

Sealing Ability of Enamel Crack using Various Dentin Desensitizers

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Abstract

Purpose: With the increase in number of remaining teeth per capita, the incidence of noncariogenic diseases other than dental caries and periodontal disease is also increasing. Among the noncariogenic diseases, microcracks in enamel (enamel cracks) may be a cause of hypersensitivity with no substance defect. The incidence of enamel cracks in healthy teeth is reported to exceed 95% in subjects aged 40 years or older. Considering sealing of enamel cracks with dentin desensitizers (desensitizers) based on their fast-acting property and convenience, we measured the permeation inhibition rate using a dentin hypersensitivity model. At the same time, the influence of the storage condition on the permeation inhibition rate was also investigated by storing specimens in either distilled water or remineralization solution.

Methods: Enamel crack specimens were prepared using healthy bovine teeth with no dental caries. A device was prepared following the method reported by Pashley et al., each specimen was connected to the device and the inner pressure was set at 25 mmHg. The desensitizers used in the experiment were SUPER SEAL (SS), MS Coat F (MS), Nano Seal (NS), Teethmate Desensitizer (TD), and G-Premio BOND (GP). After application of each desensitizer, the enamel crack permeation inhibition rate was measured. After measurement, the specimens were stored in distilled water (DW group) or remineralization solution (RS group), and the enamel crack permeation inhibition rate was measured after one week and one month and three months.

Results: The permeation inhibition rates at one month and three months were significantly higher than that immediately after application in the SS-, TD-, and NS-applied DW groups. In the SS- and NS-applied RS groups, the permeation inhibition rates at one month and three months were significantly higher than that immediately after application. In the TD-applied RS group, the permeation inhibition rate at three months was significantly higher than that immediately after application. In the MS-applied DW and RS groups, the permeation inhibition rates at one week and one month and three months were significantly higher than that immediately after application. In the GP-applied DW and RS groups, a high sealing ability was noted from immediately after application, but the differences were not significant.

Conclusion: It is suggested that although the timing of the demonstrated effect differs among the desensitizers, the effect is exhibited by repeated application.

Key words: enamel crack, sealing ability, dentin hypersensitivity model

Introduction

Population aging has advanced in Japan, but the number of remaining teeth per capita has increased due to promotion activities, such as the 8020 Campaign, and developments in dental treatment¹⁾, with which the incidence of noncariogenic diseases other than dental caries and periodontal disease has increased. Noncariogenic diseases include tooth wear, tooth fracture and cracking, hypoplasia, abnormal tooth morphology, and abnormal number of teeth²⁾. Microcracks of enamel (enamel cracks) may be one of the causes of noncariogenic diseases showing hypersensitivity despite the absence of any substance defect. It is considered difficult to macroscopically observe early-stage enamel cracks, but various factors, such as long-term occlusal force, changes in intraoral environment by food ingestion, and reduction of saliva secretion, may influence expansion of enamel cracks³⁾. The incidence of enamel cracks in healthy teeth increases with aging: 60% in the teen years, 68% in the 20s, and 90% in the 30s⁴⁾, and an incidence exceeding 95% in the 40s and older has been reported⁵⁾. The presence of enamel cracks may lead to esthetic disturbance of the tooth due to dye penetration and secondary caries, dentin hypersensitivity-like pain, and interference with whitening^{6,7)}. Enamel cracks may reach the dentin and stimulation may be transmitted to the dental pulp through the dentinal tubule. As the concept of minimal intervention (MI) dentistry has recently been spreading, noninvasive treatment is needed. In this study, we considered the application of dentin desensitizers (desensitizers) to seal enamel cracks based on their fast-acting property and convenience. To measure the permeation inhibition rate, we prepared a device capable of providing a pressure of 25 mmHg, the same as the human dental pulp pressure, and a dentin hypersensitivity model following the report of Pashley et al.⁸⁾ We also investigated the influence of the storage condition on the permeation inhibition rate by storing the teeth in either distilled water or remineralization solution after desensitizer application.

Materials and Methods

1) Preparation of specimens and device

For the specimens, healthy bovine teeth (bovine teeth) with no dental caries were stored in physiological saline at -40°C and thawed immediately before the experiment. The flat enamel surface of the bovine tooth was exposed from the labial-side maximum contour using a model trimmer, and the tooth root was cut near the tooth cervix. After removal of the dental pulp, the tooth was polished using waterproof abrasive paper up to #600, and a columnar enamel/dentin disc with an 8-mm diameter and 1-mm thickness was prepared. A 200-g weight was dropped on the tooth two times from a height of 6cm. After confirming enamel cracks using a laser microscope (Keyence VK series, x100), the disc was used as an enamel crack specimen. The dentin side of the enamel crack specimen was treated with

