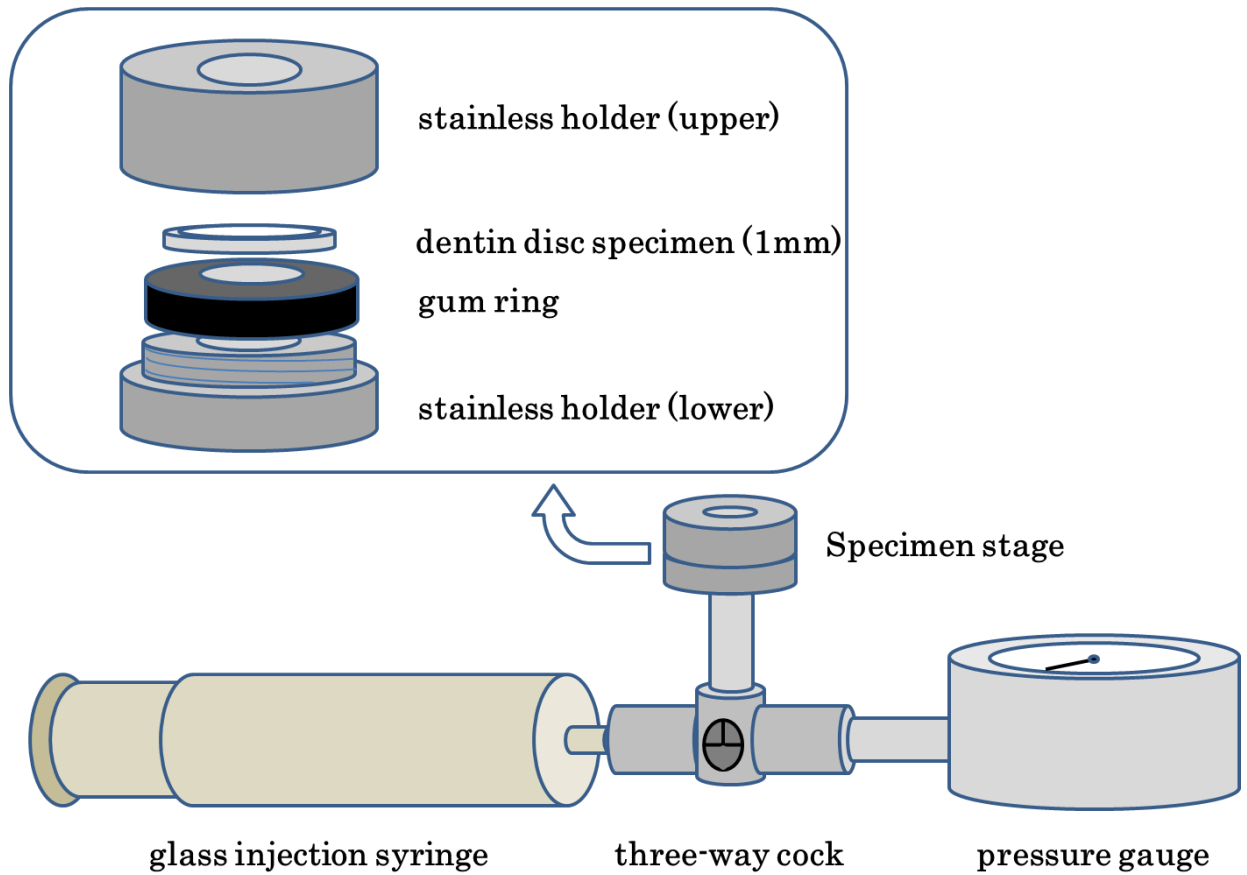
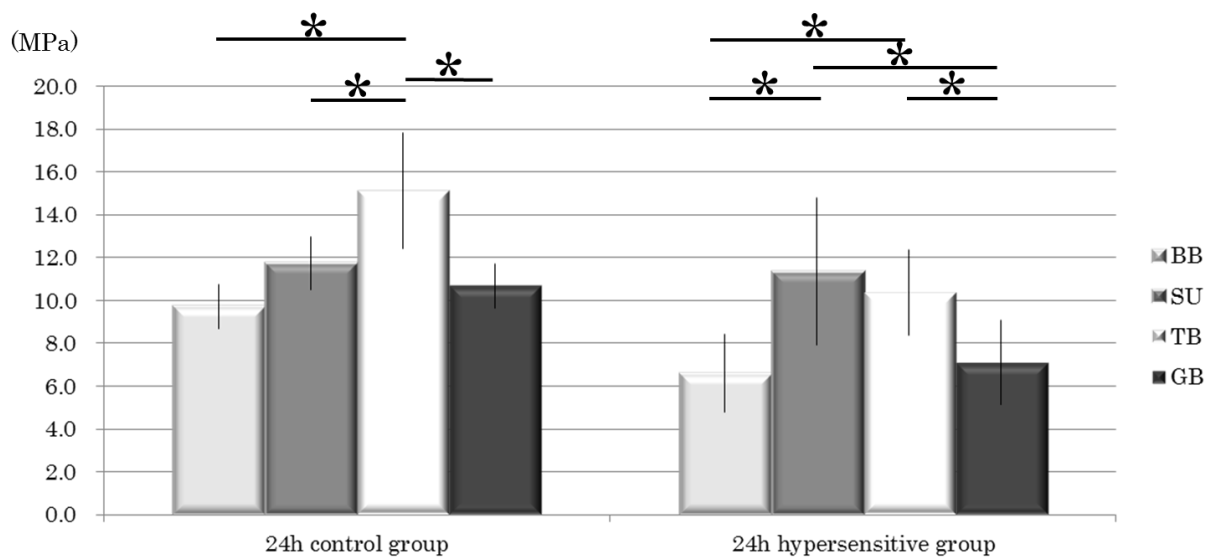


