Preventive effect of experimental polymer-based desensitizers with NaF on demineralization of root dentin—observed using micro-CT

Sho OBAYASHI¹, Hidenori HAMBA², Keiki NAKAMURA², Toru NIKAIDO³ and Junji TAGAMI¹

¹Department of Cariology and Operative Dentistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Bunkyo-ku, Tokyo 113-8510, Japan
²Department of Operative Dentistry, Cariology and Pulp Biology, Tokyo Dental College, 2-9-18 Kanda-Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan
³Department of Operative Dentistry, Division of Oral Functional Science and Rehabilitation, School of Dentistry, Asahi University, 1851 Hozumi, Mizuho, Gifu 501-0296, Japan

Corresponding author, Hidenori HAMBA; E-mail: hamba.ope@gmail.com

This study compared the effect of experimental polymer-based desensitizers with NaF and oxalic acid (OA) for preventing root demineralization via observation using micro-CT. Bovine root dentin surfaces were treated with coating materials: no treatment; MS0(+ (MS Coat One®); MS3000(+ (MS Coat®); MS0(−); MS3000(−); MS7000(+/−); sodium fluoride (NaF9000); MS; MS polymer, 0–7000 ppmF sodium fluoride in aqueous solution, has recently been developed. Oshima et al. reported that MS Coat F has a significantly higher potential for reducing dentin demineralization than MS polymer, which does not contain NaF. It has been reported that high concentrations of fluoride are effective for preventing root demineralization. Ekstrand et al. reported that a 5,000 ppmF fluoride toothpaste was more effective for preventing the progression of root caries lesions compared to a 1,450 ppmF fluoridated toothpaste. With MS polymer, it was effective in preventing dentin demineralization at 3,000 ppmF. However, it is unknown whether it is effective with MS polymer at concentrations of fluoride higher than 3,000 ppmF.

MS coat and MS coat F also contain oxalic acid as a desensitizing agent. Oxalic containing desensitizers are established as dentin desensitizers and are widely accepted among practitioners. Oxalate aqueous solution has been demonstrated to be effective in reducing the permeability of dentin tubules via calcium oxalate deposition. However, the interactions between MS polymer, oxalic acid, and F ions are unknown.

Microfocus X-ray computed tomography (micro-CT) imaging can capture three-dimensional (3D) architectural information from samples. Micro-CT has been frequently used in experiments exploring mineral density and the structure of mineral tissues, like bones and teeth. The technique is also a promising method for assessing demineralization or remineralization in enamel or dentin.

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INTRODUCTION

Dental care and knowledge on preventing dental caries are improving for the world’s aging population, resulting in more retained teeth than in past generations. Moreover, the prevalence of gingival recessions increases with age. Thus, root surfaces are more frequently exposed to the oral environment, and consequently, the risk of developing root caries lesions is rising. It is understood that caries lesions develop more rapidly in dentin than in enamel because dentin requires a higher critical pH that is more frequently reached in surrounding dental bio-film. Furthermore, access to root caries lesions for restorative management is limited or requires extensive removal of sound dental hard tissues. Therefore, several non-invasive approaches to preventing the progression of root caries have been proposed.

Methacrylate-co-p-styrene sulfonic acid (MS polymer) is a water-soluble copolymer composed of a random copolymer with methyl methacrylate and styrene sulfonic acid. According to its manufacturer, when applied on the dentin surface, it can react with calcium ions from the smear layer, producing an insolubilized polymer-Ca complex that covers the exposed dentin surface. MS polymer-containing aqueous solutions have been reported to play a unique role as the dentin desensitizer. Previous studies show that the dentin desensitizer (MS Coat) containing MS polymer can effectively reduce dentin permeability and hypersensitivity.

A fluoride-containing polymer-based desensitizer (MS Coat F), which contains MS polymer and 3,000 ppmF sodium fluoride in aqueous solution, has recently been developed. Oshima et al. reported that MS Coat F has a significantly higher potential for reducing dentin demineralization than MS polymer, which does not contain NaF. It has been reported that high concentrations of fluoride are effective for preventing root demineralization. Ekstrand et al. reported that a 5,000 ppmF fluoridated toothpaste was more effective for preventing the progression of root caries lesions compared to a 1,450 ppmF fluoridated toothpaste. With MS polymer, it was effective in preventing dentin demineralization at 3,000 ppmF. However, it is unknown whether it is effective with MS polymer at concentrations of fluoride higher than 3,000 ppmF.

