Effect of ion-releasing filler-containing gel application on dentin remineralization using optical coherent tomography and ultrasonic velocity measurement

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Contents

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Summary

New biofunctional materials have been introduced, especially those containing calcium and phosphate ions, as a strategy for aiding remineralization and improving the fluoride uptake of dental substrates. Surface reaction-type prereacted glass-ionomer (S-PRG) filler is prepared via an acid-base reaction (of the traditional glass ionomer) between fluoroboroaluminosilicate glass and a polyacrylic acid in the presence of water, whereby the preliminary product is a stable glass-ionomer phase within the glass particles. S-PRG filler has been reported to inhibit tooth demineralization and plaque formation, possibly by releasing fluoride (F^-), borate (BO_3^{3-}), strontium (Sr^{2+}), silica (SiO_3^{2-}) sodium (Na^+), and aluminum (Al^{3+}) ions. Indeed, compared with fluoroaluminosilicate glass filler materials, a resin composite with S-PRG filler was shown to release F^- at a higher rate. In the previous report, S-PRG filler also has a modulation effect on oral acidity, causing the pH of the surrounding environment to become weakly alkaline upon contact with water or acidic solutions. This effect is believed to be mediated by the released ions from the S-PRG filler. The present study principally aimed to examine the efficacy of S-PRG filler-containing gel application on the prevention of dentin remineralization using optical coherent tomography (OCT), Knoop hardness number (KHN) and ultrasonic velocity measurements.

Dentin slabs of bovine teeth were sliced and shaped into a rectangular form. Specimens were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75) for 10 min and then placed in artificial saliva (pH 7.0). This procedure was repeated three times a day for 28 days. The dentin remineralization effects of S-PRG filler-containing gel (PRG) and a high fluoride-concentration silver diamine fluoride (SDF) solution on dentin slabs of bovine teeth were examined. The specimens were divided into five different treatment groups according to the application procedures; as 1) untreated, 2) one-off application with PRG, 3) one-off application with SDF, 4) frequent-time application with PRG, 5) frequent-time application with SDF. After

treatment, the dentin slabs were immersed in 0.1 M lactic acid buffer solution and then placed in artificial saliva. This procedure was repeated three times a day for 28 days. OCT imaging was conducted on the selected location of the dentin surface. The maximum peak intensity and width at $1/e^2$ were recorded in each of the six areas on the sample and averaged. Each group had a sample size of 10. KHN and the propagation time of longitudinal ultrasonic velocities were also measured to calculate the ultrasonic velocity. And, scanning electron microscopy (SEM) observations were conducted. The data for each group were subjected to repeated-measures analysis of variance followed by Tukey-Kramer post hoc multiple comparisons test ($\alpha = 0.05$).

After the initial demineralization, some bright scattering areas on the dentin surface were observed on the B-scan images. For the untreated group after 28 days, the B-scan image was similar to that of initial demineralization group. For the one-off application with SDF and PRG, a weak and narrow signal on the dentin surface was observed after 28 days, with low intensity back-scattering. On the other hand, for the frequent-time treatment with SDF, a strong signal on the dentin surface was observed after 28 days, and the back-scattered grainy appearance was very weak. During the experimental period, the maximum peak intensities of the untreated group were increased slightly. In the one-off application specimens, the maximum peak intensities of SDF and PRG decreased dramatically on day 7 and then slightly increased during the experimental period. For the frequent-time application, the maximum peak intensities of SDF and PRG decreased on day 7, and the intensity values decreased during the experimental period.

During the initial demineralization period, the KHN of all specimens decreased. Although the KHN of the untreated specimens decreased slightly over the test period, the KHN of SDF and PRG of the one-off and frequent-time application increased significantly during the experimental period. Specimens treated with SDF had a significantly higher KHN on each measurement day when compared with those treated with PRG.

During the initial demineralization period, the ultrasonic velocities of all specimens decreased. During the experimental period, the ultrasonic velocities of the untreated specimen decreased slightly. In the one-off application specimens, the ultrasonic velocities of SDF and PRG increased dramatically on day 7 and then slightly decreased during the experimental period. For the frequent-time application specimens, the ultrasonic velocities of SDF and PRG increased during the experimental period. Specimens treated with SDF had a significantly higher ultrasonic velocity on each measurement day when compared with those treated with PRG.

The SEM images of the dentin surfaces revealed morphological differences among the treatment groups. After initial demineralization, dentinal tubules were clearly observed. At the end of the 28 day experimental period, morphological changes on the dentin surfaces were also clearly identified in the frequent-application specimens compared with those in the one-off application specimens. The dentinal tubules were totally occluded, and some precipitations were observed for SDF. Closure of the dentinal tubules and crystal precipitation were detected on the surface of the PRG.

Under the current experimental conditions, it was concluded that S-PRG filler-containing gel appears to help remineralization and inhibit demineralization of the dentin.

Introduction

Silver diamine fluoride (SDF) has been used in dentistry for more than 50 years, and research on its effectiveness is promising. SDF solution has been reported to be an effective treatment for the arrest of caries and is usually recommended for children who are at high risk of caries development1). A recent systemic review reported that an annual application of 38% SDF in elderly individuals reduced the incidence of new carious lesions on the exposed root surfaces by at least 50%, and longer interventions were even more effective2). Various concentrations have been applied in clinical trials, and the 38% solution was found to be significantly more effective in arresting dental caries than 12% SDF and no application3). When SDF was applied to the teeth, it was found to penetrate enamel and dentin and to store two to three times more subsurface fluoride on the teeth than that by other fluoride solutions4). However, one of the main disadvantages of SDF is its discoloration effect.

