

Original paper

Effects of desensitizer containing fluoroaluminocalciumsilicate glass nanoparticles on remineralization of root dentin subsurface lesions *in vitro*

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ABSTRACT

We investigated the remineralization effects of Nanoseal (NS) dentin desensitizer on demineralized root dentin. Baseline lesion specimens prepared from bovine root dentin were immersed in artificial saliva (AS) or deionized water (DW) after treatment with NS or fluoride-free Nanoseal (NS(-)). Treatment and control groups comprised: 1, AS; 2, NS/AS; 3, NS(-)/AS; 4, NS/DW; 5, NS(-)/DW; and 6, baseline demineralization. Integrated mineral loss (IML) and lesion depth (LD) were determined by transverse microradiography. Fluoride concentrations in the immersion solutions were measured. AS, NS/AS and NS(-)/AS showed higher mineral volume % at the surface and lesion body than did other groups. NS/AS showed significantly lower IML than did AS. There was no significant difference in IML between NS/AS and NS(-)/AS. The highest concentration of fluoride was in the NS/AS immersion solution. The findings suggest Nanoseal facilitated remineralization of demineralized root dentin, and fluoride and other ions included may have contributed to this effect.

INTRODUCTION

Dentin hypersensitivity is a common clinical condition characterized by transient pain extending from the cervix to the root surface when stimulated by thermal, mechanical or chemical stimulation by, for example, cold water, brushing, and fruit juice. Dentin

hypersensitivity may be induced by excessive or inappropriate teeth brushing. It was reported that the progression of non-carious cervical lesion (NCCLs) can adversely affect tooth sensitivity, plaque retention, caries incidence, structural integrity, and pulpal vitality¹⁾. Wada²⁾ reported that dentin demineralization was observed in 69% of NCCLs of extracted human teeth by using swept-source optical coherence tomography (SS-OCT). While such demineralization includes both bacterial and non-bacterial agents, desensitizers with remineralization ability would be useful. Although anti-demineralization effects of desensitizers have been studied³⁻⁵⁾, no study has reported the remineralizing effects of desensitizers using transverse microradiography (TMR) in pre-demineralized root dentin. TMR, which reveals precise levels of mineral loss, is widely recognized as the gold standard method in demineralization and remineralization studies of both enamel and dentin⁶⁻⁸⁾.

Desensitizers of various types and mechanisms have been developed, especially occlusion of dentinal tubules with microcrystals⁹⁻¹¹⁾ or bonding resin¹²⁻¹⁴⁾. Nanoseal (Nippon Shika Yakuhin, Yamaguchi, Japan), which was developed as a desensitizer, consists of two liquids. The “A” liquid is an aqueous dispersion of fluoroaluminocalciumsilicate glass nanoparticles, and the “B” liquid is 10% phosphoric acid. By mixing these liquids, fluoroaluminocalciumsilicate glass particles begin to aggregate by conventional acid-base reaction. The aggregates occlude the dentinal tubules like a type of silicate cement filling¹⁵⁾. Simultaneously, acidic aggregates demineralize the dentin surface and form an insoluble

mineral layer containing calcium fluoride, calcium phosphate, phosphor-silicate compounds, and calcium-fluoroaluminosilicate^{3,16}).

Previous studies have reported dentinal tubule occlusion, inhibition of demineralization, and reduction of sensitivity by Nanoseal^{3,16,17}). Nanoseal inhibited demineralization of specimens treated by NS followed by a longer period of immersion in artificial saliva than did a calcium-phosphate desensitizer and an NaF resin varnish¹⁸). The remineralization potential of Nanoseal on enamel subsurface lesions has been reported¹⁹), however, there has been no investigation of this material concerning dentin remineralization. It is important to not only inhibit demineralization but to remineralize demineralized dentin.

This study investigated the remineralization effects of Nanoseal on demineralized dentin *in vitro*. The null hypothesis was that Nanoseal could not facilitate root dentin remineralization.