10% phosphoric acid solution prepared with distilled water (Kishida Chemical) for 30s, followed by washing with running water for 5s. After removal of the smeared layer, the specimen was washed with distilled water using an ultrasonic cleaner for 5 min to prepare a specimen with an open dentinal tubule. Then, a dentin hypersensitivity model was prepared based on the method reported by Zennyu et al.⁹⁾ (Fig. 1). A rubber ring with a 6-mm inner diameter was placed on the dentin side of the enamel crack specimen, inserted between the upper and lower stainless holders, and used as a specimen stage. A 5-mm square fenestration (area: about 0.25 cm²) was made in the center of the specimen stage to expose the desensitizer-applied surface on the enamel side. To prevent air from entering the specimen stage, the stage was filled with dentinal tubular fluid (DF) and connected to a device capable of specifying the inner pressure prepared based on the report by Pashley et al.⁸⁾ The pressure in the specimen stage was adjusted to 25 mmHg, the same as the pressure in the human dental pulp, to reproduce the pressure in clinical practice¹⁰⁻¹³⁾. For DF, physiological saline was used. Permeability was measured at specimen selection, and each sample was regulated by unifying the amount of movement of DF.

2) Measurement of permeation inhibition rate

For the desensitizer, SUPER SEAL (SS) (Phoenix Dental), MS Coat F (MS) (Sun Medical), Nano Seal (NS) (Nippon Shika Yakuhin), Teethmate Desensitizer (TD) (Kuraray Noritake Dental), and G-Premio BOND (GP) (GC) were used. The composition of each product is shown in Table 1, and the application method of each desensitizer is shown in Table 2. First, DF from the direction of the syringe was blocked by three-way cock B, and the specimen stage and a glass capillary tube connected to the experimental device were connected. In this state, air spray was applied for 60s to the enamel surface exposed from the central fenestration of the specimen stage. After leaving it standing for 30s, DF movement in the glass capillary tube was measured¹⁴⁻¹⁶⁾. The air pressure was set at 0.3 MPa. Then, three-way cock B was adjusted to block DF flowing into the glass capillary tube and to let it flow only from the syringe direction to the specimen stage. In addition, three-way cock A was adjusted to let DF flow from the syringe toward the pressure gauge and specimen stage, and the inner pressure was adjusted to 25 mmHg. Each desensitizer was applied according to the manufacturer's instruction, followed by air spray for 60s and standing for 30s as described above, and DF movement was measured again. The permeation inhibition rate was calculated based on DF movements before and after application. Each specimen was immersed in either distilled water or remineralization solution¹⁷⁾ (1.5 mmol/l CaCl₂, 0.9 mmol/l KH₂PO₄, 130 mmol/l KCl, 20 mmol/l HEPES, 0.05% NaN₃, pH 7) and stored at 37°C. Specimens stored in distilled water were designated as the DW group, and those in remineralization solution were designated as the RS group. DF movement was similarly measured after one week, one month and three months, and the permeation inhibition rate under each condition was calculated. The desensitizer was

applied once a week, and the distilled water and remineralization solution were changed at the same time. Enamel crack specimens without desensitizer application immersed in distilled water or remineralization solution were prepared as controls. The permeation inhibition rate was calculated by numerical formula using DF movement (x) before the first desensitizer application and DF movement (y) after each desensitizer application:

Permeation inhibition rate (%) = $\{(x-y)/x\} \times 100$a

3) Statistical analysis

The calculated permeation inhibition rates were statistically analyzed using one-way layout analysis of variance and Scheffe's test ($p < 0.01$). The number of specimens was five in all groups.

4) Laser microscopic observation of enamel surface

Enamel cracks in the desensitizer-applied surface were observed under the VK laser microscope before application and one week and one month and three months after application.

5) SEM observation of longitudinal cross-sectional surface of enamel crack specimens

After completion of laser microscopic observation at three months, the specimens were embedded in epoxy resin and longitudinally cut using a low-speed diamond wheel saw (SBT-650, South Bay Technology). The sections were subjected to gold deposition following the standard method, and observed under a scanning electron microscope (JSM-5610LV, JEOL).

This study was performed with the approval of the Animal Experiment Committee of Osaka Dental University (Approval number: 1603011).

Results

1) Rate of permeation inhibition by desensitizer application

The permeation inhibition rate was measured immediately after the application of each desensitizer and after storage in distilled water or remineralization solution for one week and one month and three months. The results are shown in Fig. 2.

No change was noted in the permeation inhibition rate in either the control DW or RS group. In the SS-, TD-, NS-applied DW groups, the permeation inhibition rates at one month and three months were significantly higher than that immediately after application. In the SS- and NS-applied RS groups, the inhibition rates at one month and three months were significantly higher than that immediately after application. In the TD-applied RS group, the inhibition rate at three months was significantly higher than that immediately after application. In the MS-applied DW and RS groups, the inhibition rates at one week

and one month and three months were significantly higher than that immediately after application. In the GP-applied DW and RS groups, the permeation inhibition rate was high from immediately after application although the differences were not significant.

2) Laser microscopic observation of enamel surface

The results of laser microscopic observation of the enamel surface in the DW and RS groups are shown in Figs. 3 and 4, respectively.

Enamel cracks has slightly expanded in the control DW group, and a reaction assumed to be remineralization was noted in the superficial layer of enamel cracks in the control RS group. In the SS-, NS-, TD-, and MS-applied DW and RS groups, enamel cracks were sealed with aggregates assumed to be crystalized substances. In the GP-treated groups, the superficial layer of enamel cracks appeared covered with a resin coating in both DW and RS groups.