New biofunctional materials have been introduced, especially those containing calcium and phosphate ions, as a strategy for aiding remineralization and improving the fluoride uptake of dental substrates5). Surface reaction-type prereacted glass-ionomer (S-PRG) filler is prepared via an acid-base reaction between fluoroboroaluminosilicate glass and a polyacrylic acid in the presence of water, whereby the preliminary product is a stable glass-ionomer phase within the glass particles. S-PRG filler has been reported to inhibit tooth demineralization and plaque formation, possibly by releasing fluoride (F^-), borate (BO_3^{3-}), strontium (Sr^{2+}), silica (SiO_3^{2-}) sodium (Na^+), and aluminum (Al^{3+}) ions. Indeed, compared with fluoroaluminosilicate glass filler materials, a resin composite with S-PRG filler was shown to release F^- at a higher rate6). In the previous report7), S-PRG filler also has a modulation effect on oral acidity, causing the pH of the surrounding environment to become weakly alkaline upon contact with water or acidic solutions. This effect is believed to be mediated by the released ions from the S-PRG filler8).

The present study principally aimed to examine the efficacy of S-PRG filler-containing gel application on the prevention of dentin remineralization using optical coherent tomography (OCT), Knoop hardness number (KHN) and ultrasonic velocity measurements. The null hypothesis to be tested was that there was no difference between the specimens treated with different types of remineralization materials.

Materials and methods

Specimen preparation

An oral gel containing S-PRG filler (PRG; PRG Pro-Care Gel; Shofu, Kyoto, Japan) and a high fluoride-concentration SDF solution (Saforide; Toyo Pharmaceutical, Osaka, Japan) were used (Table 1).

Freshly extracted bovine incisors were cleaned and stored in physiological saline for up to 2 weeks. Approximately two-thirds of the apical root structure of each tooth was removed using a diamond-impregnated disk within a low-speed saw (IsoMet 1000 Precision Sectioning Saw; Buehler, Lake Bluff, IL, USA). The root dentin surfaces were ground with wet #240-grit silicon carbide (SiC) paper (Fuji Star Type DDC; Sankyo Rikagaku, Saitama, Japan) to expose flat dentin surfaces. Dentin blocks were carefully shaped into a rectangular form (4 × 4 × 1 mm) using a super-fine diamond point (SF106RD; ISO #021a, Shofu). Specimen surfaces were ground successively on wet SiC paper with grit sizes of #600, #1,200, and #2,000. A dial gauge micrometer (CPM15-25DM; Mitutoyo, Tokyo, Japan) was used to measure the thickness and size of the specimens, which were then covered with wax, except for the labial side of the dentin slab of the treatment surface.

All specimens were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM $CaCl_2 \cdot 2H_2O$, and 0.45 mM KH_2PO_4) for 10 min and then placed in artificial saliva (pH 7.0, 14.4 mM NaCl, 16.1 mM KCl, 0.3 mM $MgCl_2 \cdot 6H_2O$, 2.0 mM K_2HPO_4, 1.0

mM $CaCl_2 \cdot 2H_2O$, and 0.10 g/100 mL sodium carboxymethyl cellulose) at 37℃. These procedures were conducted three times daily (with an 8 h interval time) over 28 days. The specimens were then divided into five different treatment groups of 10 specimens each.

1. Untreated: Specimens were treated with undersaturated 0.1 M lactic acid buffer solution for 10 min and then placed in artificial saliva. This procedure was repeated three times. The specimens were then subjected to the acidic challenge during the experimental period.
2. One-off application with PRG: PRG was applied with a soft brush for 10 s and then rinsed with tap water and placed in artificial saliva. This procedure was repeated three times. The specimens were then subjected to the acidic challenge during the experimental period.
3. One-off application with SDF: SDF was applied with a microbrush and left for 3 min and then rinsed with tap water and placed in artificial saliva. This procedure was repeated three times per day.
4. Frequent-time application with PRG: PRG was applied with a soft brush for 10 s and then rinsed with tap water. This procedure was repeated once a week during the experimental period with the acidic challenge.
5. Frequent-time treatment with SDF: SDF was applied with a microbrush and left for 3 min and then rinsed with tap water. This procedure was repeated once a week during the experimental period with the acidic challenge.

The application of PRG was conducted using a micromotor handpiece (Torq tech CA-DC; J. Morita Mfg., Kyoto, Japan) and a soft brush (Merssage Brush Soft; Shofu) at 1,000 rpm and a constant pressure of 0.1 N, as monitored by a digital balance (AT200; Mettler-Toledo, Greifensee, Switzerland). All procedures were conducted by a single operator.

OCT measurements

A time-domain OCT (TD-OCT) imaging system (J. Morita Tokyo Mfg., Saitama, Japan) was used. A scanning probe connected to the time-domain OCT device was fixed 2 mm from the specimen surface. The specimen was taken out the artificial saliva, and then rinsed with distilled water for 5 s. The excess water was removed using paper (KimWipes; Nippon Paper Crecia, Tokyo, Japan) before the measurements. Imaging was performed within 30 s of mounting the specimen on the sample stage. The scanning light beam was set at right angles to the surface of the specimen, and changes in the specimen were determined based on the peak intensity values from the OCT images (B-scan images). The width between points, at which the intensity decreased to a value of $1/e^2$ and corresponded to the peak intensity of the line profile. The width of $1/e^2$ was equivalent to the distance between the two points where the intensity falls to 13.5% of the maximum value. Peak intensities and the widths at $1/e^2$ were obtained for six points around the restoration in each specimen, and these obtained data were averaged (mean values). Six specimens were examined in each treatment group. Measurements were taken at 23°C ± 1°C and 50% ± 5% relative humidity. Measurements were taken before the test and on days 0 (end of the initial demineralization period), 7, 14, 21, and 28 during the experiment period.

KHN measurement

After OCT measurement, KHN were obtained from the surfaces of each sample. The indenter was kept on the surface for 30 s with a 0.25 N load using a microhardness tester (DMH-2; Matsuzawa, Tokyo, Japan). Five measurements were conducted on each sample, and the mean hardness value was set as the KHN of the sample.

