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Effects of Various Desensitizing Agents on the Microtensile Bond Strength of a Hypersensitive Dentin Model Produced in vitro Using a One-step Self-etch System

Bayarmaa Batzorig1, Kenjiro Nakano1, Kosei Murata1, Mayumi Maesako1, Kazuho Inoue1, Takafumi Kishimoto1, Shigetaka Tomoda1, Hatsuhiiko Maeda2, Taku Horie1 and Morioki Fujitani1

1) Department of Operative Dentistry, School of Dentistry, Aichi Gakuin University, Nagoya, Japan
2) Department of Oral Pathology, School of Dentistry, Aichi Gakuin University, Nagoya, Japan

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Abstract: In the treatment of dentin hypersensitivity accompanying tooth substance defects such as wedge-shape defects, hypohesthesia can be achieved by applying a desensitizing agent before carrying out restoration using resin composite. However, almost no research has investigated the adhesion of resin to dentin coated with the latest desensitizing agents. Therefore, this study investigated the effects of various desensitizing agents on the adhesion of resin to dentin in combination with a 1-step self-etch system by using a hypersensitive dentin model in which the dentinal tubules were opened without etching and there was almost no smear layer on the intertubular dentin. Specimens with a #4000 polished dentin flat surface were ultrasonically cleaned for 60 min (15 min × 4 times). Then, the bond strength, failure modes, and micromorphology of surfaces coated with desensitizing agent to which resin was bonded immediately afterward and surfaces coated with desensitizing agent to which the resin was bonded after storage for 7 days in water were compared against a control to which no desensitizing agent was applied. The desensitizing agents used in this research did not promote adhesion of the resin immediately after application, but rather suppressed or completely obstructed it. Although deposits of microparticles and thin film material, which were observed immediately after application, tended to disappear after 7 days of storage in water, some of the desensitizing agents exhibited the same bond strength as the control, whereas other desensitizing agents did not show recovery of adhesion strength. Therefore, care is required when performing resin restoration immediately after application of a desensitizing agent, depending on the agent used, and caution must be exercised in the selection of desensitizing agents in the clinical setting.

Key words: Desensitizer, Hypersensitive dentin, Microtensile bond strength, One-step self-etch system

Introduction

In recent years, the number of patients with dentin hypersensitivity has been growing rapidly due to lifestyle changes such as dietary habits. This change has been accompanied by the commercialization of desensitizing agents with various mechanisms of action. In the treatment of dentin hypersensitivity accompanying tooth substance defects such as wedge-shaped defects, the defect is not always immediately repaired using resin. Instead, an attempt is first made to alleviate the symptoms by applying a desensitizing agent before proceeding to restoration.

Although many studies have investigated the adhesion of resin to dentin surfaces coated with desensitizing agents, the observed resin bond strengths tended to differ according to the experimental conditions, even for the same desensitizing agent. The reason for this is that almost all of the prepared dentin surfaces to which the experimental condition was applied in those studies were #600 polished using waterproof silicon carbide paper. Given that the morphology of these surfaces is nearly the same as that obtained by using a dental bur and the surface is covered by a smear layer with occluded dentinal tubules, the surface properties are thought to differ from those of dentin at sites of hypersensitivity where the dentinal tubules are open. Furthermore, phosphoric acid or EDTA is generally used to remove the smear layer and open the dentinal tubules, and these etching agents also etch the surface of the intertubular dentin. Thus, the bond strength is potentially affected. No study has been investigated the bonding surface in patients with dentin hypersensitivity in the clinical setting to determine the effect of desensitizing agents on resin–dentin bond strength, and no study has compared the resin bond strength on dentin surfaces coated with various types of recent desensitizing agents.

Apart from the reports of Yoshiyama et al., no study has been investigated in detail the biopsy data of hypersensitive dentin. According to their findings, around 75% of dentinal tubules at sites of hypersensitivity had openings, and many microscopic abrasion marks, which are thought to be due to dentifrice, were found at those positions, whereas other positions had a very smooth and glossy appearance. However, resin bond strength has not been investigated using a dentin bonding surface that faithfully reproduces these morphological properties.

Therefore, this study developed a new hypersensitive dentin model in which the dentinal tubules are opened without etching, little or no smear layer remains on the intertubular dentin, and polishing marks are
found at the openings. This model was used to investigate the effects of various types of recent desensitizing agents on the resin–dentin bond strength in combination with a 1-step self-etch system.