MATERIALS AND METHODS

Preparation of dentin specimens

Eighteen bovine incisors were obtained from a slaughterhouse and the periodontal ligaments and other soft tissues were removed. Their crowns were separated at the cementum-enamel junction (CEJ). Their roots were horizontally sectioned about 5 mm below the CEJ, then vertically sectioned into two halves (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) to produce 36 specimens. A flat experimental surface was made by

cutting the root surface with a diamond-coated wire sectioning machine (Well type 3242; Walter Ebner, Mannheim, Germany) at 3 mm from the pulp chamber. The surface of the specimens was polished with 2000-grade water resistant paper (Fuji star DCCS, Sankyo Rikagaku, Saitama, Japan), washed with deionized water, then cleaned ultrasonically (US-2R US Cleaner, AS ONE, Osaka, Japan) in deionized water at 10°C for 5 min. The 36 specimens were randomly allocated into six groups and fixed to the bottom of six plastic containers with dental wax (New Sticky Wax, GC, Tokyo, Japan). The entire surface, except a 2×3 mm experimental window, was coated with acid-resistant varnish. The study protocol is shown in Figure 1.

Preparation of subsurface demineralization lesions

To make a root subsurface lesion model, demineralization of all specimens was performed at 37°C for 10 days using the acetic acid two-layer method (24 ml lower layer: 8% Methocel MC gel (Fluka, Buchs, Switzerland); 36 ml upper layer: 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 50 mM acetic acid, 0.2 ppm F, pH 5.0)²⁰⁻²⁴). After demineralization, using a 3-way syringe that supplied a focused stream of compressed air and water, each specimen was washed for approx. 10 s to remove the gel, then was washed for a further 10 s with deionized water. The specimens were then assigned into experimental treatment and control groups.

Experimental treatment and control groups

Experimental treatment and control groups comprised: 1, artificial saliva immersion (AS); 2, AS following Nanoseal treatment (NS/AS); 3, AS following fluoride-free Nanoseal treatment (NS(-)/AS); 4, deionized water immersion (DW) following Nanoseal treatment (NS/DW); 5, DW following fluoride-free Nanoseal treatment (NS(-)/DW); and 6, a baseline demineralization group (Dem) that was not treated or immersed.

Nanoseal or fluoride-free Nanoseal (Nippon Shika Yakuhin, Yamaguchi, Japan) was applied to the demineralized dentin surface three times every 20 s with a microbrush, then washed with deionized water for 5 s after 1 min. in adherence with the manufacturer's instructions which say Nanoseal should be used within 1 min of its preparation.

Artificial saliva or deionized water immersion

The coated specimens were immersed in AS (1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 130 mM KCl, 20 mM Hepes, Casein 100 µg/ml, 0.02% NaN₃, pH 6.8, 37°C)^{18,21, 25-27} or DW for 14 days with their solution changed after 7 days. Sixty ml of solution was added to each container housing six samples.

Transverse microradiography (TMR) analysis

After the 14 days of immersion, two, 300-µm thick sections were cut perpendicularly to the

experimental surface from each specimen using a diamond-coated wire sectioning machine. Each section was placed between thin, hermetically sealed polyester sheets with 13 layers of aluminum step wedges and a droplet of water to prevent shrinkage as described²⁸). Then, each section was radiographed using a high-resolution glass plate (Konica Minolta, Tokyo, Japan), and an X-ray device (PW3830, Spectris, UK) using Cu as a radiation source and Ni as a filter (15 mA, 35 kV, 15 min). Development, fixing, washing with water and drying were performed as conventional. The X-ray image of each section was analyzed using a microscope/ video camera/ microcomputer and TMR analysis software (TMR2006, 2012, Inspektor, The Netherlands). A scan of each section was performed at the center of the lesion, and values were calculated for three parameters: mineral content profile, integrated mineral loss (IML: vol% $\times\mu\text{m}$), and lesion depth (LD: μm). The values of two sections from each specimen were averaged^{4,29,30}).

Fluoride release

Each immersion solution was recovered at day 7 and 14 to measure released fluoride. 0.3 ml of sodium acetate buffer (0.1 mol/l, pH 5.1) was added to 3 mL of each eluate. Fluoride concentrations were measured with a combination fluoride electrode (Orion 9609BNWP ionplus Sure-Flow Fluoride, Thermo Fisher Scientific, Waltham, MA, USA) connected to a fluoride-ion meter (720Aplus, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

To compare IML and LD among groups, statistical software (IBM SPSS Statistics version 21, Tokyo, Japan) was used to perform one-way ANOVA and Tukey's multiple comparison tests and differences with $p < 0.05$ were deemed significant. Fluoride concentration measurements were not statistically analyzed because only one solution was collected from each group.