Ultrasonic velocity measurement

Ultrasonic velocity was measured using a system comprising a pulser-receiver (5900PR;

Panametrics, Waltham, MA, USA), a longitudinal wave transducer (V112; Panametrics), and an oscilloscope (Waverunner LT584; LeCroy, Chestnut Ridge, NY, USA). The equipment was initially calibrated using a standard procedure with a stainless steel calibration blocks (2211M; Panametrics). The transducer was oriented perpendicular to the contact surface of each sample to obtain an echo signal. The ultrasonic waves propagated from the transducer to the tooth were transmitted through the tooth and detected by the transmitter on the opposite side. Measurements were taken before the test and on days 0 (end of the initial demineralization period), 7, 14, 21, and 28 during the experiment period.

Statistical analysis

Statistical analyses were conducted using a commercial statistical software package (Sigma Plot Ver.13; Systat Software, Chicago, IL, USA). Data from each group were first tested for homogeneity of variance using Bartlett's test and normal distribution using the Kolmogorov-Smirnov test and then analyzed using repeated-measures analysis of variance followed by Tukey-Kramer post hoc multiple comparisons test. The level of significance was set at $\alpha = 0.05$.

Scanning electron microscopy observation

Ultrastructural observation of the specimen surfaces was conducted using field-emission scanning electron microscopy (SEM, ERA 8800FE; Elionix, Tokyo, Japan). Specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 min, 75% for 20 min, 95% for 20 min, and 100% for 2 h) and then transferred to a freeze dryer (model ID-3; Elionix, Tokyo, Japan) for 30 min. Specimens were coated in a vacuum evaporator (Quick Coater Type SC-701; Sanyu Electron, Tokyo, Japan) with a thin film of gold and observed at an accelerating voltage of 10 kV.

Results

Representative B-scan images from TD-OCT in different treatment groups are shown in Fig. 1. After the initial demineralization period, some bright scattering areas on the dentin surface were observed. For the untreated group after 28 days, the B-scan image was similar to that of initial demineralization group. For the one-off application with SDF and PRG, a weak and narrow signal on the dentin surface was observed after 28 days, with low intensity back-scattering. On the other hand, for the frequent-time treatment with SDF, a strong signal on the dentin surface was observed after 28 days, and the back-scattered grainy appearance was very weak.

The maximum peak intensities and widths at $1/e^2$ in different treatment groups are shown in Figs. 2 and 3. During the initial demineralization period, all groupus showed the maximum peak intensities increased and the width of $1/e^2$ decreased. During the experimental period, the maximum peak intensities of the untreated specimens were increased slightly. In the one-off application specimens, the maximum peak intensities of SDF and PRG decreased dramatically on day 7 and then slightly increased during the experimental period. For the frequent-time application specimens, the maximum peak intensities of SDF and PRG decreased on day 7, and the intensity values decreased during the experimental period. Although the width at $1/e^2$ in the untreated specimens decreased over the test period, SDF and PRG in the one-off application specimens demonstrated an increase in the widths on day 7 followed by a slight decrease. For the frequent-time application specimens of SDF and PRG, the width at $1/e^2$ increased on day 7 as well as during the experimental period. After 14 days of treatment, significantly higher $1/e^2$ values were obtained for the SDF than those for the PRG.

The average KHN in different treatment groups are shown in Fig 4. During the initial demineralization period, the KHN of all groups decreased. Although the KHN of the untreated specimens decreased slightly over the test period, the KHN of SDF and PRG of the one-off and frequent-time application specimens increased significantly during the experimental period.

Specimens treated with SDF had a significantly higher KHN on each measurement day when compared with those treated with PRG.

The average ultrasonic velocities in different treatment groups are shown in Fig. 5. During the initial demineralization period, the ultrasonic velocities of all groups decreased. During the experimental period, the ultrasonic velocities of the untreated specimens were decreased slightly. In the one-off application specimens, the ultrasonic velocities of SDF and PRG increased dramatically on day 7 and then slightly decreased during the experimental period. For the frequent-time application specimens, the ultrasonic velocities of SDF and PRG increased during the experimental period. Specimens treated with SDF had a significantly higher ultrasonic velocity on each measurement day when compared with those treated with PRG.

Representative SEM images of the specimens are displayed in Fig. 6. The SEM images of the dentin surfaces revealed morphological differences among the treatment groups. After initial demineralization, dentinal tubules were clearly observed. At the end of the 28 day experimental period, morphological changes on the dentin surfaces were also clearly identified in the frequent-application specimens compared with those in the one-off application specimens. The dentinal tubules were totally occluded, and some precipitations were observed for SDF. Closure of the dentinal tubules and crystal precipitation were detected on the surface of the PRG.

Discussion

The present study was conducted to examine the remineralization ability of a multiple ion-releasing gel on dentin demineralization using OCT and ultrasonic measurement. OCT is a noninvasive imaging modality that uses near-infrared light to obtain tissue information at subsurface levels9). The OCT method can be used to visualize changes in dentin minerals, measure the severity of dentin demineralization nondestructively, and determine the effectiveness of interventions with fluoride-containing anticaries agents10). The accurate determination of optical constants from OCT image data is of paramount importance because it describes the interaction of the optical field with the sample. On the other hand, ultrasonic measurement is a non-invasive technique, and so it is possible to follow the process of demineralization or remineralization of the tooth structure. In addition, this method may be suitable for grasping the changes in a single specimen over time. In the demineralization process, the acid reduces the solidity of the dentin, resulting in a reduction of the ultrasonic velocity. In contrast, in the remineralization process, the increase in ultrasonic velocity is directly proportional to the volume concentration of minerals of the dentin11).

In the PRG and SDF groups of the OCT measurement, peak signal intensities were significantly decreased on day 7 (experimental period) for both application specimens and slightly decreased for frequent-time application. When comparing the peak signal intensities among the three different treatments, the values obtained for the untreated specimens were higher than those for the treated specimens. Before demineralization, the dentin surfaces were covered by a smear layer through which light could not pass, leading to lower peak signal intensities. Conversely, the smear layer was removed from specimens during the initial demineralization period because of the immersion into the acidic solution. The remineralization ions achieved remineralization of dentin surfaces by creating uniform and smooth dentin surfaces and occluding the dentinal tubules compared with that in the untreated specimens, leading to a decrease in peak signal intensity.