3) SEM observation of longitudinal cross-sectional surface of specimens

The results of SEM observation of the longitudinal cross-sectional surface of the specimens are shown in Fig. 5. No sealing of the inner region of enamel cracks was noted in the control DW or RS group. The inner region of enamel cracks was sealed with aggregates in all SS-, MS-, NS-, TD-, and GP-applied DW and RS groups.

Discussion

Enamel cracks are examined by visual observation, palpation, and radiography. Early discovery of enamel cracks was previously difficult, but the accuracy of examination has improved with the recent spread of dental microscopy, dental cone-beam CT, and intraoral imaging. Regarding the mechanism of pain development, it is considered very likely that stimulation reaches dentin through enamel cracks and causes fluid movement in the dentinal tubule following the hydrodynamic mechanism^{18,19)}, stimulating free nerve endings³⁾. It has also been reported that dye penetrated all layers of enamel and came close to the dental pulp through the dentinal tubule in an extracted tooth with enamel cracks reaching the deep region^{5,20)}.

For treatment of enamel cracks, conservative treatment, such as drug application, introduction of ions, laser therapy, and coverage with adhesive materials, and nonconservative methods such as pulpectomy, are selected similarly to treatment of dentin hypersensitivity. Among these methods, drug application is frequently selected as the first choice because of its convenience and fast-acting property. In the action mechanism of desensitizers, it is important to block the transmission of stimulation to the dental pulp through enamel cracks and inhibit the excitement of dental pulp cells. In addition, promotion of deposition of calcified substances in enamel cracks and their active closure

are considered²¹⁾. In this study, five desensitizers were selected and their ability to seal enamel cracks was investigated. In addition, the influence of the storage condition on the permeation inhibition rate was investigated by immersion of specimens in either distilled water or remineralization solution.

Enamel crack specimens (control)

Enamel crack specimens stored by immersion in either distilled water or remineralization solution without desensitizer application were prepared as controls. No change was noted in the permeation inhibition rate in either the control DW or RS group. In the DW group, enamel cracks had slightly expanded. This may have been caused by the force loaded when the specimen was fixed to the specimen stage, and it may also occur in daily occlusal force-bearing conditions. In the RS group, reactions assumed to be remineralization were observed in the superficial layer of enamel cracks under laser microscopy, but no change was noted in the permeation inhibition rate. Remineralization of enamel is induced by the oversaturation of calcium and phosphate ions in saliva²²⁾. It was assumed that the reaction was limited to the superficial layer of enamel cracks because remineralization by the remineralization solution alone takes time, and it did not reach the point of entirely sealing the enamel cracks. It was also considered that the air spray and inner dental pulp pressure loaded during the measurement of the permeation inhibition rate acted as remineralization inhibitors. Moreover, the force loaded by fixing the specimen to the specimen stage may have acted as a remineralization-inhibitory factor, as in the DW group.

SUPER SEAL (SS)

It has been reported that the main component of SS, oxalic acid, reacts with Ca in the tooth and forms insoluble calcium oxalate crystals in the dentinal tubule, sealing the tubule²³⁻²⁵⁾. In the RS group, the permeation inhibition rate markedly changed one week after application compared with that immediately after application, unlike that in the DW group. Thanatvarakorn et al.²⁶⁾ reported that when human tooth dentin was immersed in artificial saliva containing CaCl₂, KH₂PO₄, and NaN₃ after application of desensitizer, inorganic crystals formed on the dentin surface and sealed the dentinal tubule. It was assumed that the crystallization of aggregates was promoted due to the influence of ions released from CaCl₂, KH₂PO₄, and NaN₃ contained in the remineralization solution in our experiment, elevating the value to a high level earlier in the RS group. Nomura et al.²⁷⁾ reported that the inhibition rate after storage in distilled water for 24 h decreased compared with that immediately after desensitizer application to dentin, whereas the inhibition rate increased from that immediately after desensitizer application after storage in artificial saliva for 24 h, suggesting elution of components in the DW group, but repeated application may lead to dense crystal formation, increasing the permeation inhibition rate during the process. In addition, since elution of components was suppressed by applying a

desensitizer once a week, no difference was found between the DW group and the RS group.

MS Coat F (MS)

In MS-applied tooth, fluorine ion was incorporated into the tooth simultaneously with reaction of nanosize MS polymer (methyl methacrylate-styrene sulfonic acid copolymer) and oxalic acid²⁸⁾, and the formation of a macromolecular protective coating containing calcium oxalate and calcium fluoride crystals closely sealed the inner regions of micro cracks not only on the tooth surface but also in the dentinal tubule and dentin. This sealing of the dentinal tubule inhibits fluid movement in the tubule, which is the factor of hypersensitivity development, inhibiting hypersensitivity.

