

Effect of Various Materials on Dentin Permeability
for the Treatment of Dentin Hypersensitivity

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Running title : Permeability for the Treatment of Dentin Hypersensitivity

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Abstract

Purpose: In recent years, the number of patients with transient dentin hypersensitivity to cold water and abrasion pain without dental caries has been increasing. In the treatment of dentin hypersensitivity, the first choice is frequently the topical application of medicament due to its simplicity and immediate effect. A wide range of products with different action mechanisms are available for clinical use. The present study focused on dentin desensitizers and their dentinal tubular solubility. The dentin permeability inhibition ratio was measured using a model of hypersensitive dentin. In addition, the influence of post-application preservation conditions on serial changes in the permeability inhibition ratio was evaluated.

Methods: Dentin discs were prepared from extracted human molars for use as hypersensitivity model specimens. The specimens were applied to a device based on Pashley et al., with modifications, and the pulpal pressure was determined to be 25 mmHg. After applying four different dentin desensitizer products, Gluma Desensitizer (GL), Super Seal (SS), MS Coat One (MO), Nanoseal (NS), Teethmate Desensitizer (TD) and Shield Force Plus (SP), the specimens were stored in distilled water (DW group) or artificial saliva (AS group) for 24 h and for one week, and the dentin permeability was measured.

Results: In the DW group of all dentin desensitizers, the dentin permeability inhibition ratio one week after application decreased or showed a serial decrease in comparison with that

immediately after application. In the AS group of SS, MO, NS and TD, the ratio one week after application increased or showed a serial increase in comparison with that immediately after application. In the AS group of GL and SP, the ratio one week after application showed a serial decrease in comparison with that immediately after application.

Conclusion: When the specimens were stored in distilled water, all dentin desensitizers showed a decrease in the sealability of the dentinal tubules. When the specimens were stored in artificial saliva imitating the human intraoral cavity, dentin desensitizers Super Seal, MS Coat one, Nanoseal and Teethmate Desensitizer showed a serial increase in the sealability of the dentinal tubules, suggesting a bioactive action..

Key words : Dentin hypersensitivity; Sealability; Model of hypersensitive dentin

Introduction

In recent years, the development of minimally invasive dental treatment, and sustained efforts in dental-oral health promotion, have resulted in an increase in the number of remaining permanent teeth per person, leading to a higher number of patients with transient dentin hypersensitivity to cold water and abrasion pain without dental caries.

Dentin hypersensitivity is caused by physical stimulation such as changes in temperature and chemical stimulation such as changes in pH.¹⁾ Dentin hypersensitivity is classified according to the region of exposed dentin into cervical or root surface hypersensitivity and postoperative hypersensitivity due to dentin surface exposure after cavity preparation. It is recently considered that dentin hypersensitivity is aggravated by abfraction and enamel microcracks due to bruxism and clenching caused by stress. Although its subjective symptoms vary among patients from slight to unbearable pain, there are many patients who strongly desire relief from the discomfort of transient pain.

Treatment of dentin hypersensitivity consists of preservation therapy such as the topical application of medicament, iontophoresis, laser treatment, coverage using adhesive materials and removal of the dentin pulp in severe cases.²⁾ Although the optimal treatment is selected in each case, there is a possibility of recurrence. When the treatment effects of a selected method are negligible, other treatment methods with differing action mechanisms are selected. To reduce the patient's

discomfort and promote effective treatment of dentin hypersensitivity, it is important to fully understand each treatment method and the properties of each medicament, and select the optimal treatment method according to the pathologic conditions.

Among the dentin hypersensitivity treatment methods, the topical application of medicament is frequently the first choice due to its simplicity and immediate effect. The action mechanisms are varied, and many different products are available for clinical use, such as those for which the main components are potassium nitrate and aluminum lactate expecting desensitization,^{3,4)} those that react with tooth calcium to create crystals of mineral salts aiming to seal the dentinal tubules,⁵⁻⁸⁾ those for which the main component is glutaraldehyde to seal the dentinal tubules with the coagulation of tissue fluid⁹⁾ and those that seal the dentinal tubules using resin and glass ionomer cements.¹⁰⁾ However, the dislodging of medicaments due to tooth brushing and the serial decrease in effect due to the elution of the medicament in saliva have been clinically reported.¹¹⁾

The present study focused on dentin desensitizers and their dentinal tubular sealability. We established a pressure value equivalent to the internal pressure of the human dental pulp, using a diluted serum solution. Using a model of hypersensitive dentin, in which the amount of protein in the dentinal tubular fluid is near the clinical condition, we measured the dentin permeability inhibition ratio (permeability inhibition ratio), and evaluated the influence of post-application preservation conditions on serial changes in the permeability inhibition ratio.