**Materials and Methods**

**Preparation of dentin bonding surface**

Freshly extracted bovine incisors were cut perpendicular to the tooth axis at a position around 7 mm apical to the labial cervical center by using a low-speed precision cutter (IsoMet; Buehler, Lake Bluff, IL, USA). After removing the pulp, a flat dentin surface (approx. 5 mm × 10 mm) was prepared on the labial cervical part of the tooth by using an automatic grinder (Ecomet; Buehler) and waterproof silicon carbide paper (#400; Refine Tech, Tokyo, Japan) under flowing water; this was cleaned 3 times for 5 min each using an ultrasonic cleaner (40 kHz, AUC-06L; AS ONE, Osaka, Japan). For convenience in the experiments, a flat surface (#400) was also prepared on the lingual cervical part of the tooth parallel to the prepared surface.

To simulate a surface cut by a dental bur 13,14 by using the flat dentin surfaces prepared above on the labial cervical part of the teeth, #600 polished surfaces were prepared using waterproof silicon carbide paper under flowing water, and the surfaces were cleaned ultrasonically (5 min × 3). Furthermore, to simulate a hypersensitive dentin surface, polishing was performed sequentially in two perpendicular directions using #800, #1000, and #2000 waterproof silicon carbide paper under flowing water, and the surfaces were cleaned ultrasonically (5 min × 3). Next, #4000 polished surfaces were prepared using the same method but with a 3-μm aluminum oxide film (A3-3SHT, Abrasive Lapping Film #4000; 3M ESPE, St. Paul, MN), and ultrasonic cleaning was performed (15 min × 4).

The #600 and #4000 finished dentin surfaces were observed in detail by scanning electron microscopy [SEM; (VE-9800; Keyence, Osaka, Japan)], with a focus on the abrasion marks and the condition of the dentinal tubule openings. Fig. 1 shows the experimental procedure.

**Application of desensitizing agent and observation of the coated surface**

The #4000 polished dentin surfaces were used as a control. The experimental specimens were #4000 polished dentin surfaces coated using the 4 desensitizing agents shown in Table 1, namely, Superseal (Phoenix Dental, Inc., Fenton, MI), Caredyne Shield (CS; GC Corp., Tokyo, Japan), Nanoseal (NS; Nippon Shika Yakuhin Co., Ltd., Yamaguchi, Japan), and MS Coat Hys Block Gel (Sun Medical Co., Ltd., Shiga, Japan), according to the respective manufacturer’s instructions. The coated specimens were washed for 5 s in distilled water (pH 6.5) (Table 2). The samples were divided into two groups: the Immediate group, in which the bonding process was performed immediately after application of the desensitizing agent; and the 7-day group, in which the bonding process was performed after storage in distilled water (pH 6.5) at 37°C for 7 days (Fig. 2). The distilled water was replaced once every 2 days during storage to prevent changes in pH 21. In addition, the conditions of the dentin surfaces coated with the various desensitizing agents were observed by SEM focusing on the aspect of the openings of dentinal tubules in both groups.

**Bonding process and resin placement**

The bonding surface of each sample was washed in distilled water for 10 s and air-dried for 10 s, after which the Scotchbond Universal Adhesive (3M ESPE) 1-step self-etch system was applied, as shown in Table 2, and exposed to light (All VALO LED; Ultradent Products, Inc., South Jordan, UT) for 20 s.

Resin composite (Clearfil AP-X; Kuraray Noritake Dental, Inc.) was incrementally applied in a stack of 1-mm-thick layers to a thickness of 5 mm, and each layer was light-cured for 20 s. These samples were stored in distilled water (pH 6.5) at 37°C for 24 h and then subjected to a microtensile bond-strength test (Fig. 2).

**Microtensile bond strength test and statistical analysis**

Each of the specimens with resin attached was cut into 1.0-mm sec-
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In the tooth axis direction by using the Isomet, and then square prism-shaped specimens (1.0 mm × 1.0 mm) were fabricated by trimming to obtain a bonding interface surface area of $1.0 \text{mm}^2$. There were 10 samples in each group. Both ends of each of these specimens were affixed to a bond-strength testing jig by using cyanoacrylate adhesive (Zapit; Dental Ventures of America, Inc., Corona, CA) and subjected to a microtensile bond strength test (EZ Test; Shimadzu, Kyoto, Japan) at a crosshead speed of 1.0 mm/min (Fig. 2).