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Microfocus X-ray computed tomography (micro-CT) imaging can capture three-dimensional (3D) architectural information from samples. Micro-CT has been frequently used in experiments exploring mineral density and the structure of mineral tissues, like bones and teeth. The technique is also a promising method for assessing demineralization or remineralization in enamel or dentin.

Therefore, the purpose of this study is to evaluate
the effect of polymer-based desensitizers under different fluoride concentrations (0, 3,000, and 7,000 ppmF), as well as the presence or absence of oxalic acid, on the prevention of root demineralization using micro-CT. The null hypothesis of this study is that a polymer-based desensitizer with oxalic acid and 7,000 ppmF fluoride cannot inhibit dentin demineralization from an acid attack.

MATERIALS AND METHODS

Materials used in this study

The coating materials used in this study are listed in Table 1. Two commercially available dentin desensitizers: MS Coat One [MS0(+)] (Sun Medical, Shiga, Japan; US brand Pain-Free), containing both methacrylate-co-p-styren sulfonic acid polymer (MS) and oxalic acid (+); MS Coat F [MS3000(+)] (Sun Medical), containing 3,000 ppmF NaF (3000); and four experimental polymer-based desensitizers, MS0(−), MS3000(−), MS7000(−), and MS7000 (+) (Sun Medical). Fluor Jelly (Bee Brand, Osaka, Japan) containing 9,000 ppmF NaF was used as the positive control [NaF9000].

Table 1 Coating materials used in this study

| Group       | Brand name | Ingredients                                                                 | ppm F | Lot No  | Manufacturer                   | Application                              |
|-------------|------------|------------------------------------------------------------------------------|-------|---------|---------------------------------|------------------------------------------|
| MS0(−)      | Experimental | MS Polymer*, Water                                                           | 0     | 160617  |                                 |                                          |
| MS0(+)      | MS Coat One | MS Polymer*, Oxalic acid, Water                                              | 0     | MF1     |                                 |                                          |
| MS3000(−)   | Experimental | MS Polymer*, Water, Sodium fluoride                                          | 3,000 | 160617  |                                 |                                          |
| MS3000(+)   | MS Coat F   | MS Polymer*, Oxalic acid, Water, Sodium fluoride                            | 3,000 | 160617  | Sun Medical, Shiga, Japan       | Rub for 30 s, air blow for 10 s          |
| MS7000(−)   | Experimental | MS Polymer*, Water, Sodium fluoride                                          | 7,000 | 160617  |                                 |                                          |
| MS7000(+)   | Experimental | MS Polymer*, Oxalic acid, Water, Sodium fluoride                            | 7,000 | 160617  |                                 |                                          |
| NaF9000     | Fluor Jelly | Carmellose sodium, Saccharin sodium hydrate, Cetylpyridinium chloride hydrate, Phosphoric acid, etc. | 9,000 | 602 GD  | Bee Brand, Medico Dental, Osaka, Japan |                                          |

MS polymer: random copolymer of methyl methacrylate and styrene sulfonic acid

Specimen preparation

The specimen preparation procedure is schematically illustrated in Fig. 1. Eighteen bovines, stored frozen until the experiment, were thoroughly cleaned and

![Fig. 1 Schematic illustration of specimen preparation procedure.](image)
washed under running water to remove all adherent soft tissues. The bovine incisors were cut at the cement-enamel junction using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under running water. The dentin roots were embedded (EpoxiCure 2, Buehler), and the dentin root surfaces were ground flat using 600–1500 grit silicon carbide papers (Fuji Star, Sankyo Rikagaku, Saitama, Japan) under running water. The specimens were prepared by cutting into the dentin blocks (3×5×3 mm) using a low-speed diamond saw under water (with the water serving as a coolant). Each incisor was sectioned into 8 blocks to give a total of 144 specimens (n=18).

The specimens were treated with the coating materials listed in Table 1 for 30 s, and then air dried for 10 s with highly pressurized air from a dental air syringe. In the control specimens, the surface was left untreated. The surface of the specimens was then covered with nail varnish (CO11, Revlon, New York, NY, USA), with a 2×4 mm window left exposed on the polished dentin surface of each specimen.

