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The Evaluation of Dentinal Tubule Occlusion by Desensitizing Agents: A Real-time Measurement of Dentinal Fluid Flow Rate and Scanning Electron Microscopy

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Clinical Relevance

Light-curing adhesive and oxalate-type desensitizing agents exhibited better reduction of dentinal fluid flow rate than did protein-precipitation and fluoride-type desensitizing agents based on measurements by a new fluid flow measuring device of subnanoliter scale.

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SUMMARY

The aims of this study were to examine changes in dentinal fluid flow (DFF) during the application of a desensitizing agent and to compare the permeability reduction levels among different types of desensitizing agents.

A cervical cavity was prepared for the exposure of cervical dentin on an extracted human premolar connected to a subnanoliter fluid flow measuring device under 20 cm of water pressure. The cavity was acid-etched with 32% phosphoric acid to make dentin highly permeable. The different types of desensitizing agents that were applied on the cavity were Seal&Protect as the light-curing adhesive type, SuperSeal and BisBlock as oxalate types,

Gluma Desensitizer as the protein-precipitation type, and Bi-Fluoride 12 as the fluoride type. DFF was measured from the time before the application of the desensitizing agent throughout the application procedure to five minutes after the application. The characteristics of dentinal tubule occlusion of each desensitizing agent were examined by scanning electron microscopy.

The DFF rate after each desensitizing agent application was significantly reduced when compared to the initial DFF rate before application for all of the desensitizing agents ($p<0.05$). Seal&Protect showed a greater reduction in the DFF rate when compared to Gluma Desensitizer and Bi-Fluoride 12 ($p<0.05$). SuperSeal and BisBlock exhibited a greater reduction in DFF rate when compared to Bi-Fluoride 12 ($p<0.05$).

The dentin hypersensitivity treatment effects of the employed desensitizing agents in this study were confirmed through real-time measurements of DFF changes. The light-curing adhesive and oxalate types showed greater reduction in the DFF rate than did the protein-precipitation and fluoride types.

INTRODUCTION

Among several theories that explain dentin hypersensitivity (DH), the hydrodynamic theory has been the most widely accepted. This theory proposes that a stimulus applied to an affected tooth causes the movement of dentinal tubular fluid in either an outward or inward direction. This movement stimulates the mechano-receptors of the sensory nerves in the dentin or pulp.[1] Based on the hydrodynamic theory, hypersensitive dentin exhibits open dentinal tubules and high permeability so that, in theory, if dentinal tubules are partially or completely occluded, DH symptoms decrease or vanish as a result.[2] Patients who have DH usually exhibit dentin exposure caused by microleakage of a restoration, cervical abrasion, or cementum loss. Hypersensitive dentin has wider and much more permeable dentinal tubules when compared to nonhypersensitive dentin.[3]

Cervical resin composites or glass ionomer restorations are typically performed if a tooth has moderate to severe cervical tooth loss. However, in cases of slight cervical abrasion or root exposure with DH, the application of a desensitizing agent to relieve symptoms is usually preferred over restoration treatment. Although the mechanism of desensitizing agent for hypersensitive dentin has not been clearly revealed, most of the currently employed desensitizing agents in clinics are intended to seal the dentin surface or to occlude dentinal tubules by protein precipitation or calcium complex formation so that the movement of dentinal tubular fluid can be suppressed.[4] A number of studies have reported the effects of the application of desensitizing agent on dentinal tubule occlusion. However, there exist no consistent conclusions regarding which product or which mechanism is superior.[5]

Evaluations of the effects of desensitizing agent on dentinal tubule occlusion are generally performed by the observation of the occlusion of the dentinal tubules using a scanning electron microscope (SEM) or by the observation of changes in permeability by measuring the hydraulic conductance of a dentin disc.[5] However, to date no report has investigated changes in the permeability of dentinal tubules in real time throughout the desensitizing agent application process for permeable dentin.

Recently, a study[6] reported the measurement of the dentinal fluid flow (DFF) in real time during a restorative procedure on an extracted tooth using a newly fabricated subnanoliter-scaled fluid flow measuring device (NFMD), which was capable of discriminating a volume change of 0.2 nL. In the current study, the DFF was measured in real time during the desensitizing agent application process for a cervical cavity. The immediate effects of dentinal tubule occlusion were compared with the different types of desensitizing agents. The dentin surfaces and subsurfaces onto which the desensitizing agents were applied were also examined by SEM to confirm the different aspects of dentinal tubule occlusion. The null hypothesis was that there would be no difference in the DFF rates before and after desensitizing agent application and that there would be no differences in the permeability reduction among the agents, despite the fact that the agents employed different mechanisms to occlude the dentinal tubules.