Statistical analysis was performed for all of the obtained microtensile bond strengths ($\mu$TBS) by using one-way analysis of variance and Tukey’s test ($\alpha=0.05$).

Observation of failure modes after bond strength test

The failure modes of each specimen after the bond strength test were observed in detail by digital stereoscopic microscopy (UFX-II; Nikon, Tokyo, Japan, 10× magnification) and SEM. The observed failure modes were categorized as cohesive failure inside the dentin, cohesive failure inside the resin, cohesive failure inside the bonding resin, adhesive failure at the dentin–bonding resin interface, and a mixture of these modes (Fig. 3).

| Materials | Composition | Manufacturer | Lot. No | Code |
|-----------|-------------|--------------|---------|------|
| Superseal | oxalic acid, potassium salt, purified water | Phoenix Dental, Inc., Fenton, MI | 100295 | SS |
| Caredyne Shield | Liquid A: phosphoric acid, purified water Liquid B: zinc, calcium, fluoride | GC Corp., Tokyo, Japan | 1909191 | CS |
| Nanoseal | Liquid A: fluorooxaluminosilicate glass dispersion, purified water Liquid B: phosphoric acid, purified water | Nippon Shika Yakuhin Co., Ltd., Yamaguchi, Japan | J9L | NS |
| MS Coat Hys Block Gel | MS polymer, oxalic acid, sodium fluoride, potassium salt, purified water | Sun Medical Co., Ltd., Shiga, Japan | VE22 | MS |

Table 2. Application methods

| Materials | Application method |
|-----------|--------------------|
| Superseal | Take 1 drop and apply gently to the dentin surface for 5 s. |
| Caredyne Shield | Mix 2 equal proportions of liquid thoroughly using a micro-brush and apply to the dentin surface for 20 s. |
| Nanoseal | Mix 2 equal proportions of liquid thoroughly using a micro-brush and apply to the dentin surface for 20 s. |
| MS Coat Hys Block Gel | Apply gently to the dentin surface for 30 s. |
| Scotchbond Universal Adhesive | Take 2 drops and apply the adhesive to the entire prepared dentin surface with a micro-brush gently for 20 s. Gently air-blow for 5 s. Light-cure for 20 s. |
| Clearfil AP-X | Each 1-mm-thick layer is placed incrementally and light-cured for 20 s until a thickness of 5 mm is reached. |

Table 1. Materials used

Table 2. Application methods

Results

Micromorphology of surfaces coated with desensitizing agent

Fig. 4 and Fig. 5 show representative SEM images of the micromorphology of the #600 polished and #4000 polished dentin surfaces and #4000 polished dentin surfaces coated with each desensitizing agent (SS, CS, NS, and MS) in the immediate and 7-day groups.

In the immediate group, the #600 polished dentin surface was covered by a smear layer and regular abrasion marks were observed but no dentinal tubule openings were found. In contrast, on the surface of the control #4000 polished dentin, almost no smear layer was observed, with only a small amount remaining on the intertubular dentin, but open dentinal tubules and polishing marks at the openings were observed, whereas the relatively smooth intertubular dentin was observed around some occluded tubules. On the SS-coated surface, a single layer of ultrafine particles appeared to be deposited on the dentin surface, and open dentinal tubules were scattered throughout the area. The surfaces coated with CS and NS exhibited a similar condition, and a morphology resembling intertubular dentin and dentinal tubule openings covered in a single layer of fine particles was observed. However, the MS-coated surface was covered with a single layer of film material consisting of microparticles and no dentinal tubules were observed.

In the 7-day group, the morphologies of the #600 and #4000 pol-
ished dentin surfaces were nearly the same as those in the immediate group. On the SS- and MS-coated surfaces, almost no deposits and thin film material like those observed in the immediate group were observed, and the dentinal tubules were open across the entire surface. Even on the surfaces coated with CS or NS, the microparticles observed in the immediate group disappeared to varying degrees, from partially to almost completely. On the CS-coated surface, the intertubular dentin surface was smooth and there was a scattering of open dentinal tubules. On the NS-coated surface, some microparticles of relatively large diameter remained on the intertubular dentin and at the dentinal tubule openings.