The results of the present study showed that the $1/e^2$ values in the untreated specimens decreased during the initial demineralization period, and this tendency continued for the untreated specimens during the experimental period. Conversely, wider $1/e^2$ widths were recorded in the PRG- and SDR-applied specimens, and a significant increase in $1/e^2$ width was observed for the specimens receiving the frequent-time application. These results indicated that the OCT signals in the SDF- and PRG-applied specimens were generated by light that traveled over a longer path than that observed in the untreated group. The presence of F^- from SDF and ions from the PRG filler in the gel might have strengthened the eroded dentin surface12), ultimately leading to changes in the optical properties of the specimens. The SEM observations of the PRG specimens revealed that the relatively smoother dentin surfaces with the occluded dentinal tubules; these results agree with the data collected from specific OCT measurements. Thus, S-PRG-containing gel is incorporated into clinical maintenance procedures, the ions released from the glass-ionomer phase of the S-PRG filler may contribute to the remineralization of the tooth surface. These findings were supported by the ultrasonic measurement conducted in this study.

SDF has been shown to provide dentin remineralization properties via preservation and protection of the collagen matrix13). This laboratory study further supports these findings, as SDF showed a greater degree of protection against dentin demineralization when compared with untreated specimens, as detected by OCT, KHN, and ultrasonic velocity measurements. Because SDF contains a high concentration of fluoride and silver, it can be expected to have a great effect on the prevention of tooth decay in root dentin. Besides its antidemineralization effects on the mineral phase, reportedly, SDF might have significant effects on dentin collagen fibrils, reducing their degradation in an acidic environment14). SDF has also demonstrated highly effective antibacterial action against cariogenic biofilm15). Conversely, dentistry is driven by esthetics, and the potential shortcomings of SDF must be considered. One of its main disadvantages is that it causes discoloration, which occurs when silver ions in the composition of SDF change to metallic silver in the presence of light and spread deep inside the teeth16). Therefore, restoration of the tooth structure using translucent materials on SDF-treated surfaces may result in the emission of a dark hue underneath restorative materials.

The S-PRG filler acts as a fluoride-releasing material, and the combination of F^- and Sr^{2+} enhances the crystallinity of carbonated hydroxylapatite. The F^- and SiO_3^{2-} released from the S-PRG filler in the gel might have contributed to the enhancement in dentin remineralization. Additionally, Sr^{2+} may act as a substitute for Ca^{2+} during precipitation and has a synergistic caries control effect with F^-[17).

Conclusions

1. Although the B-scan image of the untreated group was similar to that of initial demineralization group, the B-scan image of the frequent-time application with SDF revealed a strong signal on the dentin surface.
2. In the untreated group, the maximum peak intensity increased and the width of $1/e^2$ decreased.
3. In the SDF and PRG of the frequent-time application specimens, the maximum peak intensities decreased and the width of $1/e^2$ increased.
4. Although the KHN of the untreated group decreased slightly, the KHN of SDF and PRG of the frequent-time application specimens increased.
5. Although the ultrasonic velocity of the untreated group decreased slightly, the ultrasonic velocity of SDF and PRG of the frequent-time application specimens increased.
6. For the frequent-time application with SDF and PRG specimens, dentinal tubules were occluded and some of the precipitations were clearly observed.

Under the current experimental conditions, it was concluded that S-PRG filler-containing gel appears to help remineralization and inhibit demineralization of the dentin.

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Tables and figures

Table 1. Materials used in this study and their main components.

Material	Code	Main component	Manufacturer	Lot No.
PRG Pro-Care Gel	PRG	glycerin, diamond powder (1 μm), aluminum oxide, S-PRG filler, purified water, viscosity agent	Shofu, Kyoto, Japan	91701
Saforide	SDF	38% silver diamine fluoride	Toyo Pharmaceutical, Osaka, Japan	808RA