The permeation inhibition rates at one week and one month and three months were significantly higher than that immediately after application in the DW and RS groups. Yoneda et al.²⁹⁾ reported that insoluble calcium was precipitated in the dentinal tubule by oxalic acid, for which a tubule-narrowing effect can be expected, suggesting that the superficial layer of enamel cracks is covered by a macromolecular protective coating and aggregates formed by oxalic acid narrow the inner regions of enamel cracks. Furthermore, repeated application may have strongly sealed covered regions and coarse inner regions. In addition, no difference was observed between DW group and RS group by applying desensitizer once a week. Fluoride is compounded in MS at a high concentration (3,000 ppm), and its incorporation into the tooth has been confirmed to improve the acid resistance of the tooth³⁰⁻³²⁾, suggesting that the antifouling property of MS polymer coating is improved and the promotion of tooth remineralization can be expected.

Nano Seal (NS)

NS comprises a dispersion of fluoroaluminosilicate glass micronized to nanosize fine particles (Solution A) and aqueous phosphoric acid solution (Solution B). When this mixture solution is applied to the tooth surface, it reacts with inorganic components of the tooth and forms a 1- μ m thick nanoparticle layer comprised of calcium fluoride, calcium phosphate, phosphate-silicate, and non-reacted fluoroaluminosilicate glass³³⁾, through which nanoparticles are deposited in defects in the microstructure of the tooth and bind to the tooth, which may repair enamel cracks³⁴⁾. NS contains tooth calcification-related elements, such as Ca and F, and induction of remineralization of Si has also been suggested³⁵⁾.

The permeation inhibition rate at one month was higher than that immediately after application in both the DW and RS groups. NS instantaneously reacts with the tooth and forms a particle deposition layer, but it is considered inappropriate for cases with large substance defects requiring physical blockage of stimulation because the nanoparticle deposition layer is thin³⁶⁾. Han et al.³⁴⁾ reported that an NS-permeated layer 2-3- μ m thick

was observed in enamel, suggesting that remineralization is also induced in the inner region of enamel cracks. However, repeated application may be necessary because remineralization requires time. Repeated application may have strengthened the covered region and inner coarse region and sealed the cracks with time. In addition, since elution of components was suppressed by applying a desensitizer once a week, no difference was found between the DW group and the RS group.

Teethmate Desensitizer (TD)

Sugawara et al.³⁷⁾ reported that Calcium Phosphate Remineralizing Slurry (CPRS) mainly comprised of tetracalcium phosphate (TTCP) and dicalcium phosphate dihydrate (DCPD) produces hydroxyapatite (HAp) in vivo. They confirmed sealing of the opening of exposed dentinal tubule with apatite crystals. TD was developed based on this phenomenon, and HAp produced in the dentinal tubule acts as a core and strongly binds to inorganic compounds of the tubular wall³⁷⁾. In addition, Ohmori et al.³⁸⁾ observed that enamel cracks were sealed with crystalloid structures on the TD-applied enamel surface, suggesting that a phenomenon similar to that observed in the dentinal tubule also occurs in enamel cracks.

The permeation inhibition rate was higher than that immediately after application at one week in the DW group and three months in the RS group, suggesting that repeated application of TD gradually sealed the inner regions of enamel cracks with time; it has been reported that the density of crystalline structures formed on the dentin surface increased with time³⁷⁾. In addition, since elution of components was suppressed by applying a desensitizer once a week, no difference was found between the DW group and the RS group. Under SEM, sealing of the inner regions of enamel cracks with aggregates assumed to be crystalloid structures was observed in both the DW and RS groups.

G-Premio Bond (GP)

GP includes a compound of 10-methacryloyloxydecyl di-hydrogen phosphate (MDP) and 4-methacryloyloxyethyl trimellitate (4-META), through which GP strongly adheres to enamel and dentin. Water is effectively removed because highly volatile acetone is used for the solvent. In addition, it does not readily absorb water because hydrophilic 2-hydroxyethyl methacrylate (HEMA) is not compounded. It is a light-cured single-solution bonding agent for which long-term bond-durability can be expected. It has been reported that resin desensitizers have high ability to seal dentinal tubules compared with those of inorganic and protein coagulation systems²⁷⁾. In the present experiment, a higher permeation inhibition rate was noted in enamel cracks immediately after application in the DW and RS groups, unlike the other desensitizers. Therefore, no difference was found between the DW group and the RS group.

Iwata et al.³⁹⁾ reported that the opening of the dentinal tubule was closed with a layer of about 5- μm thick in the longitudinal cross-sectional surface of GP-applied dentin observed

under SEM. Under laser microscopic observation, the GP-applied surface was entirely covered with a resin coating. Under SEM, GP permeated the inner region of enamel cracks and sealed it. These findings suggest that the superficial layer was covered with a resin coating about 5- μ m thick, and enamel cracks were sealed with GP that penetrated the inner region. In addition, repeated application may have strengthened the coarse resin coating, increasing the permeation inhibition rate.

Conclusion

The ability of various desensitizers to seal enamel cracks was investigated, and the following findings were obtained:

1. Repeated application of SUPER SEAL, MS Coat F, Nano Seal, and Teethmate Desensitizer to teeth stored in either distilled water or remineralization solution increased their ability to seal enamel cracks.
2. The enamel crack sealing ability was high from immediately after application in teeth applied with G-Premio Bond and stored in distilled water or remineralization solution.