**Microtensile bond strength**

Table 3 and Fig. 6 show the obtained microtensile bond strengths and the corresponding statistical analysis results. The bond strength is expressed as the mean value (standard deviation).

On the #600 polished surface and control #4000 polished surface (Cont(#4000) group), which was not coated with desensitizing agent, the bond strengths in the immediate group were 35.3 (4.1) and 36.2 (7.1) MPa, respectively, and were 41.1 (9.1) and 42.7 (8.3) MPa, respectively, in the 7-day group; there was no significant differences (p>0.05) between these four bond strengths.

In the immediate group, the bond strengths on the #4000 polished dentin surfaces coated with SS, CS, and NS (the SS group, CS group, and NS group, respectively) were 16.5 (6.2), 8.4 (3.2), and 45.5 (5.8) MPa, respectively. Compared with the bond strength of 36.2 (7.1) in the Cont(#4000) group, the bond strengths of the SS and CS groups were significantly lower (p<0.05) and those in the NS group were nearly the same (p>0.05). Furthermore, in dentin coated with MS (MS group), the resin detached during sample preparation for the bond-strength test, and thus measurement was not possible.

In the 7-day group, the bond strengths of the SS, CS, NS, and MS groups were 26.1 (6.4), 35.4 (5.7), 46.6 (7.5), and 38.8 (6.0) MPa, respectively. Compared with the bond strength of 42.7 (8.3) MPa in the Cont(#4000) group, the bond strength in the SS group was significantly lower (p<0.05), whereas those in the CS, NS, and MS groups were not significantly different (p>0.05).

In contrast, among the SS, CS, and NS groups, a significant difference in bond strength between the immediate and 7-day groups was found in only the CS group, not counting the MS group for which measurement was not possible in the immediate group (p<0.05). No differences due to storage were found in the other groups (p>0.05).

**Failure modes after bond strength test**

Fig. 7 shows the distribution of failure modes after the microtensile
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A bond strength test was performed in each experimental group. In the immediate group, cohesive failure inside the bonding resin was found in 60% to 100% of the specimens in the Cont(#4000), SS, CS, and NS groups except for the #600 group that exhibited cohesive failure inside the resin or dentin and adhesive failure between the bonding resin and dentin in nearly half of the specimens. In the Cont(#4000) and SS groups, cohesive failure inside the bonding resin was found in nearly all of the specimens, and 60% to 90% of the specimens experienced cohesive failure inside the dentin. The CS group showed failure at the interface between the bonding resin and dentin in 40% of the specimens in addition to cohesive failure inside the bonding resin in 60% of the specimens. The NS group showed almost the same failure as that in the Cont(#4000).

In the 7-day group, the #600 group exhibited cohesive failure inside the bonding resin in 80% of the specimens, cohesive resin failure in 30%, cohesive dentin failure in 30% and adhesive failure between the bonding resin and the dentin in 20% of the specimens. In the Cont(#4000) group, cohesive failure inside the bonding resin was found in 80% of the specimen, and 70% had cohesive dentin failure, whereas 30% had cohesive resin failure, exhibiting similar failure modes as those in the immediate group. The SS group exhibited almost similar failure mode as that in the Cont(#4000) group; cohesive failure inside the bonding resin was found in all of the specimens, and 60% and 30% of these also had cohesive failure inside the dentin and cohesive failure inside the resin, respectively. The CS group exhibited similar failure modes, with cohesive failure observed inside the bonding resin in 90% of the specimens, and cohesive failure inside the resin or the dentin was found in 20% of these specimens. The NS and MS groups showed similar failure modes, with cohesive failures inside the bonding resin in 80% to

| Group     | #600     | Cont(#4000) | SS       | CS       | NS       | MS       |
|-----------|----------|-------------|----------|----------|----------|----------|
| Immediate | 35.3 (4.1) | 36.2 (7.1)  | 16.5 (6.2) | 8.4 (3.2) | 45.5 (5.8) | 46.6 (7.5) |
| 7-day     | 41.1 (9.1) | 42.7 (8.3)  | 26.1 (6.4) | 35.4 (5.7) | 46.6 (7.5) | 38.8 (6.0) |

Cont, control; SS, Superseal; CS, Caredyne Shield; NS, Nanoseal; MS, MS Coat Hys Block Gel

Bond strengths are shown as the mean (SD), n=10.