RESULTS

Representative TMR images

Figure 2 shows representative TMR images for each group. Dem showed typical subsurface demineralization lesions. NS/DW and NS(-)/DW showed lesions similar to those of Dem. In contrast, the radiopacity of lesions in AS, NS/AS and NS(-)/AS increased, further NS/AS showed markedly narrower demineralization zones than those of AS and NS(-)/AS.

Averaged mineral profile and IML, LD

Figure 3 shows averaged mineral profiles appeared to fall into two groups: Dem, NS / DW and NS (-) / DW group; and AS, NS / AS and NS (-) / AS group. The former group had slight surface layers of about 30 mineral vol% and severe lesion bodies of less than 20 mineral

vol%. In contrast, the latter group had surface layers exceeding 40 mineral vol% and lesion bodies exceeding 30 mineral vol%. Table 2 shows the IML and LD of each group. The IML of NS/AS (721.4 vol% $\times\mu\text{m}$) was the lowest among the groups ($p<0.05$). AS showed lower IML (1,212.2) than did Dem (2,575.8) ($p<0.05$), but higher than that of NS/AS ($p<0.05$). The IML of NS/DW and NS(-)/DW (2,861.1, 2,663.9) did not statistically differ from those of Dem ($p>0.05$). The LD of AS, NS/AS and NS(-)/AS (103.3, 87.7, 94.0 μm) were significantly lower than those of Dem, NS/DW and NS(-)/DW (133.9, 152.4, 152.6) ($p<0.05$).

Fluoride release

Table 3 shows the concentration of released fluoride in each group. The highest value was found in NS/AS immersion solution at day 7. Solutions recovered from other groups at day 7 and all groups at day 14 showed lower values than the detection limit (0.02 ppm F).

DISCUSSION

Hypersensitivity was previously found in 40% of non-carious cervical lesions (NCCLs) and demineralization was found in 69% of NCCLs by using swept-source optical coherence tomography¹). Those findings indicated that cervical hypersensitive areas are associated with a high prevalence of dentin demineralization. Therefore, it is important to not only inhibit

demineralization but promote remineralization to treat dentin hypersensitivity. The present study investigated the remineralization effects of Nanoseal on pre-demineralized dentin *in vitro*.

It is widely known that fluoride treatment is effective in prevention of root caries³¹⁾. In one clinical trial, Nyvad reported that active root caries were rendered inactive by normal daily brushing with fluoride toothpaste³²⁾. Also, Sudjalim³³⁾ reported that 9,000 ppm F toothpaste effectively prevented demineralization around orthodontic brackets. High-concentration ionic fluoride acts as a fluoride reservoir on the tooth surface to cause the deposition of calcium fluoride, releasing fluoride over a long period due to its slow dissolution³⁴⁾. Other studies have reported the facilitating effects of fluoride on remineralization of dentin^{7,8, 35)}. Using an *in situ* model, it was shown - in comparison with non-treatment - that daily plaque removal and application of 2% NaF, inhibited the progression of demineralization and facilitated partial remineralization of dentinal lesions³⁶⁾. Fluoride present in solution at the crystal surface during a pH rise following demineralization can combine with dissolved calcium and phosphate ions to precipitate or grow fluorapatite-like crystalline material within the tooth. Fluoride enhances mineral gain (remineralization) and provides a material which is more resistant to subsequent acid attack³¹⁾. The main component of Nanoseal is fluoroaluminocalciumsilicate glass nanoparticles. To elucidate the degree to which fluoride enhances remineralization, we included an NS(-)/AS group. Nanoseal showed least mineral

loss among the groups and enabled less mineral loss compared with fluoride-free Nanoseal after remineralization, however there was no significant difference between these groups. These results indicate the fluoride ions may contribute to remineralization, but other ions included are also involved.

Artificial saliva is a remineralizing solution containing calcium ions and phosphate ions, which has been used in several experiments^{18, 21, 25-27}). In this study, a solution containing casein was used. It was reported that the protein-free remineralization solution causes excessive mineral deposition on the surface and leaves less mineral recovery in the lesion body³⁷). By adding casein to the remineralization solution, remineralization of the lesion is improved²⁷). The influence of artificial saliva on remineralization is significant, but without artificial saliva, one cannot simulate an oral environment. In this study, it was considered that Nanoseal had a remineralization-promoting effect, as samples treated with Nanoseal showed significantly higher remineralization than those without.