Materials and methods

1. Production of the specimens

The subject teeth were healthy human molars without dental caries (human teeth) that were extracted at the Department of Oral Surgery in the hospital attached to our university, and stored in physiological saline at -40°C . They were defrosted immediately before being used in the experiments. This study was approved by the Medical Ethics Committee of Osaka Dental University (approval number:Daishi-irin 110767). After exposing human dentin from the occlusal surface side using a model trimmer, and cutting the tooth root around the cervical area, the dental pulp was removed. Next, polishing was performed up to #600 using water-resistant polishing papers to make the dentin surface flat, and exposed cylindrical dentin discs of 8 mm in diameter and 1 mm in thickness were produced. On the occlusal surface side of the dentin disc, a phosphate solution (Kishida Chemical) adjusted to 10 % using distilled water was applied for 30 s, and the specimen was washed for 5 s under running water. On the pulp side, sodium hypochlorite (Kishida Chemical) adjusted to 10 % using distilled water was applied for 10 s, and the specimen was washed for 5 s under running water to remove the smear layers on both sides. Ultrasonic cleaning in distilled water was performed for 5 min, and specimens with the dentinal tubules open were used as dentin disc specimens.

Next, a model of hypersensitive dentin was produced according to the method of Zennyu et

aL¹²⁾(Fig.1). A gum ring of 6 mm in internal diameter was placed on the pulp side of the dentin disc specimen, and a specimen stage was set, with insertion performed by using the upper and lower sides of a stainless steel holder. To expose the dentin on the enamel side, the central area of the specimen stage was fenestrated in a circular form of 7mm in diameter (area: approximately 0.39 cm²). Dentinal tubular fluid (DF) was injected into the specimen stage using a glass syringe with a Teflon tube. The specimen stage was filled with DF without containing air, and connected to a device configured to adjust the internal pressure according to Pashley et al.¹³⁾ The internal pressure of the specimen stage was adjusted to 25 mmHg, which is equivalent to the internal pressure of human dental pulp, to reproduce the clinical dental pulp's internal pressure.¹⁴⁻¹⁷⁾ The clinical amount of protein in DF was reproduced by using bovine serum diluted four times with distilled water.¹⁸⁻²⁰⁾

2. Measurement of the dentin permeability inhibition ratio

Table 1 lists the dentin desensitizers used in the experiment. Table 2 shows the application method for each dentin desensitizer. DF from the injection syringe was shut at three-way cock B, and a glass capillary tube connected with the experimental device was connected to the specimen stage. Under these conditions, air blowing on the exposed dentin surface in the central fenestrated area of the specimen stage was performed for 60 s, and after leaving it for 30 s, the amount of DF movement in the glass capillary tube was measured.²¹⁻²³⁾ The air pressure was set at 3.0 kgf/cm².

Then, DF flowing in the glass capillary tube was shut, adjusting three-way cock B so that DF only flowed from the injection syringe in the specimen stage direction. Furthermore, three-way cock A was adjusted so that DF flowed from the injection syringe in the pressure gauge and specimen stage direction, and the internal pressure was set at 25 mmHg. After applying each dentin desensitizer according to the manufacturer's instructions, air blowing on the specimen dentin surface was performed for 60 s, as was done before applying the dentin desensitizers, and after leaving it for 30 s, the amount of DF movement in the glass capillary tube was measured again. The amount of DF movement before and after application was used to measure the permeability inhibition ratio. Each specimen was immersed in distilled water and artificial saliva (Saliveht Aerosol, Teijin Pharma, Table 3), and stored at a constant temperature of 37°C. The specimens stored in distilled water were classified as the DW group, and those stored in artificial saliva were classified as the AS group. Thereafter, the amount of DF movement was measured at 24 h and at one week after application, using the same method, and the permeability inhibition ratio was measured.