S-PRG: surface reaction-type pre-reacted glass-ionomer

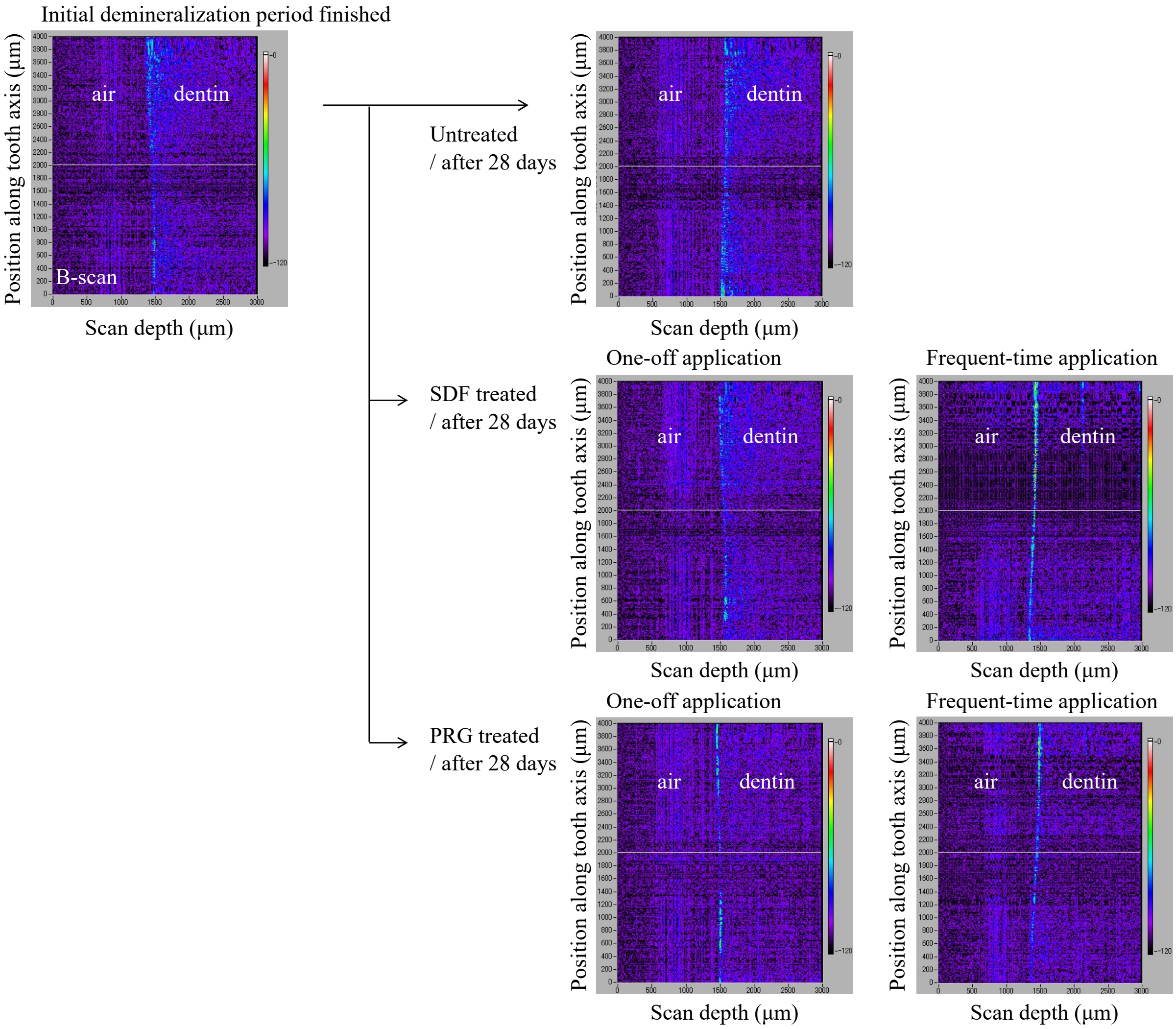
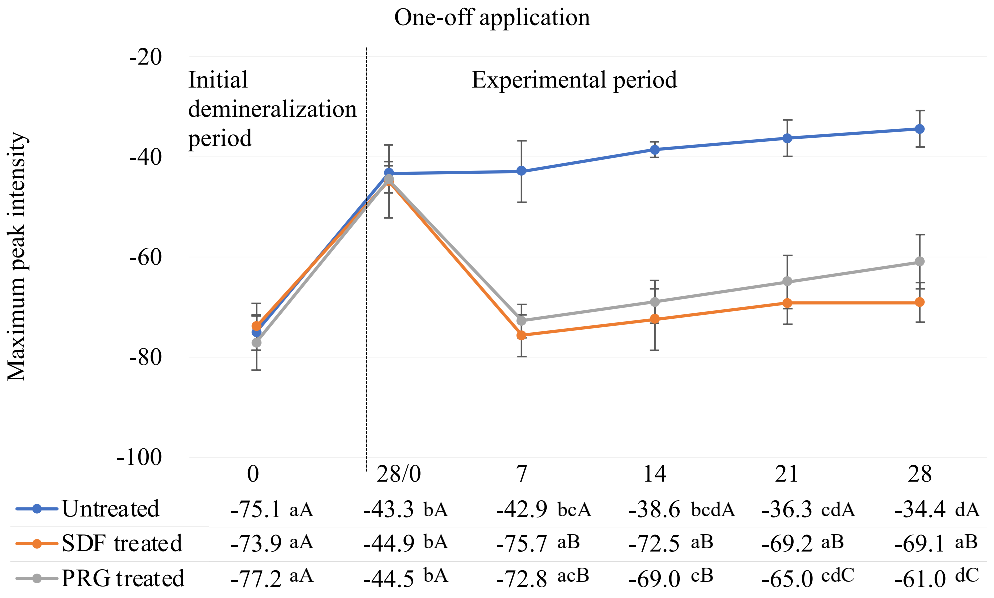
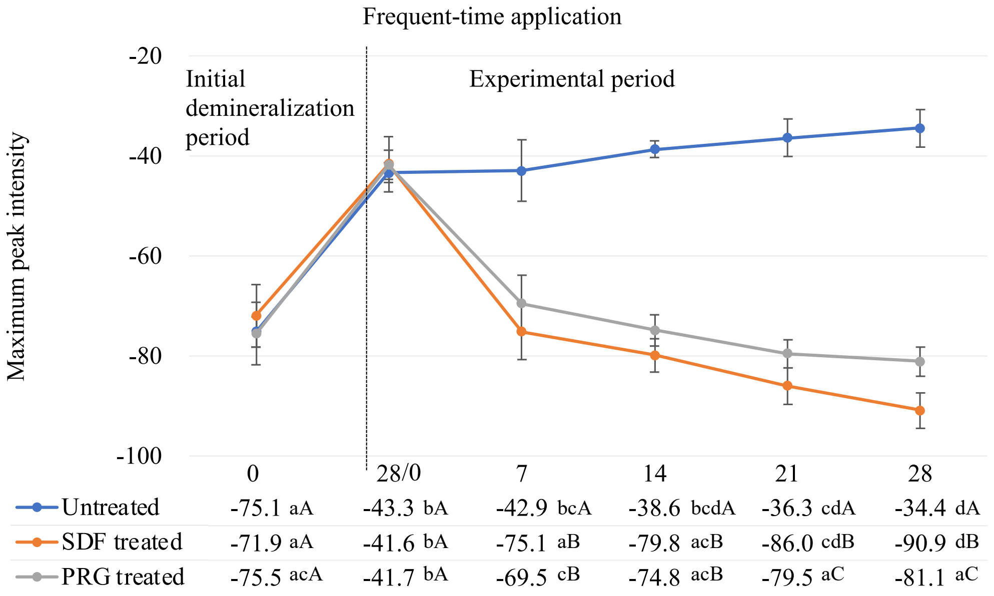


Fig. 1 Representative B-scan images using TD-OCT in different treatment groups. For the frequent-time application, a signal on the dentin surface was observed after 28 days in both SDF and PRG treated groups, however the signal intensity of the PRG group was weak compared to that of the SDF group.


