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

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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.

Keywords: Remineralization, Desensitizer, Fluoride, Root dentin, Transverse microradiography

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 vitality15. Wada et al.2 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 studied15-17, no study has reported the remineralization 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 microcrystals6-13) 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-fluoroaluminosilicate8-16).

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 Fig. 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) (Table 1) 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 milliliters of solution was added to each container housing six samples.

TMR analysis
After the 14 days of immersion, two, 300-µm thick

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**Table 1  Materials used**

| Materials          | Abbreviations | Ingredients                                      | Lot number | Manufacturer       |
|--------------------|---------------|-------------------------------------------------|------------|-------------------|
| Nanoseal           | NS            | Solution A: fluoroaluminocalciumsilicate glass  | F48        | Nippon Shika      |
|                    |               | Solution B: 10 % phosphoric acid                | F4C        | Yakuhin           |
| Nanoseal (fluoride free) | NS (−)  | Solution A: aluminocalciumsilicate glass       | —          | Nippon Shika      |
|                    |               | Solution B: 10 % phosphoric acid                | —          | Yakuhin           |
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 described\(^ {29}\). Then, each section was radiographed using a high-resolution glass plate (Konica Minolta, Tokyo, Japan), and an X-ray device (PW3830, Spectris, Surrey, 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, Amsterdam, 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%×µm), 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).

**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. C 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%.

![Fig. 2 Representative TMR images of each group.](image1)

![Fig. 3 Average mineral profiles of each group.](image2)
Table 2: Integrated mineral loss (IML) and lesion depth (LD)

| Groups    | IML (vol%×µm) | LD (µm) |
|-----------|---------------|---------|
| Dem       | 2,575.8 (192.0)* | 133.9 (7.7)* |
| 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)* | 152.4 (14.8)* |
| NS(-)/DW  | 2,663.9 (198.3)* | 152.6 (16.5)* |

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

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 NCCLs and demineralization was found in 69% of NCCLs by using SS-OCT[1]. 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 caries[31]. In one clinical trial, Nyvad reported that active root caries were rendered inactive by normal daily brushing with fluoride toothpaste[32]. Also, Sudjalim et al.[33] 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 dissolution[34]. 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 lesions[36]. 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 attack[31]. 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\(^{1,2,3,4,5}\). 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 body\(^{27}\). By adding casein to the remineralization solution, remineralization of the lesion is improved\(^{27}\). 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 matrix\(^{38}\). Silicate ions at a concentration as low as 5 µM played a significant role in initiating mineral phase in dentin\(^{39}\).

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 body\(^{40}\).

In summary, our null hypothesis was rejected, i.e., Nanoseal may promote remineralization. However, this study was an \textit{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 \textit{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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