3. Statistical test

The permeability inhibition ratio was tested using oneway layout analysis of variance and TUkey's analysis ($p < 0.05$). The number of specimens was five.

4. SEM observation of the dentin surface

Gold evaporation was performed according to the conventional method, and the specimens used in the experiment were observed using a scanning electron microscope (JSM-5610LV, JEOL, SEM).

Results

Fig.2 shows the measurements of the permeability inhibition ratio immediately, 24 h and one week after applying each dentin desensitizer. Fig.3 shows the results of SEM observation of each dentin disc.

In the DW group of GL, MO, TD and SE the permeability inhibition ratio 24 h and one week after application decreased significantly in comparison with that immediately after application. In the DW group of SS, the ratio one week after application decreased significantly in comparison with that immediately after application. In the DW group of NS, there was no significant difference between the rate immediately after application and that 24 h after application. Furthermore, the ratio one week after application decreased significantly in comparison with that 24 h after application. In the AS group of MO and TD, the ratio one week after application increased significantly in comparison with that immediately after application. In the AS group of SS and NS, the ratio 24 h and one week after application showed a serial increase in comparison with that immediately after application. In the AS group of GL and SP, the ratio 24 h and one week after application showed a serial decrease in comparison with that immediately after application.

SEM observation of the dentin surface showed that, in GL, the dentinal tubules were sealed with aggregates immediately after application. In both the DW and AS groups, the sealability of the dentinal tubules 24 h and one week after application was lower than that immediately after

application. In SS, MO, NS and TD, aggregates sealed the dentinal tubules immediately after application, covering the dentin surface. In the DW group, the sealability of the dentinal tubules 24 h and one week after application was lower than that immediately after application. In the AS group, although the aggregates covering the dentin surface 24 h and one week after application decreased in comparison with that immediately after application, a decrease in the sealability of the dentinal tubules was not noted. In SP, aggregates of polymers sealed the dentinal tubules immediately after application, covering the dentin surface. In both the DW and AS groups, although exposure of dentinal tubules 24 h and one week after application was not observed, the aggregates covering the dentin surface had become rough.

Discussion

Dentin hypersensitivity is caused by physical stimulation such as changes in temperature and chemical stimulation such as changes in pH for exposed dentin. It is recently considered that dentin hypersensitivity is aggravated by abfraction and enamel microcracks due to bruxism and clenching caused by stress.²⁴⁾ The mechanism of dentin hypersensitivity has not yet been clearly elucidated. There are several hypotheses regarding the sensory transmission mechanism of dentin hypersensitivity. The hydrodynamic theory, in which the movement of the fluid in the dentinal tubules with external stimulation stimulates the free nerve endings in the dental pulp and causes pain, is convincing^{25,26)} because opening of the dentinal tubules on the hypersensitivity-contracted dentin surface is noted.^{27,28)} In dentin hypersensitivity cases based on the hydrodynamic theory, pain can be reduced by preventing the movement of the tissue fluid in the dentinal tubules, using appropriate methods to seal the orifices of the dentinal tubules.

Treatment of dentin hypersensitivity consists of preservation therapy such as topical application of medicament, iontophoresis, laser treatment, coverage using adhesive materials and removal of the dentin pulp in severe cases, and the optimal treatment is selected in each case. Among the types of treatment for dentin hypersensitivity, the topical application of medicament is frequently the first choice due to its simplicity and immediate effect. However, the dislodging of medicaments due to tooth brushing, and the serial decrease in effect due to the elution of the medicament in saliva have