One-off application

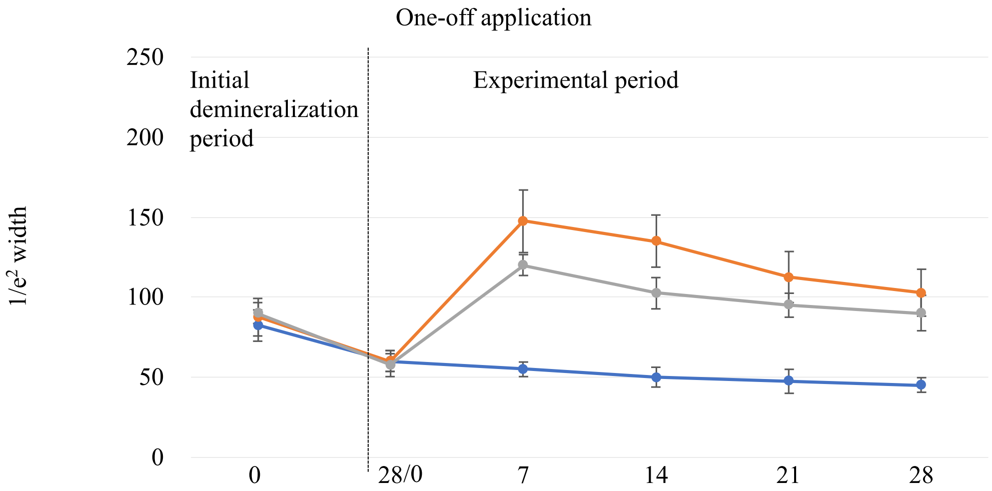
	0	28/0	7	14	21	28
Untreated	-75.1 aA	-43.3 bA	-42.9 bcA	-38.6 bcdA	-36.3 cdA	-34.4 dA
SDF treated	-73.9 aA	-44.9 bA	-75.7 aB	-72.5 aB	-69.2 aB	-69.1 aB
PRG treated	-77.2 aA	-44.5 bA	-72.8 acB	-69.0 cB	-65.0 cdC	-61.0 dC


Frequent-time application

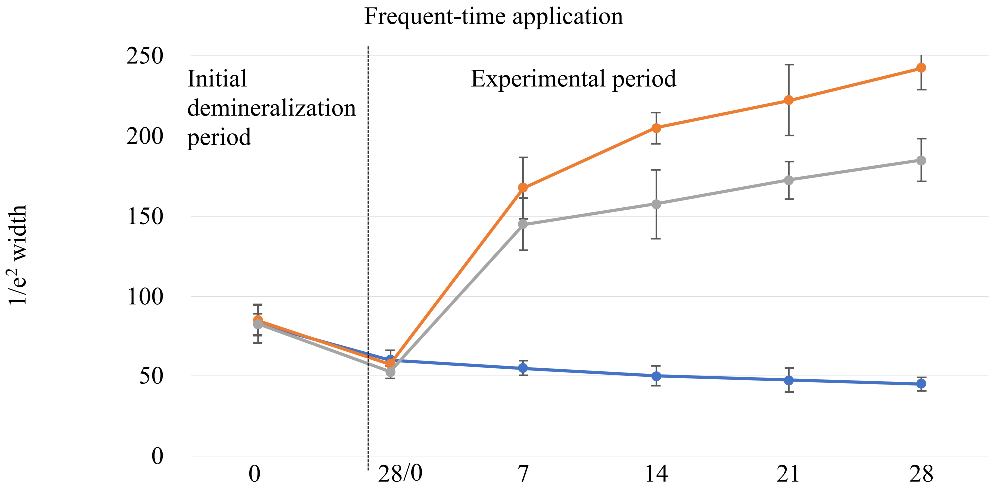
	0	28/0	7	14	21	28
Untreated	-75.1 aA	-43.3 bA	-42.9 bcA	-38.6 bcdA	-36.3 cdA	-34.4 dA
SDF treated	-71.9 aA	-41.6 bA	-75.1 aB	-79.8 acB	-86.0 cdB	-90.9 dB
PRG treated	-75.5 acA	-41.7 bA	-69.5 cB	-74.8 acB	-79.5 aC	-81.1 aC

Unit: dB, n = 10 teeth per group.
Within groups, means with the same lower-case letter are not significantly different ($p > 0.05$).
Between groups at the same periods, means with the same upper-case letter are not significantly different ($p > 0.05$).

Fig. 2 Changes in the maximum peak intensity of different treatment groups.



	0	28/0	7	14	21	28
Untreated	82.5 aA	60.0 bA	55.0 bcA	50.0 bcA	47.5 cA	45.0 cA
SDF treated	87.5 aA	60.0 bA	147.5 cB	135.0 cdB	112.5 adB	102.5 aB
PRG treated	90.0 aA	57.5 bA	120.0 cC	102.5 aC	95.0 aB	90.0 aB