MATERIALS AND METHODS

Structure of the NFMD

The NFMD used in this study consisted of three parts: a glass capillary and photosensor to detect the fluid movement; a servomotor, lead screw, and ball nut to track the fluid movement; and a rotary encoder and computer software to record the data. The minimum measurable volume of water movement was 0.196 nL. Details of the working mechanism of the NFMD were described in a previous study.[6]

Specimen Preparation

Upper and lower premolars that were extracted for orthodontic reasons were used in this study. The project was approved by the Institutional Review Board of the Seoul National University Dental Hospital (CRI 09005). Extracted teeth were stored in a 1% chloramine-T solution at 4°C and were used within three months following their extraction.

Each root was removed 5 mm below the cemento-enamel junction using a low-speed diamond saw (Isomet, Buehler, IL, USA). The pulp tissue in the pulp chamber was carefully removed without altering the pre-dentin surface using thin tissue forceps and endodontic files. A sandblasted Plexiglas square (10 mm per side and 2 mm thick) with a hole drilled at its center was used to mount each tooth. A metal tube with a diameter of 0.9 mm was inserted into the hole, and the Plexiglas was attached to the tooth using an adhesive (Adper Scotchbond MultiPurpose, 3M ESPE, St Paul, MN, USA) and a flowable composite (Denflow, Vericom, Anyang, Korea) to ensure that one end of the metal tube was located in the pulp chamber. The exposed root surface and outer surface of the bonded interface between the Plexiglas and the tooth on the top surface and between the Plexiglas and the metal tube on the bottom surface were covered with nail varnish.

The prepared specimen was stored in distilled water and was connected to a water reservoir containing distilled water. A hydrostatic pressure of 20 cm H_2O was applied to the specimen 24 hours before the experiment was conducted.[7]

Cervical Cavity Preparation and the Measurement of the DFF During Desensitizing Agent Application

The prepared specimen was connected to a glass capillary by silicone tubing filled with distilled water (Figure 1). A hydrostatic pressure of 20 cm H_2O was applied throughout all of the procedures with a water reservoir to simulate physiological pulp pressure. The temperature and relative humidity of the environment were 24°C ± 0.5°C and 30% ± 5%, respectively. Each specimen underwent a stabilizing time of 10 minutes after it was connected to the NFMD. After confirming that the fluctuation level of the DFF was within ±5 nL for another 10 minutes, the cavity preparation was conducted.

A V-shaped cervical cavity with a mesio-distal width of 5 mm, an occluso-cervical height of 3 mm, and a depth of 2 mm was prepared with a round-end tapered diamond bur of 106-125-μm grit size (Mani, Tochigi, Japan) using an air-driven, high-speed handpiece (MACH-QD, NSK, Tokyo, Japan) at 200,000–300,000 rpm under water coolant. Acid-etching for 15 seconds using 32% phosphoric acid was performed to remove the smear layer formed during cavity preparation and to make dentin highly permeable. The cavity was then rinsed with water and blot-dried with a wet cotton pellet. Different types of desensitizing agents were applied in the prepared cavity according to the manufacturer's instructions (Table 1).

DFF measurement was performed continuously from 60 to 100 seconds after blot-drying the cavity, throughout the desensitizing agent application, and five minutes after desensitizing agent application in real time.

The average DFF rate, as measured before the desensitizing agent application, was set as the baseline flow rate. This baseline flow rate referred to the permeability of each tooth specimen and served as an internal reference for comparison with the subsequent flow rate measurements after desensitizing agent application. To determine the DFF rate after desensitizing agent application, the average flow rate was calculated for five minutes after applying the desensitizing agent. Reductions in the flow rate were indicated as a percentage of the decreased flow rate after desensitizing agent application with respect to the baseline flow rate [% reduction in flow rate $= 100 \times (\text{flow rate}_{\text{baseline}} - \text{flow rate}_{\text{postapplication}})/\text{flow rate}_{\text{baseline}}$].

The number of specimens required for each desensitizing agent was six, which was determined by a power analysis (78% power, 0.05 type 1 error level). A paired *t*-test was conducted to analyze whether there were any differences in the flow rate before and after desensitizing agent application. One-way analysis of variance was conducted to analyze whether there were differences in the reduction of the flow rate among desensitizing agents. A multiple comparison test was conducted using the Duncan test. The level of significance was $\alpha = 0.05$.