It was reported that silica and hydroxyapatite nanoparticles infiltrated into demineralized dentin, acting as seeds within the collagen matrix³⁸). Silicate ions at a concentration as low as 5 μM played a significant role in initiating mineral phase in dentin³⁹).

Although the two experimental groups, NS/DW and NS(-)/DW groups, are situations that do not occur in the actual oral cavity, we included them to clarify whether decrease of IML was due to remineralization and not simple invasion of silica or aluminum. Our results

showed that NS/DW groups in which deionized water was used in substitution for artificial saliva did not show remineralization, however NS(-)/AS slightly enhanced remineralization. This means the increase of radiopacity of the NS/AS groups on TMR was not simply due to infiltration of the nanoparticles, although silica might be capable of nucleating for dentin remineralization.

NS/DW and NS(-)/DW groups showed higher IML and LD than did Dem group (albeit not significantly). In NS, immediately after mixing, the pH is 2.0 to 2.5 whereas at 1 min after mixing it is 3.5 to 4.0. NS(-) is about the same. The findings suggest that: demineralization occurred by applying acidic NS and NS(-); or the treated dentin minerals dissolved in DW due to increased solubility; or a combination of both. To determine which occurred, measuring IML just after applying NS and NS(-) to Dem specimens should be performed in a later study.

LD of AS, NS/AS and NS(-)/AS were significantly less than those of other groups ($p < 0.05$), however there was no significant difference among these three groups. Unlike remineralization of enamel, remineralization of dentin is less likely to change the depth of demineralized lesions, and minerals are acquired from the lowest mineral part of the lesion body⁴⁰.

In summary, our null hypothesis was rejected, i.e., Nanoseal may promote remineralization. However, this study was an *in vitro* investigation of what components affect remineralization.

The TMR profiles, revealed presence of lesions so it cannot be said that the remineralization was complete. In the future, we plan to investigate the progress of remineralization under conditions closer to an actual oral cavity, including the number of applications, the amount of AS per sample, and the use of human extracted teeth.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that Nanoseal facilitated remineralization of demineralized root dentin, and fluoride and other ions included may have contributed to this effect.

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REFERENCES

- 1) Aw TC, Lepe X, Johnson GH, Mancl L. Characteristics of noncarious cervical lesions: a clinical investigation. J Am Dent Assoc 2002; 133: 725-733.

- 2) Wada I, Shimada Y, Ikeda M, Sadr A, Nakashima S, Tagami J, et al. Clinical assessment of non carious cervical lesion using swept-source optical coherence tomography. *J Biophotonics* 2015; 8: 846-854.
- 3) Miyajima H, Ishimoto T, Ma S, Chen J, Nakano T, Imazato S. In vitro assessment of a calcium-fluoroaluminosilicate glass-based desensitizer for the prevention of root surface demineralization. *Dent Mater J* 2016; 35: 399-407.
- 4) Kawamura K, Kunimatsu Y, Nakano T, Hasegawa H, Arakawa H, Mukai Y. Anti-demineralization effect of desensitizer containing copolymer and sodium fluoride on root dentin – a transverse microradiographic study. *Acta Biomater Odontol Scand* 2019; 5: 38-43.
- 5) Saad A, Nikaido T, Abdou A, Matin K, Burrow MF, Tagami J. Inhibitory effect of zinc-containing desensitizer on bacterial biofilm formation and root dentin demineralization. *Dent Mater J* 2019; 38: 940-946.
- 6) Lagerweij MD, de Josselin de Jong E, ten Cate JM. The video camera compared with the densitometer as a scanning device for microradiography. *Caries Res* 1994; 28: 353-362.
- 7) Mukai Y, Lagerweij MD, ten Cate JM. Effect of a solution with high fluoride concentration on remineralization of shallow and deep root surface caries in vitro. *Caries Res* 2001; 35: 317-324.