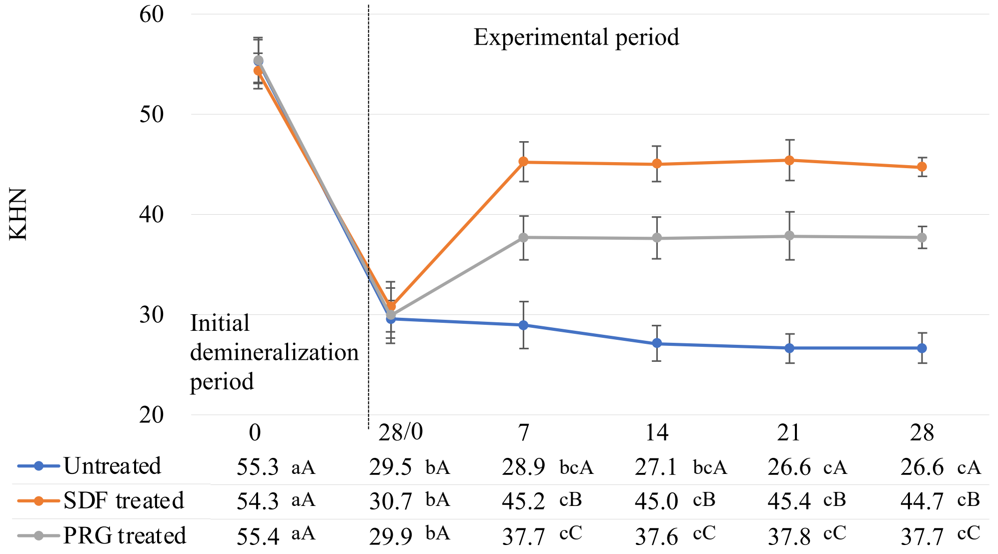


	0	28/0	7	14	21	28
Untreated	82.5 aA	60.0 bA	55.0 bcA	50.0 bcA	47.5 cA	45.0 cA
SDF treated	85.0 aA	57.5 bA	167.5 cB	205.0 dB	222.5 deB	242.5 eB
PRG treated	82.5 aA	52.5 bA	145.0 cB	157.5 cdC	172.5 deC	185.0 eC

Unit: µm, n = 10 teeth per group.
Within groups, means with the same lower-case letter are not significantly different ($p > 0.05$).
Between groups at the same periods, means with the same upper-case letter are not significantly different ($p > 0.05$).

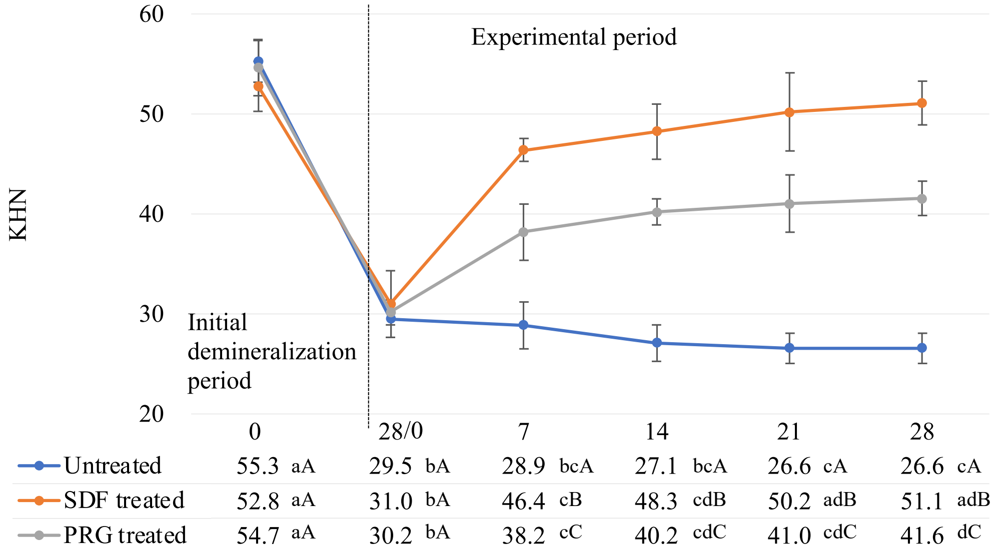
Fig. 3 Changes in the 1/e^2 width of different treatment groups.

One-off application



	0	28/0	7	14	21	28
Untreated	55.3 aA	29.5 bA	28.9 bcA	27.1 bcA	26.6 cA	26.6 cA
SDF treated	54.3 aA	30.7 bA	45.2 cB	45.0 cB	45.4 cB	44.7 cB
PRG treated	55.4 aA	29.9 bA	37.7 cC	37.6 cC	37.8 cC	37.7 cC