SEM Analysis

Premolar teeth extracted for orthodontic reasons were prepared in the same manner as described above to simulate physiologic pulp pressure. One

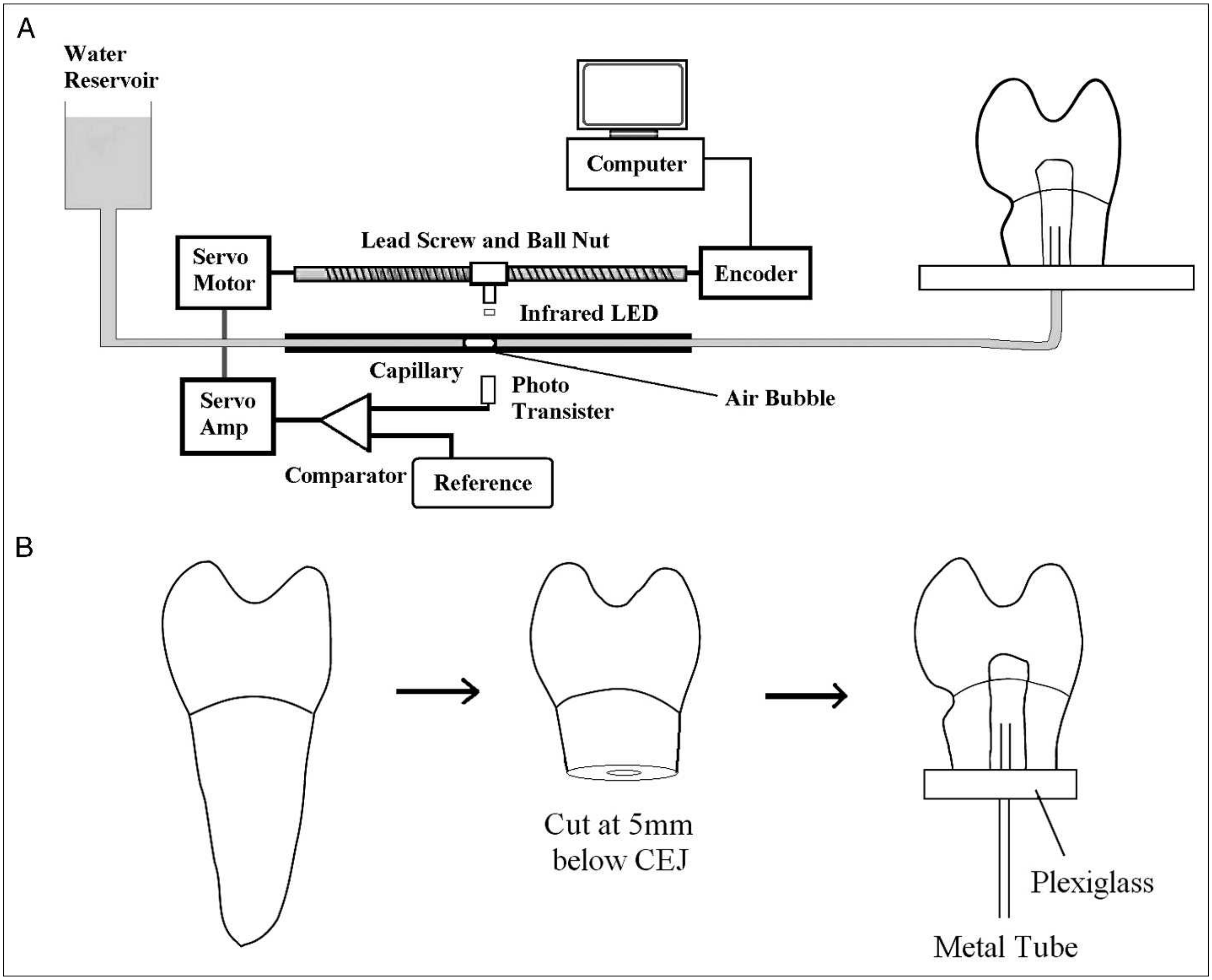

Figure 1. *(A) Schematic diagram of the subnanoliter-scaled dentinal tubular fluid flow measurement device (NFMD). (B) Specimen preparation.*

premolar tooth was representatively prepared for every individual desensitizing agent. After horizontal crown reduction was performed to expose the dentin surface, the specimen was connected to a water reservoir that contained distilled water at a hydrostatic pressure of 20 cm H_2O 24 hours before the experiment.