- 8) Mukai Y, ten Cate JM. Remineralization of advanced root dentin lesions in vitro. *Caries Res* 2002; 36: 275-280.
- 9) Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Suh BI. Integrating oxalate desensitizers with total-etch two-step adhesive. *J Dent Res* 2003; 82: 703-707.
- 10) Geizer S, Matalon S, Blasbalg J, Tung M, Eichmiller FC. The clinical effect of amorphous calcium phosphate (ACP) on root surface hypersensitivity. *Oper Dent* 2003; 28: 496-500.
- 11) Frechoso SC, Menéndez M, Guisasola C, Arregui I, Tejerina JM, Sicilia A. Evaluation of the efficacy of two potassium nitrate bioadhesive gels (5% and 10%) in the treatment of dentine hypersensitivity. A randomised clinical trial. *J Clin Periodontol* 2003; 30: 315-320.
- 12) Dondi dall'Orologio G, Lone A, Finger WJ. Clinical evaluation of the role of glutardialdehyde in a one-bottle adhesive. *Am J Dent* 2002; 15: 330-334.
- 13) Momoi Y, Akimoto N, Kida K, Yip KH, Kohno A. Sealing ability of dentin coating using adhesive resin systems. *Am J Dent* 2003; 16: 105-111.
- 14) Baysan A, Lynch E. Treatment of cervical sensitivity with a root sealant. *Am J Dent* 2003; 16: 135-138.
- 15) Wilson AD, Batchelor RF. Dental silicate cements. II. Preparation and durability. *J Dent Res* 1967; 46: 1425-1432.

- 16) Han L, Okiji T. Effects of a novel fluoride-containing aluminocalciumsilicate-based tooth coating material (Nanoseal) on enamel and dentin. *Am J Dent* 2013; 26: 191-195.
- 17) Miyaji H, Kato A, Tanaka S. Suppression of root caries progression by application of Nanoseal®: A single-blind randomized clinical trial. *Dent Mater J* 2020; 22: doi: 10.4012/dmj.2019-038.
- 18) Lodha E, Hamba H, Nakashima S, Sadr A, Nikaido T, Tagami J. Effect of different desensitizers on inhibition of bovine dentin demineralization: micro-computed tomography assessment. *Eur J Oral Sci* 2014; 122: 404-410.
- 19) Hamba H, Nakamura K, Nikaido T, Furusawa M, Tagami J, Muramatsu T. Remineralization potential of a calcium-fluoroaluminosilicate glass-based desensitizer on artificial human enamel subsurface lesions. *J J Conserv Dent* 2020; 63: 156-164.
- 20) Ingram GS, Silverstone LM. A chemical and histological study of artificial caries in human dental enamel in vitro. *Caries Res* 1981; 15: 393-398.
- 21) Hoppenbrouwers PM, Driessens FC, Borggreven JM. The mineral solubility of human tooth roots. *Arch Oral Biol* 1987; 32: 319-322.
- 22) Nyvad B, ten Cate JM, Fejerskov O. Microradiography of experimental root surface caries in man. *Caries Res* 1989; 23: 218-223.
- 23) Tatevossian A. *J Dent Res* 1990 Feb;69 Spec No:645-652; discussion 682-683.
- 24) Shiiya T, Mukai Y, Tomiyama K, Teranaka T. Anti-demineralization effect of a novel

- fluoride-releasing varnish on dentin. *Am J Dent* 2012; 25: 347-350.
- 25) Kuramochi E, Iizuka J, Mukai Y. Influences of bicarbonate on processes of enamel subsurface remineralization and demineralization: assessment using micro-Raman spectroscopy and transverse microradiography. *Eur J Oral Sci* 2016; 124: 554-558.
- 26) ten Cate JM, Timmer K, Shariati M, Featherstone JD. Effect of timing of fluoride treatment on enamel de- and remineralization in vitro: a pH-cycling study. *Caries Res* 1988; 22: 20-26.
- 27) Romero MJ, Nakashima S, Nikaido T, Ichinose S, Sadr A, Tagami J. Inhibition of hydroxyapatite growth by casein, a potential salivary phosphoprotein homologue. *Eur J Oral Sci* 2015; 123: 288-296.
- 28) van Strijp AJP, Buijs MJ, ten Cate JM. Contact microradiography of dentine under wet conditions to protect lesion shrinkage. *Caries Res* 1995; 29: 107-110.
- 29) Mukai Y, Kamijo K, Fujino F, Hirata Y, Teranaka T, ten Cate JM. Effect of denture base-resin with prereacted glass-ionomer filler on dentin demineralization. *Eur J Oral Sci* 2009; 117: 750-754.
- 30) Shiiya T, Tomiyama K, Iizuka J, Hasegawa H, Kuramochi E, Fujino F, et al. Effects of resin-based temporary filling materials against dentin demineralization. *Dent Mater J* 2016; 35: 70-75.
- 31) ten Cate JM, Featherstone JDB. In: Fejerskov O, Ekstrand J, Burt BA, editors. Fluoride