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There is no conflict of interest to be disclosed in the execution of this study and the preparation of this report.

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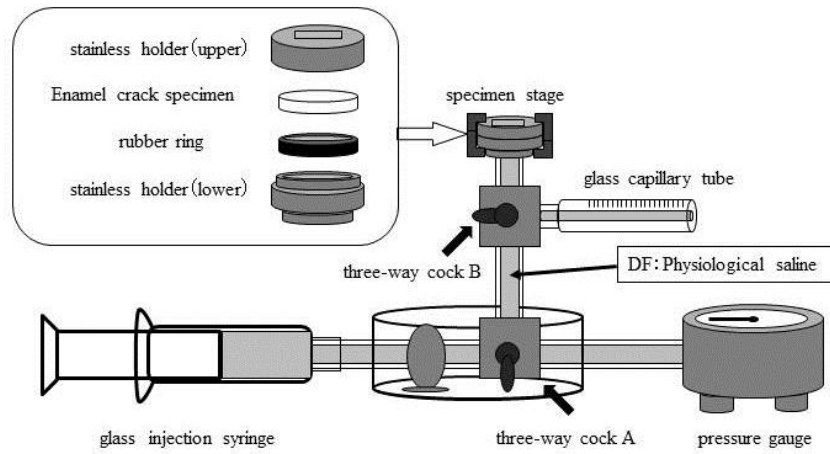