been clinically reported.¹¹⁾

In the present experiment, using various dentin desensitizers with differing action mechanisms, we evaluated the influence of preservation conditions on each inhibitory effect on dentin permeability. Regarding the action mechanism of each dentin desensitizer, GL is a solution of 2-hydroxyethyl methacrylate and glutaraldehyde, aiming at the coagulation of tissue fluid. It fixes and stabilizes collagen in the intratubular dentin, the orifices of the tubules are sealed by the aggregating action of protein in the fluid in the dentinal tubules, membranous films are formed on the dentin surface, sealing the dentinal tubules, and dentin permeability is inhibited.⁹⁾ SS, NS, and TD aim at sealing the dentinal tubules with crystals of mineral salts. In SS, oxalic acid reacts with tooth calcium, the dentinal tubules are sealed with crystals of calcium oxalate developing in the tubules, and dentin permeability is inhibited.⁵⁾ In NS, a liquid mixture of fluoroaluminosilicate glass dispersing liquid and phosphoric acid solution reacts with the tooth substance through an acid-base reaction, acid-resistant nanoparticle precipitates (calcium fluoride, calcium phosphate, and silica phosphate) are created, the precipitated nanoparticles are integrated with the intertubular dentin and internal wall of the dentinal tubules, sealing the dentinal tubules, and dentin permeability is inhibited.⁷⁾ In TD, hydroxyapatite is created after hardening of a mixture of tetracalcium phosphate, dicalcium phosphate anhydrous, and purified water, sealing the dentinal tubules, and dentin permeability is inhibited⁸⁾. Particularly in NS and TD, an action with hydroxyapatite and specific

reactions are expected in cases of enamel microcracks due to bruxism and clenching aggravating hypersensitivity. Furthermore, a clinical characteristic is that only washing with water or rinsing is necessary after their application, and air drying is unnecessary. MO is a solution of methyl methacrylate-styrenesulfonic acid copolymer emulsion and oxalic acid, aiming at the sealing of dentinal tubules by crystals of mineral salts and polymers. Copolymers of methyl methacrylate and styrenesulfonic acid react with hydroxyapatite in the intertubular dentin, macromolecular stratified films are formed on the dentin surface, inorganic polymer plugs containing calcium oxalate are deposited in the dentinal tubular orifices, sealing the dentinal tubules, and dentin permeability is inhibited.⁶⁾ It is clinically necessary to perform repeated application, and drying with air after application. SP aims at dentinal tubular sealing with resin. Polymerization of its main components, which are phosphate monomers, Bis-GMA, TEGDMA, and HEMA, forms resin tags in the dentinal tubules, macromolecular polymer films are formed on the dentin surface, and the dentinal tubules are sealed.²⁹⁾ It is considered that since the current composite resin tooth bonding system is applied, SP is excellent in terms of its sealability and long-term stability.

Regarding differences in the permeability inhibition ratio between preservation methods of the specimens, in the DW group, all dentin desensitizers (GL, SS, MO, NS, TD and SP) showed a decrease in the permeability inhibition ratio one week after application in comparison with that immediately after application. Furthermore, SEM observation in the DW group showed a decrease

in the sealability of the dentinal tubules with all dentin desensitizers. Therefore, in the DW group, it was considered that the elution of the sealing component of each dentin desensitizer resulted in the decrease in the permeability inhibition ratio. On the other hand, in the AS groups, GL and SP showed a decrease in the permeability inhibition ratio one week after application in comparison with that immediately after application. It was considered that this was because actions such as the recharging of ions in saliva were absent, and bioactive effects did not occur. However, SS, MO, NS and TD showed an increase in the permeability inhibition ratio one week after application in comparison with that immediately after application. In SEM observation one week after application in the AS group, GL and SP showed a decrease in the sealability of the dentinal tubules and rough aggregates covering the dentin surface, whereas SS, MO, NS and TD showed the favorable sealability of tubules. It was considered that this was because crystallization of aggregates formed on the dentin surface was facilitated by the ions in Salivhet Aerosol, sealing the dentinal tubules. Thanatvarakorn et al.³⁰⁾ reported that, when human dentin after applying dentin desensitizers was immersed in artificial saliva containing CaCl_2 , KH_2PO_4 , and NaN_3 , inorganic crystals occurred on the dentin surface, and the dentinal tubules were sealed. Salivhet Aerosol used in the present study is artificial saliva whose main components are NaCl , KCl , CaCl_2 , MgCl and KH_2PO_4 . Also, in the actual oral cavity, there is a possibility that a bioactive action occurs due to the crystallization of aggregates with ions released from CaCl_2 , KH_2PO_4 and NaN_3 contained in saliva, and a reaction

similar to that in the AS group can be expected.

Conclusions

The following conclusions were obtained from the present experimental results:

When the specimens were stored in distilled water, all dentin desensitizers showed a decrease in the sealability of the dentinal tubules.