The specimens were then immersed in 125 mL of a buffered demineralizing solution containing 2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄, and 50 mmol/L acetic acid adjusted at pH 4.5. They were removed from the solution after 10 h of demineralization, then rinsed and stored in distilled water for one day, after which micro-CT scanning was performed.

**Micro-CT scanning**

The mineral density values (MD) of the specimens were evaluated using an X-ray micro-CT system (InspeXio SMX-100CT, Shimadzu, Kyoto, Japan). The same scanning parameters used by Oshima et al. were applied to each specimen as follows: 100 kV voltage, 140 μA current, 400 s integration time, and 7.5 μm isotropic resolution. The specimens were rotated through 360° in 0.6° steps. The distance from the X-ray source to the specimen and from the X-ray source to the detector was 73.5 and 300.0 mm, respectively. A 0.2 mm thick brass filter was placed in the beam path to cut off low-energy X-rays to reduce any beam-hardening effects. An MD calibration curve was obtained by scanning three reference phantoms; two hydroxyapatite disks (0.60 and 0.80 gHAp cm⁻³) (Phantoms S/N-1001-39, Ratoc System Engineering, Tokyo, Japan) and one pure aluminum wire (0.9 mm in diameter, 1.55 gHAp cm⁻³). These phantoms were scanned using the same setup and parameters used for the tooth specimens. Scanning was performed under 100% humidity to avoid dehydration of the specimens, where the specimens were placed in a plastic vial tube with wet cotton.

For the micro-CT data analysis, a digital 3D object was reconstructed from 140 two-dimensional images (16-bit TIF) at a resolution of 1,024×1,024 pixels, using software (TRI/FCS-BON ver.10.01, DIF, TMD, Ratoc System Engineering). The computed tomography (CT) values were converted to MD (gHAp cm⁻³) using a calibration curve derived from a linear regression (R²>0.999) based on the phantom scans. The assessment was performed in a volume of interest (VOI) of 600×600×600 voxels at the center of the test window.

The MD values were calculated from the VOI at every 7.5 μm of depth, and then the MD profile was plotted in relation to lesion depth. The mean integrated mineral loss (ML) (vol%·μm) in each group was calculated from the MD profiles by subtracting the area under the curve for the treated dentin from that for the healthy dentin. The reference point of the depth axis (0 μm) in the mineral profile was set at the axial position of the healthy dentin surface at the baseline (Fig. 2). These calculations were performed by importing the MD data into a spreadsheet software package (Microsoft Excel 2016 for Windows, Microsoft, Redmond, WA, USA).

**Scanning electron microscope (SEM) observation**

The conditioned dentin surfaces of each group, the NaF9000 group, were observed under an SEM before and after demineralization. The specimens were prepared solely for SEM and the different specimens were used before and after demineralization. The specimens were left for 24 h on filter paper placed in a covered glass vial at room temperature to desiccate. After gold-sputter coating (SC-701AT, Elionix, Tokyo, Japan), the dentin surfaces were observed using an SEM (JSM-IT100, JEOL, Tokyo, Japan) at ×5,000 magnification (n=12). In addition to the treated surface, dentin structure was also observed cross-sectionally. For this purpose, prior to gold-sputter coating, the conditioned specimens were fractured longitudinally at the center using a cutting plier and examined through an SEM at ×5,000 magnification (n=12).

**Statistical analysis**

The calculated ML values in the micro-CT analysis were statistically analyzed using a Mann Whitney U test with Bonferroni correction. All statistical tests were performed with a computerized statistical program (SPSS for Windows 15.0, Chicago, IL, USA). The significance level for all tests was set at α=0.05.
RESULTS

**Micro-CT analysis**
A typical 2D image of the control group after demineralization was shown in Fig. 2. The mean ML values of each group are presented in Table 2. The Mann Whitney U test with Bonferroni correction reveals that the ML values are significantly different among the groups (p<0.05). The ML values of Control and MS0(−) were significantly higher than the other groups (p<0.05). However, there was no significant difference between Control and MS0(−) (p>0.05). MS0(+) and MS3000(−) indicated moderate ML values in the tested groups, in which the ML value of MS0(+) was significantly higher than that of MS3000(−) (p<0.05). The ML values of MS3000(+), MS7000(−), MS7000(+) and NaF9000 were significantly lower than the other groups (p<0.05). However, there were no differences in ML among the four groups (p>0.05).