- in Dentistry. 2nd ed. Munksgaard, Copenhagen, 1996, p 252-272.
- 32) Nyvad B, Fejerskov O. Active root surface caries converted into inactive caries as a response to oral hygiene. *Scand J Dent Res* 1986; 94: 281-284.
- 33) Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. *Am J Orthod Dentofacial Orthop* 2007; 131: 705. E1-9.
- 34) ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. *Eur J Oral Sci* 1997; 105: 461-465.
- 35) Arends J, Ruben JL, Christoffersen J, Jongebloed WL, Zuidgeest TG. Remineralization of human dentine in vitro. *Caries Res* 1990; 24: 432-435.
- 36) Nyvad B, ten Cate JM, Fejerskov O. Arrest of root surface caries in situ. *J Dent Res* 1997; 76: 1845-1853.
- 37) Fujikawa H, Matsuyama K, Uchiyama A, Nakashima S, Ujiie T. Influence of salivary macromolecules and fluoride on enamel lesion remineralization in vitro. *Caries Res* 2008; 42: 37-45.
- 38) Besinis A, van Noort R, Martin N. Infiltration of demineralized dentin with silica and hydroxyapatite nanoparticles. *Dent Mater* 2012; 28: 1012-1023.
- 39) Saito T, Toyooka H, Ito S, Crenshaw MA. In vitro study of remineralization of dentin: effects of ions on mineral induction by decalcified dentin matrix. *Caries Res* 2003; 37: 445-449.

- 40) Arends J. Mechanism of remineralization of enamel and dentine lesions. J Dent Hlth
1993; 43: 384-389.

Figure captions

Fig.1 Study protocol

Fig. 2 Representative TMR images of each group.

Fig. 3 Average mineral profiles of each group.