When the specimens were stored in artificial saliva imitating the human intraoral cavity, dentin desensitizers Super Seal, MS Coat One, Nanoseal and Teethmate Desensitizer showed a serial increase in the sealability of the dentinal tubules, suggesting the possibility of a bioactive action.

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各種知覚過敏抑制材の象牙細管封鎖性について

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抄録

目的：近年，齧蝕を伴わない一過性の冷痛または擦過痛を主とした象牙質知覚過敏症を罹患する患者が増加してきている．象牙質知覚過敏症の治療法のうち，薬物塗布による治療法は，簡便性と即効性の点から象牙質知覚過敏症の治療において第一選択となることが多く，その作用機序も多岐にわたり，多数の製品が臨床応用されている．今回，薬物塗布に用いられる象牙質知覚過敏抑制材の象牙細管封鎖性について，知覚過敏症罹患モデル象牙質を用いて，象牙質透過抑制率の測定を行うことに加えて，知覚過敏抑制材塗布後の保管環境が透過抑制率の経時的な変化に与える影響について検討を行った．

材料と方法：象牙質ディスクは抜去したヒト臼歯から作製した．試料は Pashley により報告された装置を模して作製した装置に用いられ，試料を装置に接続して内圧が 25 mmHg になるように規定した．各象牙質知覚過敏抑制材を塗布後，試料を蒸留水中または人口唾液中に 24 時間，1 週間保管し，象牙質の透過性を測定した

結果：全ての象牙質知覚過敏抑制材の DW 群で，塗布直後の象牙質透過抑制率と比べて，1 週間後の象牙質透過抑制率は有意な低下，または低下傾向が認められた．SS, MO, NS, TD の AS 群では，塗布直後の象牙質透過抑制率と比べて，1 週間後の象牙質透過抑制率は有意な上昇，または経時的な上昇傾向が認められた．GL, SP の AS 群では，塗布直後の象牙質透過抑制率と比べて，1 週間後の象牙質透過抑制率は経時的な低下傾向が認められた．

結論：以上より，ヒト口腔内を模倣した人工唾液中に保管した場合，象牙質知覚過敏抑制材 Super Seal，MS Coat One，Nano seal，Teethmate Desensitizer について経時的な象牙細管封鎖性の向上が認められ，バイオアクティブな作用がおこる可能性が示唆された。