Fig.1 Schematic diagram of model of hypersensitive dentin



\* : P < 0.05

Fig. 2 Results of TBS (24h)



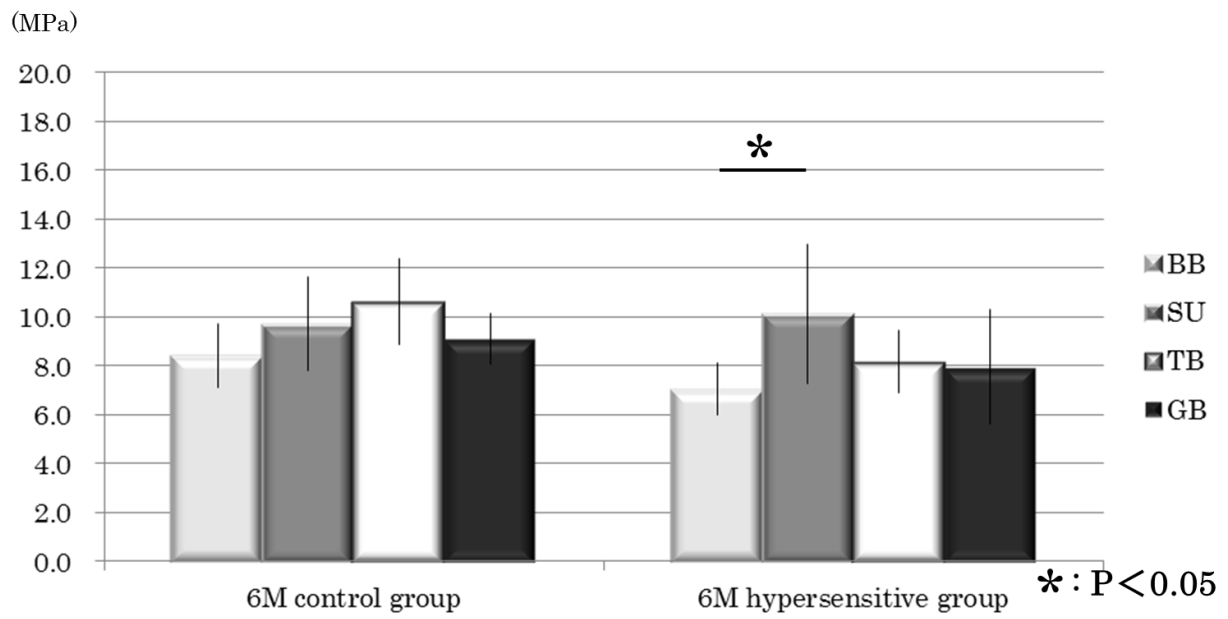


Fig. 3 Results of TBS (6M)