Acid-etching for 15 seconds using 32% phosphoric acid was performed to remove the smear layer that had formed during the preparation and to expose the highly permeable dentinal tubules. The dentin surface was then rinsed with water and blot-dried with a wet cotton pellet. Desensitizing agent was applied to half of the dentin surface, while the other half of the dentin surface was left exposed to observe the differences in both the areas *via* SEM. After the specimen was dried, a dentin disk was obtained by cutting 1–2 mm below the dentin surface using a high-speed handpiece. The dentin disk was fractured perpendicular to the border between the surface on which the desensitizing agent was applied and the surface on which the desensitizing agent was not applied. The specimens were then dried and sputter-coated with gold. Each specimen was subsequently examined using SEM (S-2300, Hitachi, Tokyo, Japan).

RESULTS

The specific behaviors of DFF during each desensitizing agent application are shown in Figure 2. The average DFF rate before desensitizing agent application indicates the baseline flow rate, which was

Table 1: *The Components and Application Procedures of the Desensitizing Agents Used in This Study*

Desensitizing Agent	Components	Procedure	Manufacturer
Seal&Protect (Lot No. 0909002823)	Di-and trimethacrylate resin, PENTA, Ffunctionalized amorphous silica, photoinitiators, butylated hydroxytoluene, cetylamine hydrofluoride, triclosan, acetone	Apply (dwell for 20 s), gentle air, light-cure[a] (10 s), reapply, gentle air, light-cure (10 s)	Dentsply, Milford, DE, USA
SuperSeal (Lot No. 991583)	Oxalate, potassium salt	Apply 30 s, gentle air-dry	Phoenix Dental, Fenton, MI, USA
BisBlock (Lot No. 0900000453)	Ferric oxalate	E&R[b], apply (dwell for 30 s), rinse	Bisco, Schaumburg, IL, USA
Gluma Desensitizer (Lot No. 010082)	Glutaraldehyde, HEMA, purified water	Apply (dwell for 60 s), air-dry, rinse	Heraeus, Hanau, Germany
Bi-Fluoride 12 (Lot No. 0941489)	Sodium and calcium fluoride	Apply (dwell for 20 s), air-dry	Voco, Cuxhaven, Germany

Abbreviations: E&R, acid-etching and rinse; HEMA, hydroxyethyl methacrylate; PENTA, dipentaerythritol penta acrylate mono monophosphate.
[a] LED light-curing unit (Elipar FreeLight 2, 3M ESPE, St Paul, MN, USA) of 600 mW/cm^2 intensity was used.
[b] E&R were omitted from this study because it had already been performed during specimen preparation.

used as an internal reference to compare the changes in the flow rate throughout the desensitizing agent application process. The postapplication flow rate decreased significantly compared to the baseline flow rate with all of the desensitizing agents used in this study ($p<0.05$). The application procedure for each desensitizing agent was reflected by the specific curve of the DFF. Regarding the SuperSeal and Bi-Fluoride 12, which require a simple "application-and-dry" procedure, even though there was a single fluctuation due to a decrease in the flow rate through the application itself and an abrupt increase in the flow rate through drying, a gradual decrease in the flow rate was observed on average. For Gluma Desensitizer and BisBlock, which have similar application procedures to SuperSeal and Bi-Fluoride 12 except for the water rinsing step, the water rinsing step led to a transient negative flow rate. For Seal&Protect, which involved two "application-and-light-curing" steps, two negative slopes by light-curing were reflected in the DFF characteristics during the application.

Figure 3 shows the mean reductions in flow rate as a percentage after individual application of desensitizing agents, when compared to the baseline flow rate. Seal&Protect showed a greater reduction in flow rate when compared to either Gluma Desensitizer or Bi-Fluoride 12 ($p<0.05$). SuperSeal and BisBlock showed a greater reduction in the flow rate when compared to Bi-Fluoride 12 ($p<0.05$).

Typical SEM images of the surface and the subsurface on which each desensitizing agent was applied are shown in Figure 4. For Seal&Protect, a thick resinous layer covering the treated area was observed when compared to the nontreated area. Resin plugs of approximately 5-10 μm that had formed in the dentinal tubules were also observed in the subsurface view. In the SuperSeal-treated area, many tiny crystals were filled in the dentinal tubules, and the depth of crystal penetration was at a maximum of approximately 20 μm. For BisBlock, larger and rounder crystals were present in the dentinal tubules when compared to SuperSeal, and the depth of crystal penetration was at a maximum of approximately 40 μm. On the Gluma Desensitizer–treated area, amorphous particles of the precipitation were observed on the surface, but not as frequently in the dentinal tubules. It was difficult to determine the precipitation characteristics in the dentinal tubules in a subsurface view. On the Bi-Fluoride 12–treated area, although many resinous plugs in the dentinal tubules were observed, they appeared porous and did not appear to fill the dentinal tubules completely.