キーワード：象牙質知覚過敏症，封鎖性，知覚過敏症罹患モデル象牙質

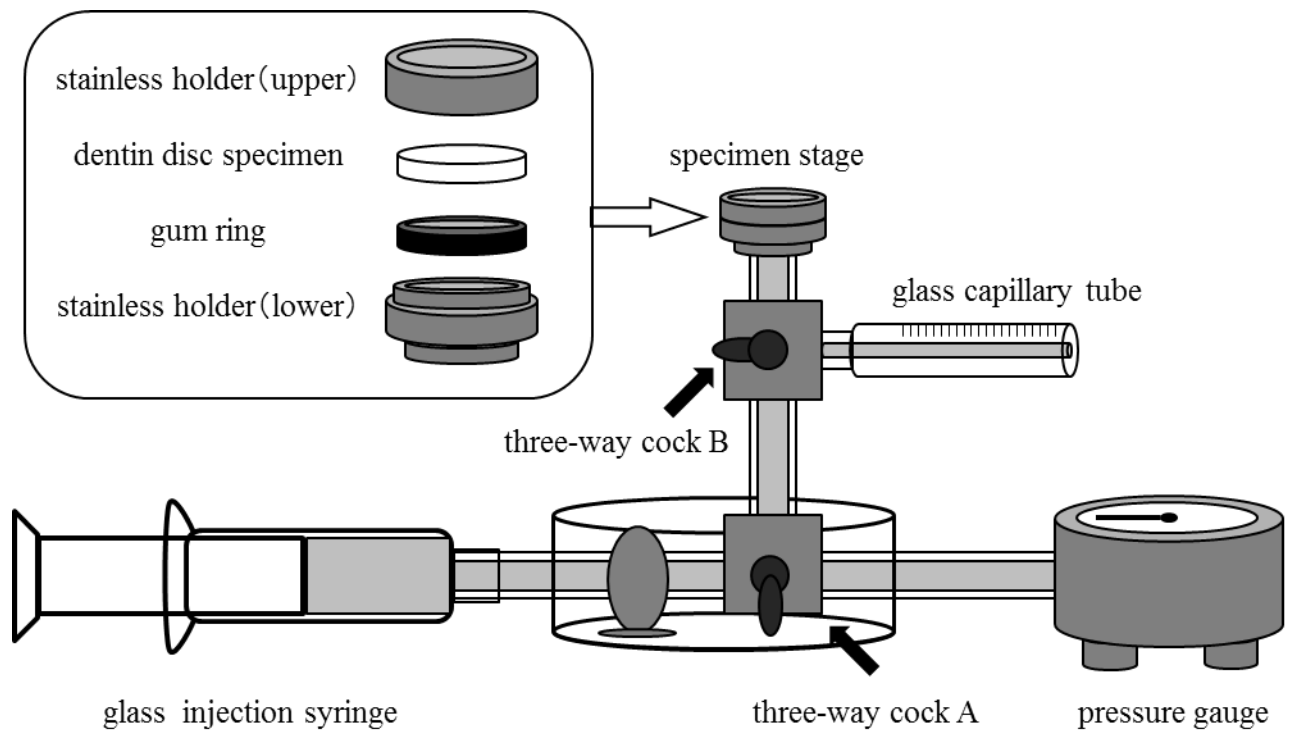


Fig. 1 Schematic diagram on model of hypersensitive dentin

Figure 1 Schematic diagram on model of hypersensitive dentin

A specimen stage was put a dentin disc specimen and a gum ring placed on the pulp side between the upper and lower sides of a stainless steel holder. A specimen stage was connected with a three-way cock B, a glass capillary tube, a three-way cock A, a glass injection syringe and a pressure gauge. The model of hypersensitive dentin was filled with dentinal tubular fluid, and the internal pressure of the model of hypersensitive dentin was adjusted to 25 mmHg.