Fig.1

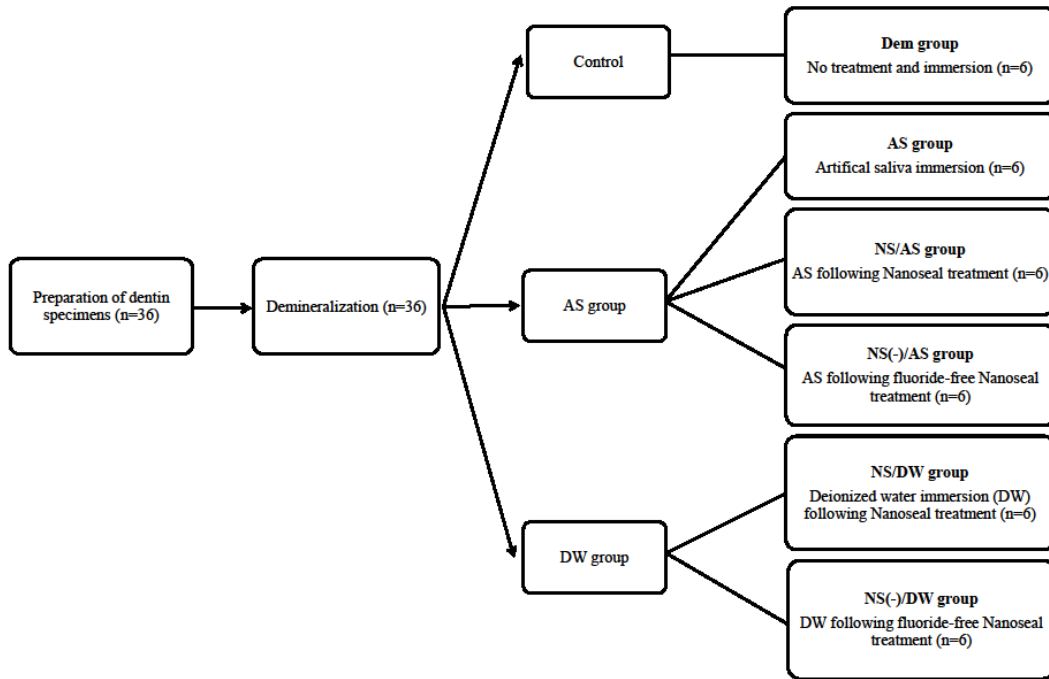


Fig.2

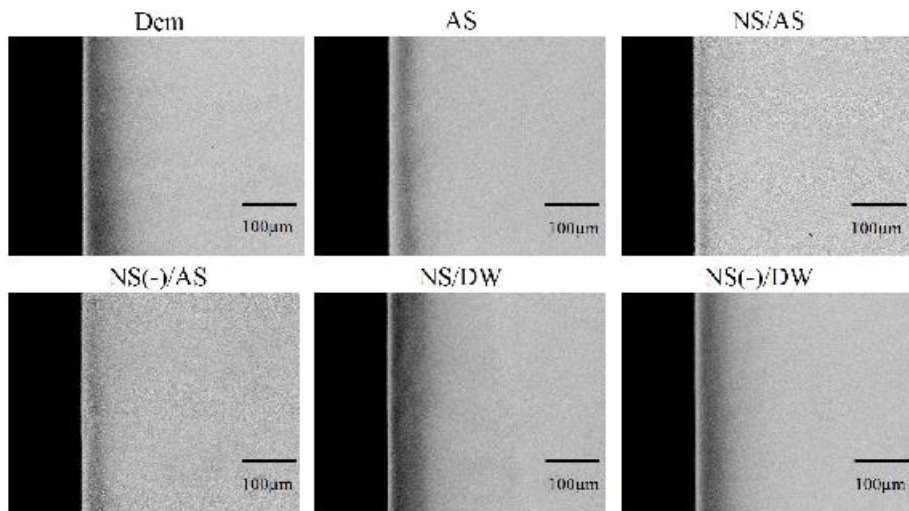


Fig.3

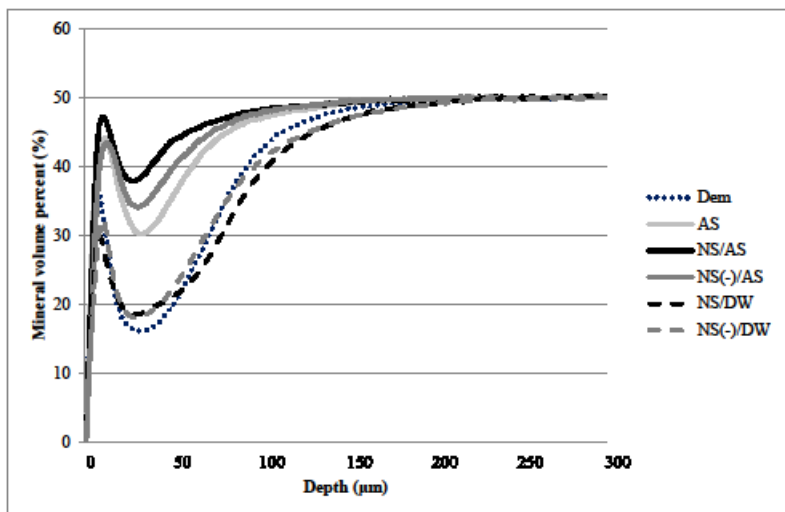


Table 1 Materials used

Materials	Abbreviations	Ingredients	Lot number	Manufacturer
Nanoseal	NS	Solution A: fluoroaluminocalciumsilicate glass	F48	Nippon Shika Yakuhin
		Solution B: 10 % phosphoric acid	F4C	
Nanoseal (fluoride free)	NS (-)	Solution A: aluminocalciumsilicate glass	—	Nippon Shika Yakuhin
		Solution B: 10 % phosphoric acid	—	

Table 2 Integrated mineral loss (IML) and lesion depth (LD)

Groups	IML (vol%×μm)	LD (μm)
Dem	2,575.8 (192.0) ^a	133.9 (7.7) ^a
AS	1,212.2 (177.6) ^b	103.3 (8.5) ^b
NS/AS	721.4 (170.9) ^c	87.7 (10.1) ^b
NS(-)/AS	991.1 (263.3) ^{b, c}	94.0 (6.1) ^b
NS/DW	2,861.1 (245.5) ^a	152.4 (14.8) ^a
NS(-)/DW	2,663.9 (198.3) ^a	152.6 (16.5) ^a

Mean (±SD), *n*=6

Values with the same superscript letters did not show significant differences between groups.

Dem: demineralization, AS: artificial saliva (remineralization solution), DW: deionized water

Table 3 Fluoride concentrations (ppm F)

Groups	Day 7	Day 14
NS/AS	0.024	n.d
NS(-)/AS	n.d	n.d
NS/DW	n.d	n.d
NS(-)/DW	n.d	n.d

n.d : not detectable