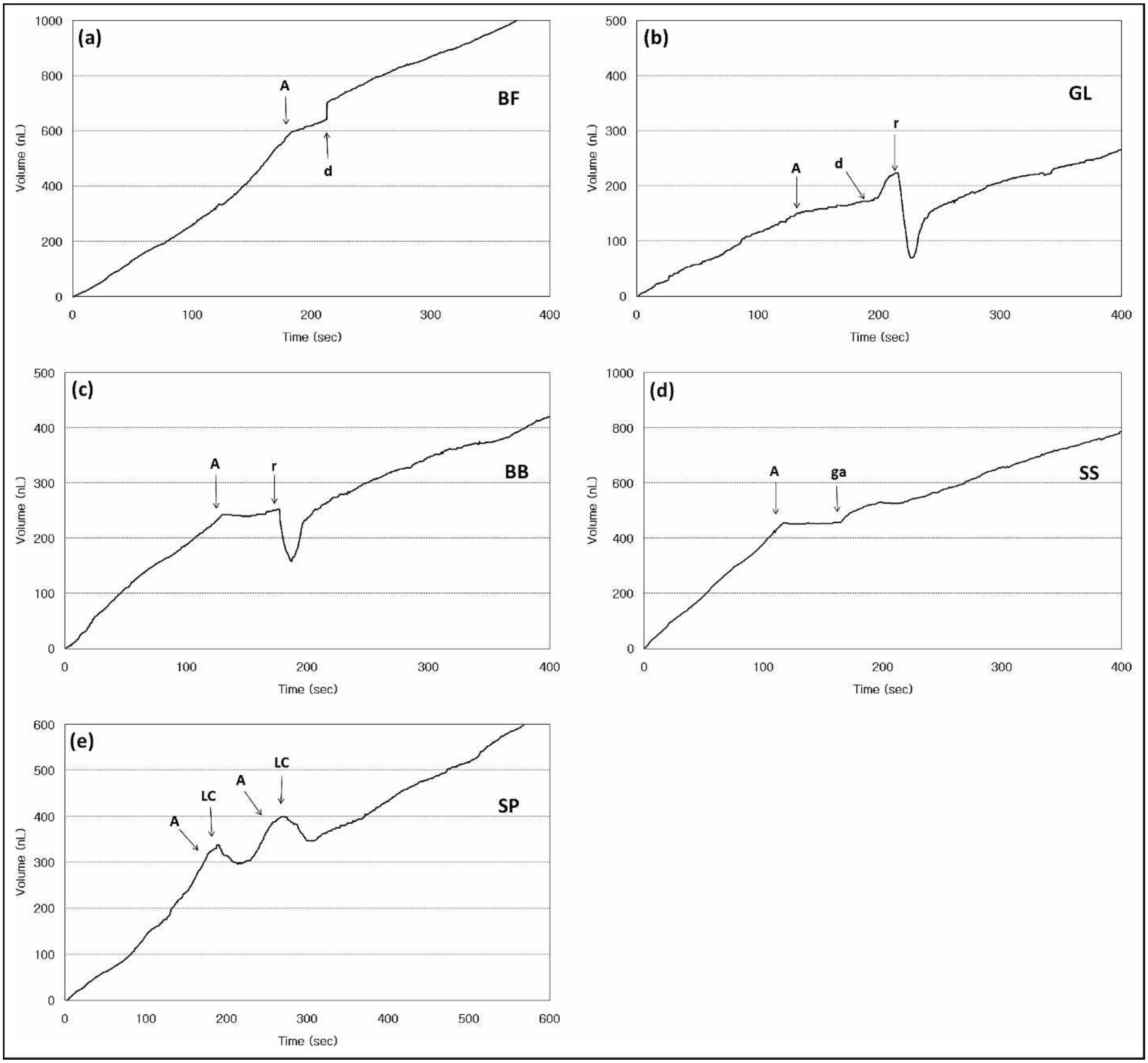

Figure 2. *A representative graph of consecutive dentinal fluid flow (DFF) during the application of each desensitizing agent. (a-e) The representative DFF graphs of Bi-Fluoride 12, Gluma Desensitizer, BisBlock, SuperSeal, and Seal&Protect, respectively. Upward (positive slope) movement vs the time on the graph indicates outward DFF, whereas downward (negative slope) movement indicates inward DFF. A, Application of a desensitizing agent; d, air dry; r, rinse; ga, gentle air-dry; LC, light-curing.*