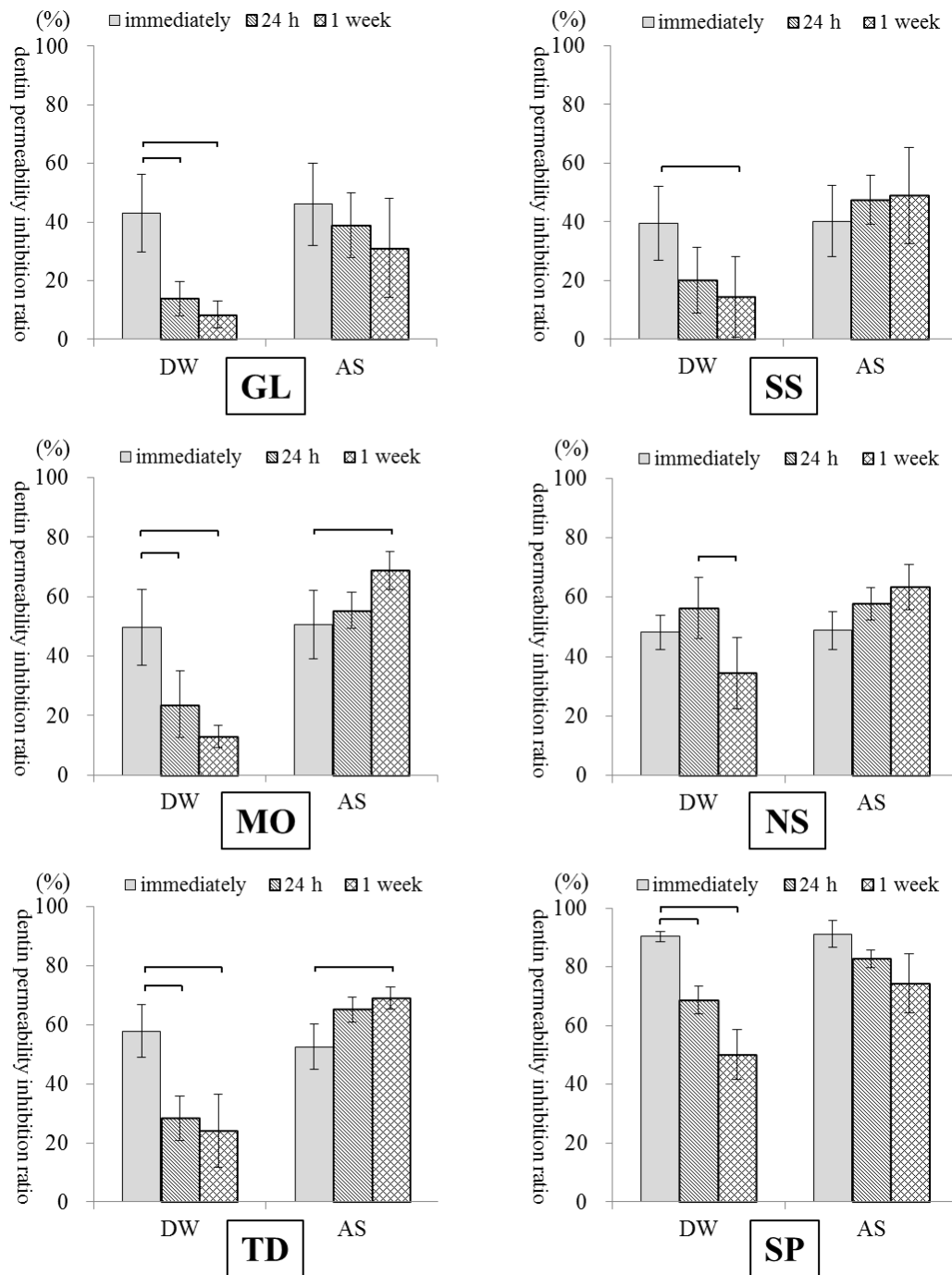


Fig. 2 Measurements of the permeability inhibition ratio of each dentin desensitizer

Figure 2 Measurements of the permeability inhibition ratio of each dentin desensitizer

The dentin specimens stored in distilled water were classified as the DW group, and those stored in artificial saliva were classified as the AS group. The permeability inhibition ratio immediately

was measured immediately after the application of dentin desensitizers. After having immersed dentin specimens with applied dentin desensitizer in DW and AS for 24 h and for one week, the permeability inhibition ratio at 24 h and at one week was measured. The ratio was tested using one-way layout analysis of variance and Tukey's analysis ($p < 0.05$). Significant differences in each group were represented by the bar.