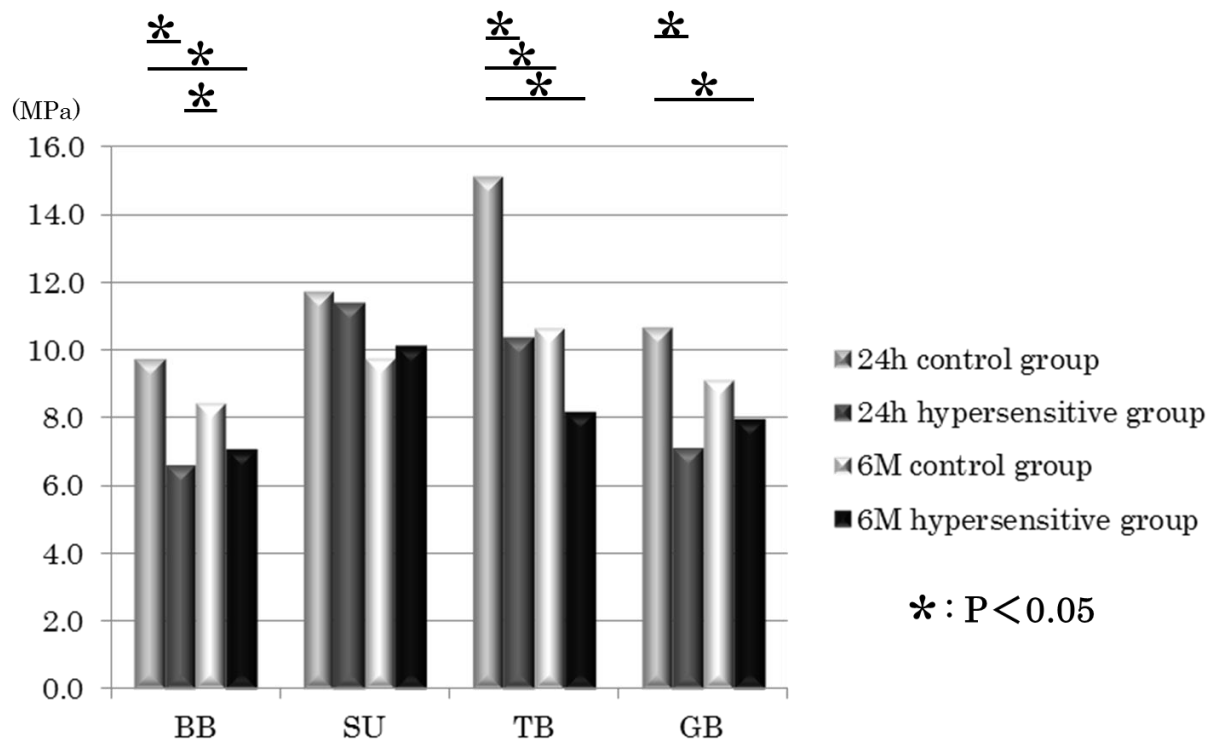


Fig. 4 Results of TBS

<b>Bonding systems</b>	<b>Manufacturer</b>	<b>Code</b>	<b>Lot No.</b>
BeautiBond Multi	Shohu	BB	111336
Scotchbond™ Universal Adhesive	3M	SU	529681
CLEARFIL® S <sup>3</sup> BOND ND Plus	Kuraray Noritake Dental	TB	AF0001
G-BOND PLUS	GC	GB	1306141

<b>Code</b>	<b>Main component</b>	<b>surface treatment</b>
<b>BB</b>	4-AET, Phosphonic acid monomer, TEGDMA, Bis-GMA, Aceton, Water	Apply(10s)-air(3s)-light(10s)
<b>SU</b>	MDP, ethanol	Apply(20s)-air(5s)-light(10s)
<b>TB</b>	MDP, HEMA, Bis-GMA, Silica filler, water, ethanol	Apply-air(5s)-light(10s)
<b>GB</b>	4-MET, Phosphonic acid monomer,UDMA, Silica filler, Aceton, Water	Apply(10s)-air(5s)-light(10s)