DISCUSSION

There are two methods of treating DH: the first is to reduce the sensory nerve activity by increasing the K^+ ion concentration, and the second method involves reducing the dentinal tubular fluid flow by occluding the dentinal tubule.[2] Ever since the wide acceptance of Brannstrom's hydrodynamic theory, desensitizing agents that effectively occlude the dentinal tubule have been typically used in clinics. Numerous laboratory studies[5,8] have investigated the effects of desensitizing agent on dentinal tubule occlusion by comparing the permeability difference before and after the application of desensitizing agent on a dentin disk connected to a capillary in a split chamber. Although this method has been used because of the convenience of specimen preparation

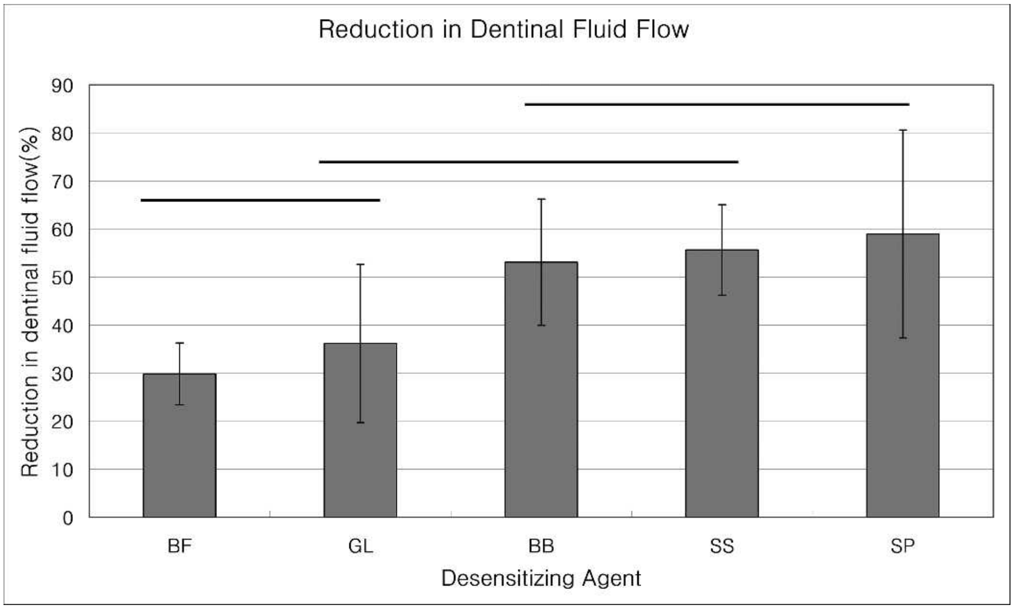

Figure 3. *Reduction in dentinal fluid flow by desensitizing agents (%). Desensitizing agents under the same bar did not show statistically significant differences (n=6).*

and desensitizing agent application, most of the studies loaded a much higher pressure than the physiologic pulp pressure to accelerate the movement of the air bubble in the capillary of the flow measurement device. The designs of previous studies were very different from clinical scenarios in terms of the location and shape of the exposed dentin. However, when compared to the preexisting and other reported studies, the design employed in the present study is very much similar to the clinical situation, because rather than employing a dentin disk, an original tooth form was used, a physiologic pulp pressure of 20 cm H_2O was applied, and the cervical dentin, which is the area that most frequently undergoes DH, was exposed. Moreover, this study is the first of its type to measure DFF change during desensitizing agent application in real time.

The change in DFF caused by each desensitizing agent during the application was reflected as a specific curve on the graph of the NFMD (Figure 2). For example, for SuperSeal and Bi-Fluoride 12, the simple treatment procedure of "application-and-dry" can be seen as a "step-like" figure on the graph. The desensitizing agent application itself caused a decrease in the DFF rate, and air-drying caused an abrupt increase in the DFF rate, even when it was performed gently. After the application period, the DFF graph eventually showed a decreased flow rate when compared to the baseline flow rate. Gluma Desensitizer and BisBlock reflect the water rinsing step as a transient negative flow rate on the DFF graph. In the case of Seal&Protect, which involved two light-curing steps, the DFF rate changed negatively when light-curing was performed. This indicates that dentinal tubular fluid was forced into the pulp as a result of the thermal expansion caused by the heat from the light-curing unit.[6] There was an abrupt increase in the DFF rate due to rebounding effect at the end of the light-curing process. Shortly after this rebounding increase in the flow rate, Seal&Protect returned to a consistent flow rate that was lower than the baseline flow rate.

All of the desensitizing agents used in this study resulted in significant reductions in flow rate following application when compared to the baseline, although all of the agents did not stop the DFF completely ($p<0.05$). Generally, the flow rate under the same pressure depends mainly on the radius of the dentinal tubules. Therefore, a reduction in the flow rate reflects the effect of dentinal tubule occlusion. As such, the desensitizing agents used in this study were expected to demonstrate the effect of treating DH to a certain degree based on the results that all the desensitizing agents occluded dentinal tubules.