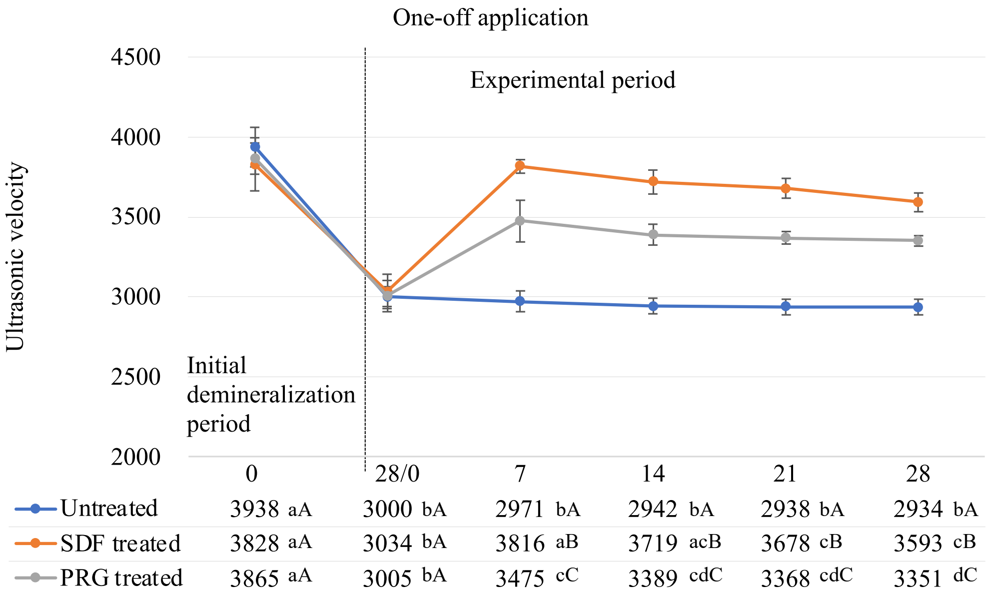
Frequent-time application



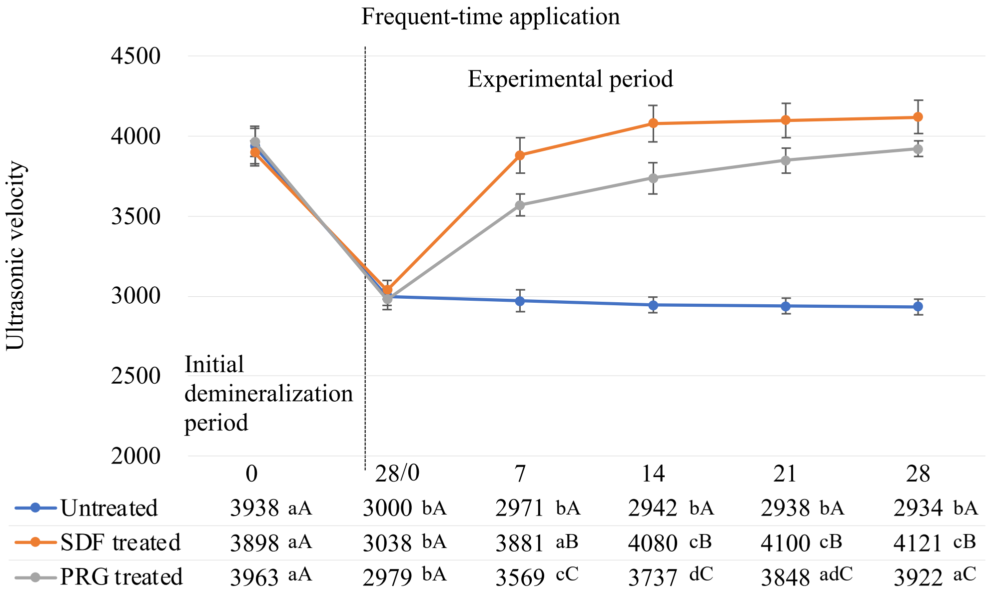
	0	28/0	7	14	21	28
Untreated	55.3 aA	29.5 bA	28.9 bcA	27.1 bcA	26.6 cA	26.6 cA
SDF treated	52.8 aA	31.0 bA	46.4 cB	48.3 cdB	50.2 adB	51.1 adB
PRG treated	54.7 aA	30.2 bA	38.2 cC	40.2 cdC	41.0 cdC	41.6 dC

Unit: KHN, n = 10 teeth per group.
Within groups, means with the same lower-case letter are not significantly different ($p > 0.05$).
Between groups at the same periods, means with the same upper-case letter are not significantly different ($p > 0.05$).

Fig. 4 Changes in the KHN of different treatment groups.



	0	28/0	7	14	21	28
Untreated	3938 aA	3000 bA	2971 bA	2942 bA	2938 bA	2934 bA
SDF treated	3828 aA	3034 bA	3816 aB	3719 acB	3678 cB	3593 cB
PRG treated	3865 aA	3005 bA	3475 cC	3389 cdC	3368 cdC	3351 dC



	0	28/0	7	14	21	28
Untreated	3938 aA	3000 bA	2971 bA	2942 bA	2938 bA	2934 bA
SDF treated	3898 aA	3038 bA	3881 aB	4080 cB	4100 cB	4121 cB
PRG treated	3963 aA	2979 bA	3569 cC	3737 dC	3848 adC	3922 aC

Unit: m/s, n = 10 teeth per group.
Within groups, means with the same lower-case letter are not significantly different ($p > 0.05$).
Between groups at the same periods, means with the same upper-case letter are not significantly different ($p > 0.05$).

Fig. 5 Changes in the ultrasonic velocity of different treatment groups.

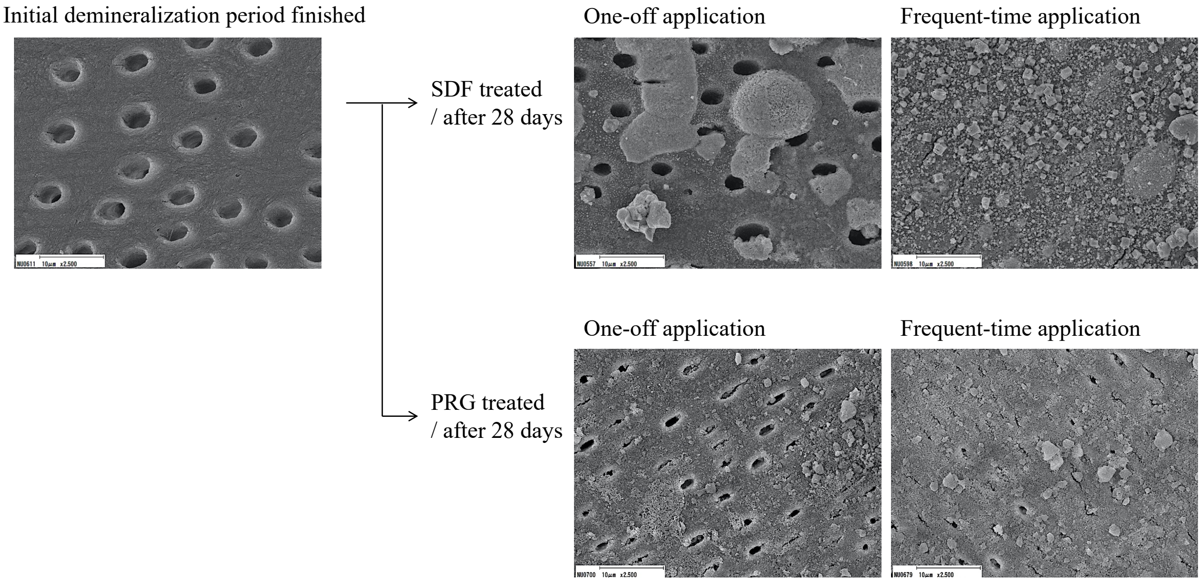


Fig. 6 Representative SEM images of the dentin surface of each group (2,500×). After initial demineralization, dentinal tubules opening were clearly observed. After 28 days of experimental period, morphological changes on the dentin surfaces were noticeably observed in the frequent-time application groups when compared with those in one-off application groups. For the frequent treatment of SDF and PRG treated groups, dentinal tubules were occluded and some of the precipitations were clearly observed.