§: Test could not be performed because of interfacial dissociation that occurred during sample preparation.

Note: Significant differences between values marked with the same letter (Tukey's test, p<0.05). Letters marked with single underlines (−) in the immediate group, double underlines (=) in the 7-day group, and a triple underline (≡) between the immediate and 7-day groups indicate significant differences.
90% of the specimens, cohesive failure inside the resin in 30% to 40% of the specimens and cohesive failure inside the dentin in 40% to 50% of the specimens.

**Discussion**

**Development of hypersensitive dentin model**

Yoshiyama et al. investigated the morphological details in biopsy data of hypersensitive dentin, and reported three features: 75% of the dentinal tubules were open at the sites of hypersensitivity; microscopic abrasion marks were found at these sites; and the surface at other sites was relatively smooth and glossy. In this research, we newly fabricated a hypersensitive dentin model in which the dentinal tubules were opened without using etching, there was almost no smear layer remaining on the intertubular dentin, and polishing marks were present at the openings, whereas the surface of the intertubular dentin around some occluded tubules were relatively smooth, in order to investigate resin adhesion after coating with various types of desensitizing agents by using a dentin surface that faithfully reproduces the morphological properties of the hypersensitive dentin.

The #4000 polished surface was prepared using 3-μm aluminum oxide film and it was shown that the dentin surface, which was cleaned ultrasonically 4 times for 15 min, reproduced the above three features. This was used as the hypersensitive dentin model. The following is an overview of the process up to that point. Distinct polishing marks were...
Established experimental groups and statistical evaluation

This research evaluated the bond strength of resin to dentin immediately after applying each of the various desensitizing agents (immediate group) and after storage in water for 7 days (7-day group). In the clinical setting, if symptoms are immediately alleviated by application of a desensitizing agent to a tooth substance defect accompanied by hypersensitivity, the defect can possibly be restored right away depending on the wishes of the patient. In such cases, it is necessary to investigate the bond strength of the resin to dentin as in the Cont(#4000) group, and the interfacial failure was found in some specimens. Microparticles were found on all of the coated surfaces, and were thought to be fluorosilicate glass in the NS group. Although these microparticles are considered to suppress the etching effect of the bonding resin and reduce the bond strength, we directly applied two droplets of bonding resin and stirred the liquid, which appears to have achieved a sufficient etching effect, and thus the bond strength was not reduced. Furthermore, in the MS group, a “MS polymer emulsion” film that formed on the dentin surface is thought to have obstructed the adhesion of the resin.

In the 7-day group, cohesive failure inside the resin and mixed failure with both cohesive resin failure and cohesive bonding resin failure were characterizedly exhibited in all groups regardless of the obtained bond strengths. The SS group exhibited a significantly lower bond strength compared with the Cont(#4000) group, but there were almost no differences in failure modes between immediately after application and 7 days later except for cohesive resin failure in the 7-day group. Although the calcium oxalate crystals observed immediately after coating disappeared and the dentin tubules were open, it is not currently known why the bond strength did not recover. The bond strength was lower in the CS group than in the Cont(#4000) group immediately after application but was nearly the same 7 days later; the difference in the failure modes between the two was significant. The failure modes observed in some specimens after 7 days were both cohesive resin failure and cohesive failure as well as cohesive failure inside the bonding resin. Calcium fluoride and calcium phosphate, which are thought to degrade the bond strength immediately after application, almost completely disappeared after 7 days, with only a small amount remaining. Zinc ions were considered to have had almost no effect on the recovery of the resin’s adhesive properties. The NS and MS groups exhibited bond strength and failure modes (cohesive resin and dentin failures as well as cohesive inside the bonding resin) that were nearly the same as in the Cont(#4000) group except for a smaller number of specimens with cohesive failure inside the dentin. In the MS group in particular, the thin film material observed in the immediate group disappeared after storage in water for 7 days, and the bond strength is thought to have recovered because of this.

The results of this study indicate that caution is required when performing resin restoration immediately after application of a desensitizing agent, depending on the agent used, and thus great care must be exercised when selecting desensitizing agents in the clinical setting. Furthermore, when one type of desensitizing agent is ineffective, additional desensitizing agents may be used in the clinical setting. Therefore, caution is warranted because the effect on resin adhesion is complex and not well understood.

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Conflicts of Interest

The authors have declared that no COI exists.

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