**SEM observation**
The SEM micrographs of the conditioned dentin surfaces of each group, except for the the NaF9000 group, before and after demineralization are presented in Fig. 3. In the control group, the dentinal tubules were exposed before and after demineralization (Figs. 3a, b). For the MS0(−) group, the dentin surface was covered with a smooth surface structure and the dentinal tubules were not exposed before demineralization (Fig. 3c) and the structure was maintained even after demineralization (Fig. 4d). For the MS0(+) group, some crystals, probably MS polymer-calcium oxalate emulsions, and for the MS3000(+) group, some crystals, probably MS polymer-calcium oxalate-CaF$_2$ emulsions formed on the dentin surface (Figs. 3e, i). However, the roughness of the surface texture decreased, the dentinal tubules were sealed with the crystals after demineralization (Figs. 3f, j). For the MS3000(−) group and the MS7000(−)

| Group       | Mineral loss (vol%•µm) | Mean±SD   |
|-------------|------------------------|-----------|
| Control     | 19.0±2.4*              |           |
| MS0(−)      | 21.9±3.5c              |           |
| MS0(+)      | 17.4±3.5c              |           |
| MS3000(−)   | 13.2±2.4*              |           |
| MS3000(+)   | 10.3±3.3a,c,e,f        |           |
| MS7000(−)   | 9.5±4.0a,s,i           |           |
| MS7000(+)   | 7.3±3.1d,g,h           |           |
| NaF9000     | 7.8±2.0j,i             |           |

Similar lower case letters indicate that there is no significant difference amongst the values (p>0.05).
Fig. 4 Cross-sectional SEM micrographs of dentin specimens of each group, except for the NaF9000 group, before and after demineralization. (a) Control group before demineralization and (b) after demineralization. (c) MS0(−) group before demineralization and (d) after demineralization. (e) MS0(+) group before demineralization and (f) after demineralization. (g) MS3000(−) group before demineralization and (h) after demineralization. (i) MS3000(+) group before demineralization and (j) after demineralization. (k) MS7000(−) group before demineralization and (l) after demineralization. (m) MS7000(+) group before demineralization and (n) after demineralization.

group, small crystals, which seems to be MS polymer-calcium fluoride emulsions, were observed before demineralization (Figs. 3g, k). However, the crystal structure disappeared and the dentinal tubules were exposed after demineralization (Figs. 3h, l). For the MS7000(+) group, relatively large crystals comparable to the MS0(+), MS3000(+), MS7000(−) group were observed before demineralization, and dentinal tubules were not observed (Figs. 3e, j, k, m). Although the crystal structure disappeared after demineralization, dentinal tubules were not clearly visible and the surface seems to have been covered with a membrane-like structure (Fig. 3n).

The cross-sectional SEM images of the conditioned specimens of each group, except for the the NaF9000 group, before and after demineralization, are shown in Fig. 4, supporting the SEM observations presented in Fig. 3.

In the control group, before and after demineralization, the dentinal tubules were open and not occluded (Figs. 4a, b). For the MS0(−) group, the dentin surface was covered with MS polymer before demineralization (Fig. 4c) that is retained even after demineralization (Fig. 4d). For the MS0(+) group and the MS3000(+) group, the dentin tubules were occluded with some crystals before demineralization (Figs. 4e, i) and the crystals are maintained after demineralization (Figs. 4f, j). For the MS3000(−) group and the MS7000(−) group, before and after demineralization, the dentinal tubules were not occluded (Figs. 4g, h, k, l). For the MS7000(+) group, before demineralization, the dentinal tubules were occluded, and the surface was thickly covered (Fig. 4m). After demineralization, occlusion in the tubules is maintained, and the surface was covered with a membrane-like structure (Fig. 4n).

DISCUSSION

Micro-CT is a powerful tool for obtaining 3D information on hard tissues, allowing dynamic detection of mineral change after demineralization and remineralization of enamel and dentin. Micro-CT can be used as a nondestructive alternative to the gold standard method of transverse microradiography, with comparable parameters for the study of mineral content and lesion depth23). Furthermore, its accuracy can be increased by reducing beam-hardening effects and using appropriate mineral density calibration curves21).