	<b>BB</b>								<b>SU</b>			
	<b>control group</b>		<b>hypersensitive group</b>		<b>control group</b>		<b>hypersensitive group</b>		<b>control group</b>		<b>hypersensitive group</b>	
	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>
<b>Interfacial failure</b>	6	5	6	6	5	6	5	6	5	6	5	6
<b>Coheasive failure of dentine</b>					2	1	1					
<b>Coheasive failure of adheasive</b>		2										1
<b>Mixed failure</b>	1		1	1			1					
<b>Mann-Whitney U-test's group</b>	a	b	c	d	a	b	c	d	a	b	c	d
	<b>TB</b>				<b>GB</b>							
	<b>control group</b>		<b>hypersensitive group</b>		<b>control group</b>		<b>hypersensitive group</b>		<b>control group</b>		<b>hypersensitive group</b>	
	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>	<b>24h</b>	<b>6M</b>
<b>Interfacial failure</b>		2	2	4		4	1	5				
<b>Coheasive failure of dentine</b>												
<b>Coheasive failure of adheasive</b>	2	5	3	3	3	3	3	2				
<b>Mixed failure</b>	5		2		4		3					
<b>Mann-Whitney U-test's group</b>	ab	c	a	b	ac	bd	ab	bd				

The same lower-case letters indicate significant difference in the Code(p < 0.05).

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# 知覚過敏症罹患モデル象牙質への 光重合型コンポジットレジンの接着性について

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## 和文抄録

目的：ヒトの歯髄内圧と同様の圧を設定し，象牙細管内の湿潤状態を臨床の状態に近づけた知覚過敏症罹患モデル象牙質を作製し，光重合型コンポジットレジン充填を行い，象牙質知覚過敏症治療における光重合型コンポジットレジン被覆時における接着状態の研究を行った。

材料と方法：被験歯としてヒト大白歯を使用した。実験には歯冠部象牙質を用い，モデルトリマーにて面出し後，歯冠側面を耐水紙 #600 まで研磨した厚さ 1mm の象牙質ディスクを作製した。研磨後，ヒト歯髄内圧とされている 25mmHg の圧を象牙質ディスクにかかるように設定し，知覚過敏症罹患モデル象牙質とした。ボンディングシステムとして，BeautiBond Multi (以下 BB)，G-BOND PLUS (以下 GB)，Scotchbond™ Universal Adhesive (以下 SU) と CLEARFIL® S<sup>3</sup>BOND ND Quick (以下 TB) を使用した。知覚過敏症罹患モデル象牙質被着面に接着操作を行い，罹患象牙質修復群とした。また，厚さ 1mm の象牙質ディスクを装置に装着せずに接着操作を行ったものをコントロール群とした。接着後 37°C 水中に 24 時間保管後と 6 か月保管後に引張接着試験を行い，それぞれ 24h コントロール群，24h 罹患象牙質修復群，6M コントロール群および 6M 罹患象牙質修復群とした。接着試験は引張強さの測定を行い，平均値および標準偏差を算出した (n = 7)。

成績：24 時間後の試料では，コントロール群において TB が，罹患象牙質修復群では SU と TB が他の製品に対し有意に高い接着強さを示した。6 か月後の試料では，コントロール群では各製品間に有意な差は認められず，罹患象牙質修復群では SU が BB に対し有意に高い接着強さを示した。各製品における 24h コントロール群，24h 罹患象牙質修復群，6M コントロール群および 6M 罹患象牙質修復群間の比較では，TB のみで，24h コントロール群と 6M コントロール群間で有意な差が認められた。

結論：象牙質知覚過敏症を接着性レジンで治療する場合，リン酸エステル系モノマー (MDP) 含有のボンディングシステムの有効性が高い。また象牙細管からの水分の浸潤によって，接着が経時的に破壊される可能性がある。

## 和文キーワード

象牙質知覚過敏症，歯髄内圧，接着試験