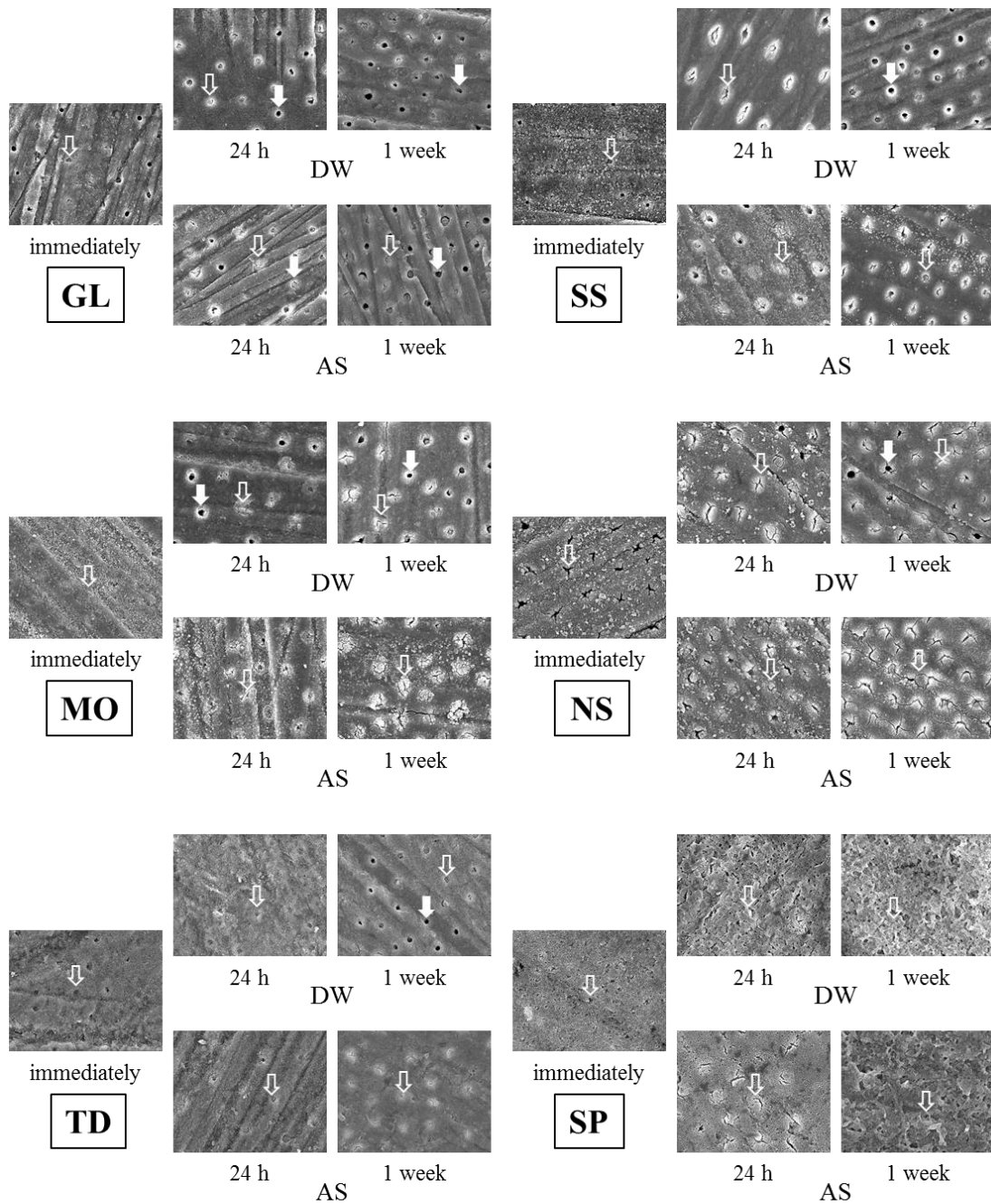


Fig. 3 SEM images of each dentin disc

Figure 3 SEM images of each dentin disc

The dentin specimens stored in distilled water were classified as the DW group, and those stored in artificial saliva were classified as the AS group. SEM micrographs of the dentin surface

were observed immediately after the application of dentin desensitizers. After having immersed dentin specimens with applied dentin desensitizers in DW and AS for 24 h and for one week, SEM micrographs of the dentin surface were observed. The precipitates were deposited on the dentin surface (blank arrows). Partially open dentin tubules were observed (white arrows).

Table 1 Materials used

Materials	Composition	Lot No.	Manufacture	Code
Glua Desensitizer	2-Hydyoxyethylmethacrylate, Glutaraldehyde, Purified water	010205	Heraeus Kulzer, Germany	GL
Super Seal	Oxalic acid, Others	991625	Phoenix Dental, USA	SS
MS Coat One	Methyl methacrylate / styrene sulfonic acid copolymer, Oxalic acid, Water	EG1	Sun Medical	MO
Nano seal	A Liquid: Fluoroaluminosilicate glass dispersion liquid, Paraben, Purified water B Liquid: Phosphoric acid, Purified water	A Liquid: A1Y B Liquid: A1Z	Nippon Shika Yakuhin	NS
Teethmate Desensitizer	Powder: Tetracalcium phosphate, Dicalcium phosphate anhydrous, Others Liquid: Purified water, Others	Powder: 000003 Liquid: 000003	Kuraray Noritake Dental	TD
Shield Force Plus	Phosphate monomer, Bis-GMA, TEGDMA, HEMA, Alcohol, Purified water, Camphorquinone, Others	0268	Tokuyama Dental	SP

Table 2 Application method for each dentin desensitizers

GL	Rub for 15 sec	Wait for 45 sec	Dry	Wash
SS	Rub for 5 sec	Dry		
MO	Rub for 30 sec	Dry	Wash	
NS	Mix	Apply for 20 sec	Wash	
TD	Mix for 15 sec	Rub for 30 sec	Wash	
SP	Apply for 10 sec	Dry	Light cure for 10 sec	

Table 3 Compositoin of Saliveht Aerosol

component	Sodium chloride, potassium chloride, calcium chloride hydrate, magnesium chloride, dipotassium phosphate
additive	Carmellose sodium, D-sorbitol, sodium benzoate, sorbic acid, sodium hydroxide, carbon dioxide