However, significant differences were found among the desensitizing agents used in this study in terms of permeability reduction. Seal&Protect, SuperSeal, and BisBlock showed a greater dentinal tubule occlusion effect when compared to Bi-Fluoride

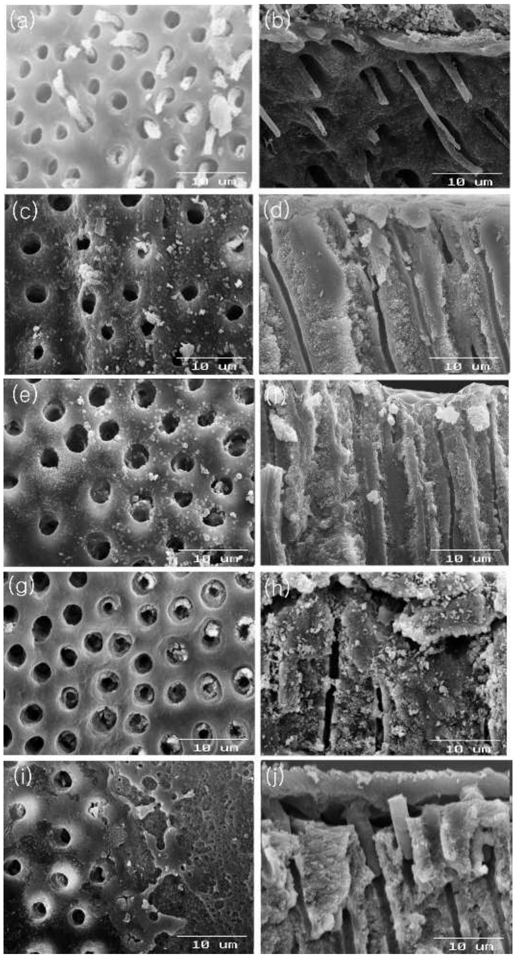

Figure 4. *Representative scanning electron microscopy (SEM) images of occlusal surface (left panels) and fractured subsurface (right panels) following application of different desensitizing agents, as follows: (a, b) Bi-Fluoride 12, (c, d) Gluma Desensitizer, (e, f) BisBlock, (g, h) SuperSeal, and (i, j) Seal&Protect. The occlusal surface images of desensitizing agents (a), (c), (e), (g), and (i) contain both the desensitizing agent-applied surface in the right side and an unapplied surface in the left side. The left side of each occlusal surface image showing wide-open dentinal tubules is an area in which desensitizing agent was not applied.*

12 and Gluma Desensitizer. Seal&Protect consists of methacrylate resins, photoinitiators, fillers, and dipentaerythritol penta acrylate monophosphate (PENTA), which is a partially acidic monomer. Therefore, the working mechanism of Seal&Protect is likely to occlude the entrance of the dentinal tubules by forming a hybrid layer in a manner similar to that of the all-in-one adhesive. The resin plug in the SEM images of Seal&Protect also indicates that dentinal tubule occlusion by Seal&Protect is similar to that of all-in-one adhesive. If an exposed dentin surface is given only one layer of Seal&Protect, it would be difficult to effectively occlude the dentinal tubules through which fluid flows out, considering that the thin all-in-one adhesive works as a permeable membrane, even after polymerization.[9] The second layer of Seal&Protect is considered to strengthen the sealing effect of the first layer. This mechanism may explain why the manufacturer suggests applying two layers of Seal&Protect. Seal&Protect showed good dentinal tubule occlusion, illustrating its feasibility for DH treatment in previous laboratory and clinical studies.[10,11]

SuperSeal may have two mechanisms in the treatment of DH. First, potassium ions inhibit pulp sensory nerve excitation, and second, oxalate binds with the calcium ions of the dentin surface or the dentinal tubules to form calcium oxalate crystals, which occlude the dentinal tubules. The manufacturer of SuperSeal claims that the formation of calcium oxalate crystal occurs within two minutes, which is proven to some extent by the reduction in the flow rate as measured during the application in this study. Potassium oxalate has long been used because it was known from previous studies[12,13] to have a great effect on dentinal tubule occlusion and is considered as a viable treatment for DH. Considering that the SuperSeal exhibited good dentinal tubule occlusion in this study, consistent with the results of previous studies, a high level of DH treating effect would be expected with SuperSeal, especially if it is used in conjunction with the inhibition mechanism of potassium ions on nerve excitation.