In this study, the test materials, MS0(+) and MS3000(+), were dentin desensitizers and based on the application method, they were applied only once. The conditioned specimens were then subjected to demineralization immediately after the application of the materials. There are similarities between acetate buffer generated lesions and natural root caries lesions, so we conducted under similar condition27). Also, in this experiment initial demineralization on the root surface is assumed, so a relatively short time of demineralization was set. In a preceding pilot study, a demineralization time of 10 h was found to be optimal for detecting the
effects of the test materials using micro-CT. This informed the relatively short time selected for the in vitro setting in this study. A fluoride concentration of 7,000 ppmF is selected for the experimental coating materials because precipitates appear at concentrations higher than 7,000 ppmF. Therefore, we selected this concentration as the upper limit for the experimental coating materials. Furthermore, conditions were set up with and without the presence of oxalic acid to evaluate the role of oxalic acid in preventing dentin demineralization and to evaluate the interaction between oxalic acid and fluoride.

The MS polymer maintained a smooth surface before and after demineralization. However, there were no differences in the ML values for MS0(−) and the control group. That MS polymer does not contribute to preventing dentin demineralization suggests that the polymer structure is sparse.

Oxalate-containing desensitizers are widely accepted as dentin desensitizers by practitioners\(^{14}\). The oxalate aqueous solution has been demonstrated to effectively reduce the permeability of dentin tubules through calcium oxalate (CaC\(_2\)O\(_4\)) deposition\(^{15,28,29}\). It is evident that oxalic acid treatment can decrease dentin hypersensitivity via calcium oxalate deposits forming on the dentin surfaces\(^{15,28,29}\). However, the occlusion of dentin tubules by the deposits is superficial and temporary because the crystals are either partially dissolved in oral fluids or lost during tooth brushing\(^{30}\). Furthermore, calcium oxalate is easily soluble in acid and oxalic acid does not have the ability to prevent dentin demineralization\(^{14}\). The ML values of MS0(+), which contain oxalic acid, were significantly lower than those of MS0(−). Through the SEM observation, some crystals (probably MS polymer-calcium oxalate emulsions) formed on the dentin surface before demineralization (Fig. 3e). After demineralization, however, the roughness of the surface texture decreased and the dentinal tubules were occluded with the crystals (Fig. 3f). MS Coat One [MS0(+)] is sufficiently acidic (pH 2.3) in that it does not require etching of the dentin surface and forms calcium oxalate crystals through the release of calcium ions\(^{30}\), and the calcium oxalate crystals aggregate into a mass through calcium oxalate deposits forming via calcium oxalate formation\(^{15,28,29}\). It is assumed that the sulfone group of the MS polymer reacts with both calcium fluoride and calcium oxalate on the dentin surface to produce relatively large crystals.

Overall ML values were lower when oxalic acid was present and dentin demineralization was more effectively prevented at higher concentrations of fluoride. Furthermore, MS7000(+) containing both oxalic acid and fluoride had an almost equal ability to prevent demineralization as NaF9000, the positive control, and when observed under the SEM the membrane-like structure on the dentin surface was maintained before and after demineralization. Dentinal tubules were occluded by oxalic acid, and fluoride and calcium ions from the emulsion of MS polymer, oxalic acid, and fluoride were constantly supplied to the dentin around dentinal tubules and the surface. Consequently, it is proposed that the dentin gradually acquires acid resistance. Essentially, the emulsion in the dentinal tubule is assumed to play the role of a CaF\(_2\)-like deposit. Furthermore, the caries prevention effect can be expected on exposed dentin root surface when an experimental MS coat such as MS7000(+) is applied for 30 s. Thus, it is clinically useful considering that there is no waiting time. Further study is required to evaluate clinical factors, surface analysis, and ML values under different experimental conditions.

**CONCLUSION**

This in vitro study suggested that the ability to prevent dentin demineralization increases with an increase in fluoride concentration from 0 to 3,000 and 7,000 ppmF. Furthermore, this effect appeared to be enhanced in the presence of oxalic acid.

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CONFLICT OF INTEREST
The authors declare that there are no potential conflicts of interest with respect to the publication of this article.

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