Fig.1 Schematic diagram of a tooth model of hypersensitivity

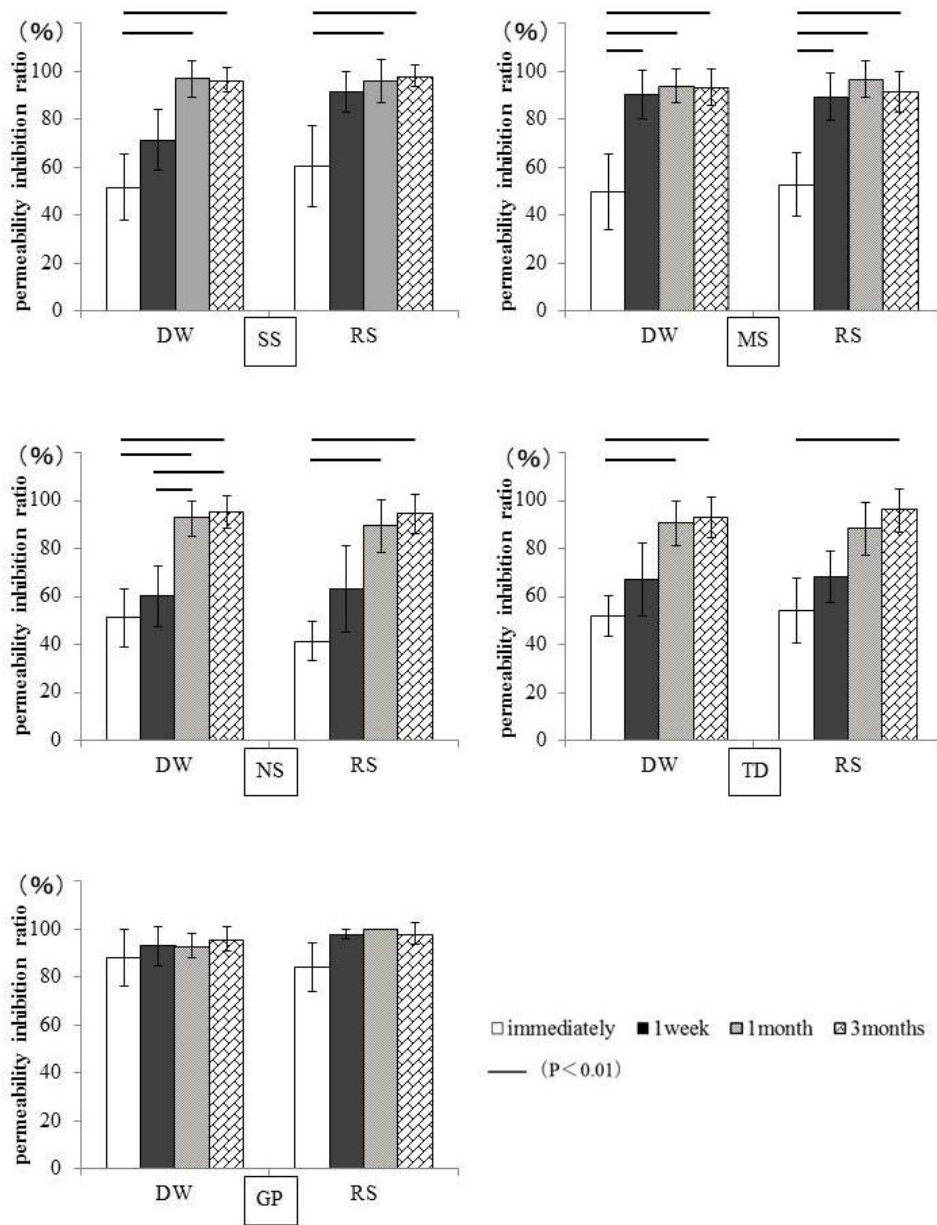