The clinical procedure for BisBlock, as suggested by the manufacturer, is to acid-etch, oxalate-apply, rinse, apply prime-and-adhesive, and light-cure. However, in this study, the adhesive application and light-curing process steps were not performed in order to examine the dentinal tubule occlusion effect of ferric oxalate itself and to exclude the adhesive effect. BisBlock had a substantial effect on the reduction in the flow rate and in causing a decrease in the dentinal tubule diameter through oxalate action without dentin sealing by a cured adhesive. Larger and rounder crystals were observed in the SEM images when compared to SuperSeal. Further studies need to be carried out to examine how the dentin tubule was occluded through double action by both the oxalate and adhesive and to determine whether it would last for a longer period of time when the adhesive application and light-curing were additionally performed according to the manufacturer's suggestion.

The Bi-Fluoride 12 of the fluoride agent exhibited the lowest reduction in flow rate in this study.

Fluoride is known to occlude the dentinal tubule by forming CaF_2 crystals.[14] A number of studies[2,12,15] reported that the dentinal tubule occlusion effect of fluoride is lower than that of oxalate agents and that the crystal structure cannot be observed in SEM images of fluoride-applied dentin specimens. Other clinical studies,[16,17] on the other hand, reported good DH treatment effects of using fluoride. Fluoride appears to be a subject of some debate with regard to the mechanism and effect of DH treatment. In this study, the Bi-Fluoride 12 specimen did not show a crystal structure but instead showed a resinous plug in the dentinal tubules.

Determination of the ingredients of a resinous plug could not be done solely on the basis of the manufacturer's disclosure of the chemical compositions. The crystal structure could not be detected, possibly because fluoride does not easily form crystals with calcium ions and/or because CaF_2 can be easily dissolved as a result of its chemical instability in a moisture-rich environment.

Gluma Desensitizer has the longest history of use as a desensitizing agent in clinical settings. The desensitizing mechanism of Gluma Desensitizer is based on the reaction of glutaraldehyde with a protein in the dentinal tubules, which in turn causes precipitation, which decreases the diameter of the dentinal tubule. Subsequently, this precipitation promotes hydroxyethyl methacrylate (HEMA) polymerization, which also causes dentinal tubule occlusion.[18,19] In contrast to previous studies[20-22] that have reported good DH treatment effects of Gluma Desensitizer, the present study showed a lower reduction in the permeability when compared to the other employed desensitizing agents. In the SEM images, decrease in the dentinal tubule diameter was not clearly observed. Moreover, only small dispersed particles that appeared to be polymerized HEMA particles were observed on the dentin surface. The lack of good dentinal tubule occlusion effects of Gluma Desensitizer, as observed in this study, was likely due to the use of distilled water as a dentinal tubular fluid (distilled water does not contain proteins). Such an application of distilled water would greatly limit the role of glutaraldehyde. In fact, previous studies[10,23] in which serum albumin was used as dentinal tubular fluid showed a highly effective reduction in permeability with Gluma Desensitizer.

A physiologic solution containing protein could not be used in this study because protein can cause sedimentation in the capillary tube. This change in the tubular diameter can reduce the consistency of the NFMD. Future studies are demanded to establish an elaborate method that will enable the NFMD to consistently measure fluid flow using physiologic fluid.

Occlusal dentin was employed to observe the occluding characterization of each desensitizing agent in SEM analysis because occlusal dentin is easier to use to standardize the specimen preparation than is cervical dentin. However, occlusal dentin and cervical dentin have different aspects with respect to direction and amount of occlusal force and may have different direction of dentinal tubules. Further study may be needed to investigate if the occluding effect of each desensitizing agent would have different aspects for the cervical dentin and occlusal dentin.

We investigated immediate dentinal tubule occlusion effects by measuring the DFF from the preapplication of desensitizing agent to postapplication in real time. In fact, it may be difficult for desensitizing agent to be retained on an exposed dentin surface because of interactions with saliva and cyclic brushing. Desensitizing agent containing a resin component may also not be retained on the dentin surface as a result of differences in thermal expansion from the dentin according to thermal changes in the oral cavity. It would be interesting to investigate how long desensitizing agent can maintain the dentinal tubule occlusion effect after application through measurements of DFF under different simulated oral cavity conditions.

CONCLUSION

Within the results of this study, based on measurements by a new fluid flow measuring device of subnanoliter scale, the following conclusions could be drawn. All of the desensitizing agents employed in this study led to a significant reduction in DFF rate following application of each desensitizing agent. Light-curing adhesive and oxalate-type desensitizing agents exhibited better reduction in dentinal fluid flow rate than did protein-precipitation and fluoride-type desensitizing agents.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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