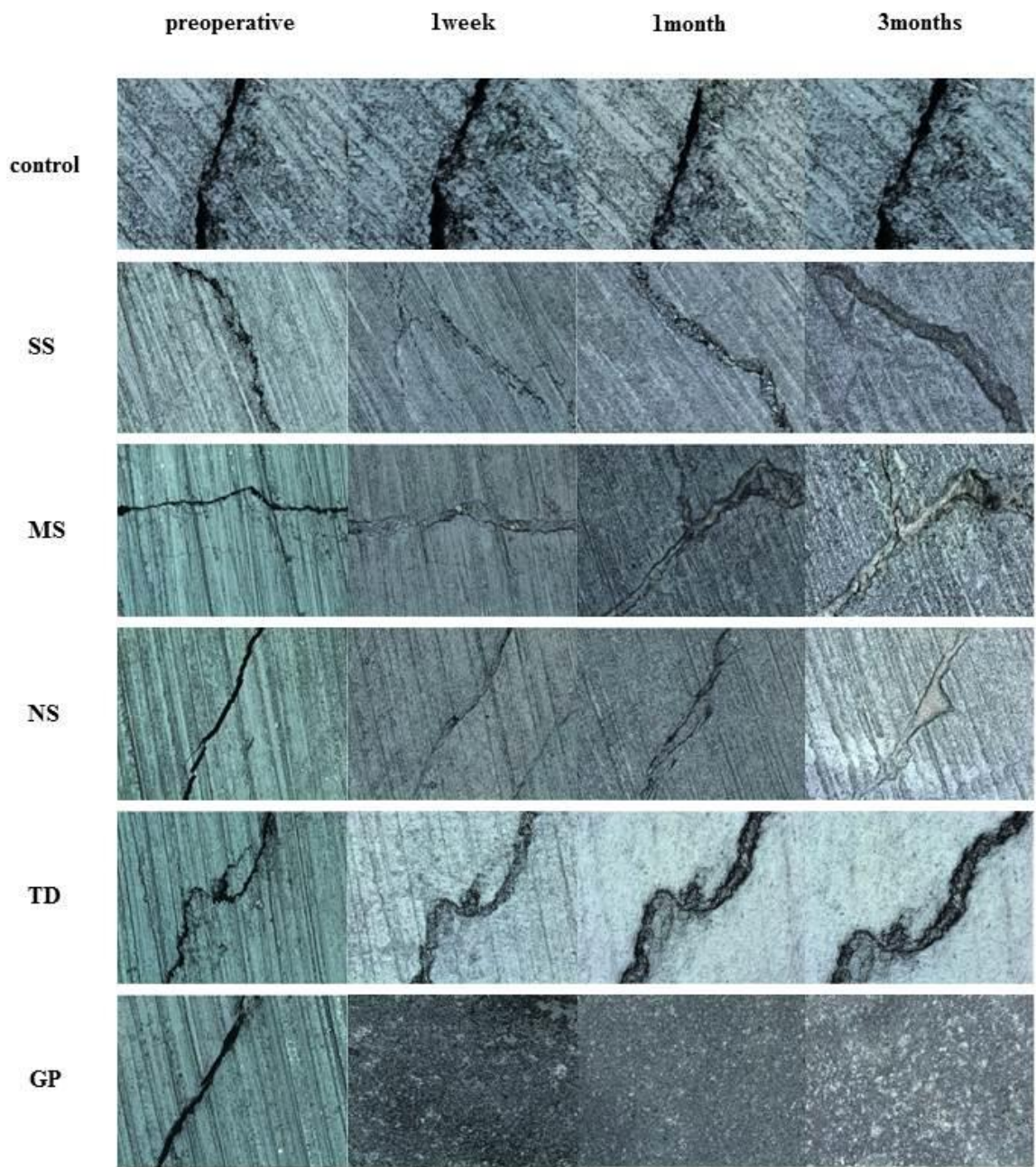
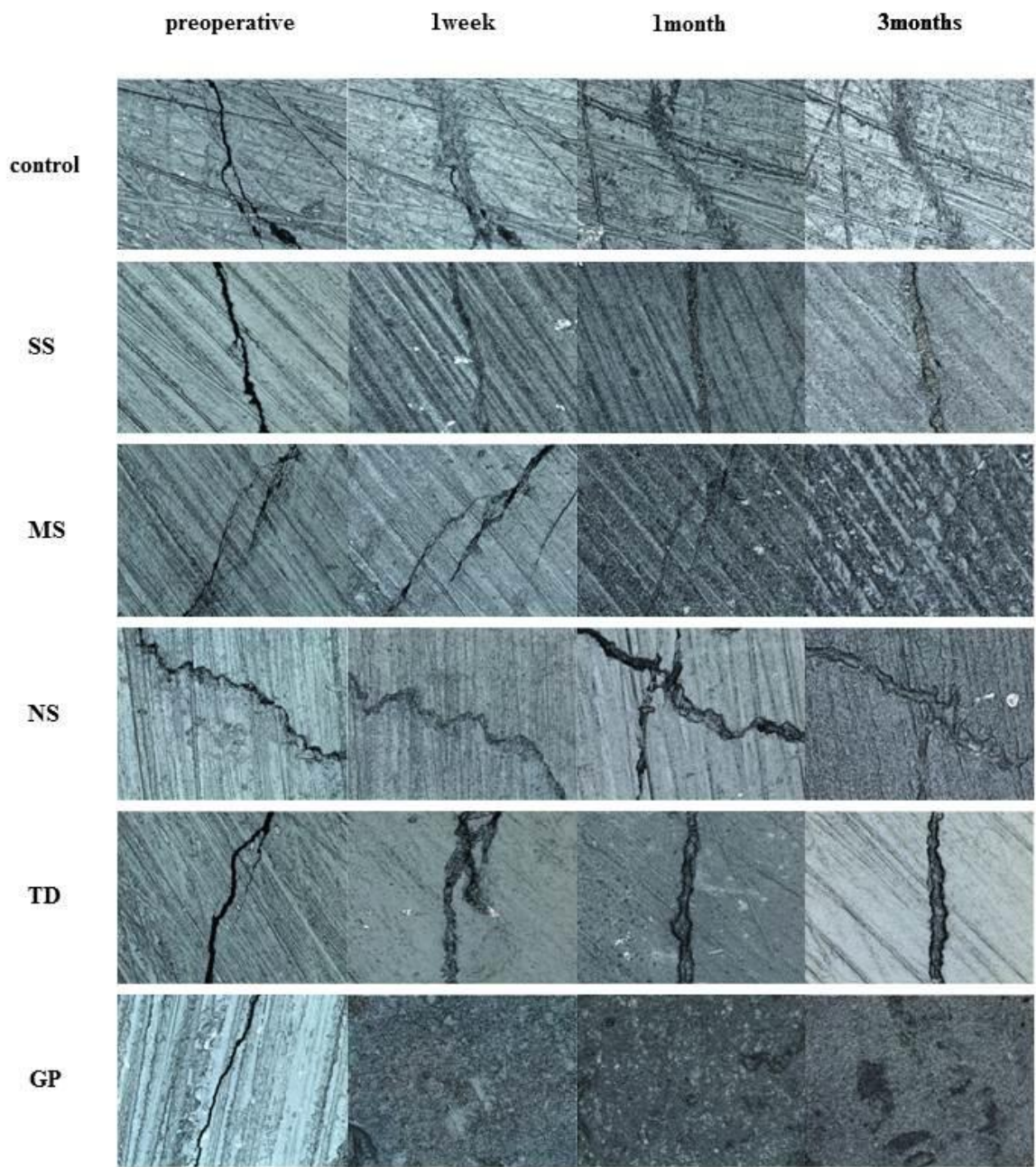


Fig. 2 Measurements of the permeability inhibition ratio of dentin desensitizers



(magnification: $\times 1000$)

Fig. 3 Laser microscopic observation of enamel surface (DW group)



(magnification: $\times 1000$)

Fig. 4 Laser microscopic observation of enamel surface (RS group)

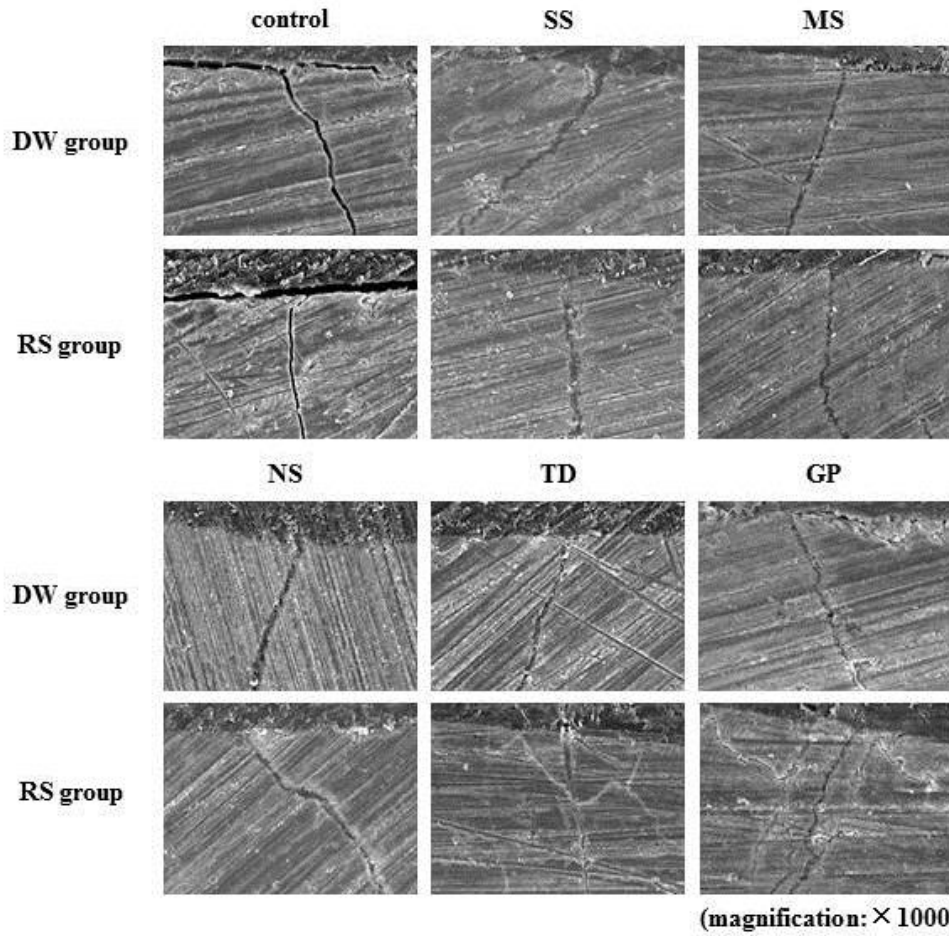


Fig. 5 SEM observation of longitudinal cross-sectional surface of enamel crack specimen

Table 1 Materials used

Materials	Composition	Lot No.	Manufacturer	Code
SUPER SEAL	Oxalic acid, Others	991662	Phoenix Dental USA	SS
MS Coat F	Methyl methacrylate / styrene sulfonic acid copolymer, Oxalic acid, Water, sodium fluoride	LF1	Sun Medical	MS
Nano Seal	A Liquid: Fluoroaluminosilicate glass dispersion liquid, Paraben, Purified water	A Liquid: EAH	Nippon Shika Yakuhin	NS
	B Liquid: Phosphoric acid, Purified water	B Liquid: EAL		
Teethmate Desensitizer	Powder: Tetracalcium phosphate, Dicalcium phosphate anhydrous, Others	Powder: 000001	Kuraray Noritake Dental	TD
	Liquid: Purified water, Others	Liquid: 000001		
G-Permio BOND	10-MDP, 4-META, methacrylate adic ester, 10-methacryoyloxydecyl dihydrogen thiophosphate, distilled water, acetone, photo initiators, silica fine powder	1503102	GC	GP

Table 2 Method of applying each dentin desensitizer

SS	Rubbing for 5 sec	Dry	
MS	Rubbing for 30 sec	Dry	Wash
NS	Mix	Apply for 20 sec	Wash
TD	Mix for 15 sec	Rubbing for 30 sec	Wash
GP	Apply for 10 sec	Dry	Light cure for 10 sec

エナメル質の微細亀裂に対する各種知覚過敏抑制材の封鎖性

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抄録

緒言：日本では高齢化が進んでいるが、8020 運動などの普及活動や歯科治療の発展により、国民 1 人あたりの残存歯数が増加している。それに伴う蝕や歯周病以外の非う蝕性疾患、特に、実質欠損を認めず、知覚過敏症を呈する患者が増加している。その原因の一つとしてエナメル質の微細亀裂（以下；エナメルクラック）が考えられる。健全歯のエナメルクラック発生率は、40 歳以降では 95% を超えるとの報告もある。本研究では、即効性や簡便性などの点から、象牙質知覚過敏抑制材（以下；知覚過敏抑制材）を用いてエナメルクラックを封鎖することはできないかと考えた。そこで、知覚過敏症罹患モデル歯質を用いて、透過抑制率の測定を行い検討した。また、知覚過敏抑制材塗布後、蒸留水と再石灰化溶液に保管することにより、環境が封鎖性に与える影響についても同時に検討を行った。

材料及び方法：エナメル亀裂試料をう蝕のない健全ウシ歯を用いて作製した。Pashley らの報告に準じて作製した装置を用いて、試料を装置に接続して内圧が 25mmHg になるように規定した。実験に使用した知覚過敏抑制材として、Super Seal (SS), MS Coat F (MS), Nanoseal (NS), Teethmate Desensitizer (TD), G-Premio BOND (GP) を用いた。各知覚過敏抑制材を塗布後、エナメルクラックの透過抑制率を測定した。測定後、試料を蒸留水中 (DW 群) または再石灰化溶液 (RS 群) に保管し、1 週間後、1 ヶ月後ならびに 3 ヶ月後にも エナメルクラックの透過抑制率を測定した。

結果：SS, TD, NS の DW 群では、塗布直後の透過抑制率に比べ、1 ヶ月後、3 ヶ月後の透過抑制率は有意に高い値を示した。SS, NS の RS 群では、塗布直後の透過抑制率に比べ、1 ヶ月後、3 ヶ月後の透過抑制率は有意に高い値を示した。TD の RS 群では、塗布直後の透過抑制率に比べ、3 ヶ月後の透過抑制率は有意に高い値を示した。MS の DW 群, RS 群では、塗布直後の透過抑制率に比べ、1 週間後、1 ヶ月後、3 ヶ月後の透過抑制率は有意に高い値を示した。GP の DW 群, RS 群ともに塗布直後より高い封鎖性が得られたことにより、1 週間後、1 ヶ月後、3 ヶ月後と比べ有意差は認められなかった。

結論：以上より、各知覚過敏抑制材において効果発現の時期などに差はみられるものの、繰り返し塗布することにより効果があらわれることが示唆された。

キーワード：エナメルクラック 封鎖性 知覚過敏症罹患モデル歯質