Effects of pastes containing ion-releasing particles on dentin remineralization

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We investigated the effects of the weekly application of pastes containing a surface reaction-type pre-reacted glass-ionomer (S-PRG) filler on dentin remineralization. Human dentin blocks were demineralized and polished using pastes containing S-PRG filler (0, 5, and 30%), and then immersed in remineralizing solution for 1 month. Nanoindentation testing was carried out during the immersion period, and the dentin surfaces were examined using scanning electron microscopy. A nano-hydroxyapatite-containing paste was used for comparison. Immersion in demineralization solution had a marked negative effect on the mechanical properties in all specimens. The mechanical properties of specimens polished with S-PRG filler-containing pastes recovered significantly after immersion in remineralization solution for 1 month compared with the other specimens. After remineralization, the open dentinal tubules were filled with a remineralization layer in specimens polished with S-PRG filler-containing and nano-hydroxyapatite-containing pastes. S-PRG filler-containing pastes can aid dentin remineralization.

Keywords: Dentin remineralization, Nanoindentation, Professional tooth polishing

INTRODUCTION

The use of acid etching and conditioning of dentin surfaces to improve adhesion is widely accepted in restorative dentistry¹. On the other hand, the incidences of dentin hypersensitivity and root caries have increased recently^{2–5}, and the development of effective methods to prevent these common clinical problems is important. The use of increased calcium and fluoride concentrations in oral fluids seems to be a reasonable approach to enhance the remineralization of demineralized dentin surfaces^{6,7}.

Various dental products and applications have been introduced in the last half century to increase the remineralization of enamel and dentin structures. As fluoride enhances hydroxyapatite (HA) mineralization and reduces solubility, fluoride-containing products such as toothpaste, mouth rinse, fluoride-releasing adhesives, and sealants have been introduced widely^{6–8}. Recently, the use of nano-HA, which is similar to the apatite crystal of dental enamel, has been proposed for the remineralization of early carious lesions^{9,10}. A surface reaction-type pre-reacted glass-ionomer (S-PRG) filler forms a stable glass-ionomer phase by pre-reacting acid-reactive glass-containing fluoride with polycarboxylic acid in the presence of water^{11–15}, and it can release Al^{3+} , BO_3^{3-} , F^- , Na^+ , SiO_3^{2-} , and Sr^{2+} . The release of SiO_3^{2-} and Na^+ into the surrounding environment leads to buffering^{16,17}; in addition, Sr^{2+} and F^- are known to be strong inducers of remineralization of etched dentin and enamel surfaces^{18,19}. The development of effective

methods of dentin mineralization to prevent dentin hypersensitivity and root caries is highly desirable. Tooth polishing with S-PRG filler-containing paste may be a promising approach to enhance dentin remineralization.

The purpose of this *in vitro* study was to investigate the effect of the weekly professional application of pastes containing S-PRG filler on dentin remineralization. Nano-HA-containing pastes were used for comparison. The null hypothesis was that the polishing of etched dentin samples with an S-PRG filler-containing paste would not enhance remineralization.

MATERIALS AND METHODS

Materials

Human non-carious premolars that were extracted from patients who underwent orthodontic treatment were subjected to nanoindentation tests to investigate the remineralization of etched dentin surfaces. Figure 1 is a schematic diagram of specimen preparation and paste application. Each tooth was sectioned at the cementum–enamel junction and the middle region of the root using a low-speed water-cooled diamond saw (IsoMet, Buehler, Lake Bluff, IL, USA), and then the portion was sectioned parallel to the long axis of the tooth to obtain root slabs. The slabs were encapsulated in epoxy resin (EpoFix, Struers, Copenhagen, Denmark). After 24 h, the specimens were ground using 600-grit sandpaper and polished using diamond suspensions with particle sizes of 3, 1, and 0.25 μm (Buehler) to obtain surfaces suitable for nanoindentation. This polishing procedure removed root cementum from the tooth surfaces. Acid-

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Fig. 1 Schematic illustration of the specimen preparation sequence for *in vitro* remineralization of dentin surfaces.

Table 1 Materials used in the present study

Materials	Brand name/ Manufacturer	Composition
S-PRG filler-containing paste		Hydrated silica, Carboxymethylcellulose sodium, Glycerol Sorbitol, Sodium dodecyl sulfate, Mint flavoring S-PRG filler (0, 5, or 30 wt%)
Nano-HA-containing paste	Renamel/Sangi	Water, Hydroxyapatite, Glycerin, Diglycerin, Xylitol, Butylene glycol Cellulosegum, Silica, Essence, Methylparaben, Propylparaben Xanthanegum, Sodium lauroyl sarcosine, Cetylpyridinium chloride
Artificial saliva		CaCl_2 , NaH_2PO_4 , Ca/PO_4 pH adjusted to 4.3 and 6.8 by CH_3COOH and NaOH

resistant nail varnish was then applied to the dentin surfaces, leaving a test surface area of 3 mm².

We created a paste containing hydrated silica (mean particle size, 6.4 μm, Evonik Industries, Essen, Germany), carboxymethylcellulose sodium (molecular weight, 725,000 Da, Nacalai Tesque, Kyoto, Japan), glycerol (84–87% pure, Nacalai Tesque), sorbitol (97% pure, Nacalai Tesque), sodium dodecyl sulfate (93.5% pure, Nacalai Tesque), mint flavoring (Yamamoto Perfumery, Osaka, Japan), and an S-PRG filler (0, 5, or 30 wt%). The S-PRG filler (mean particle size, 1.0 μm) was produced as described elsewhere¹². The nano-HA-containing paste (Renamel, Sangi, Tokyo, Japan) was used for comparison. Table 1 lists the composition of the materials used in this study. This study was approved by the ethics committee of the Health Sciences University of Hokkaido.

Dentin remineralization ability and changes in mechanical properties

The specimens (embedded dentin blocks with polished surface areas of 3 mm²) were divided into five groups of 15 specimens each. Artificial saliva containing CaCl_2 and NaH_2PO_4 with a Ca/PO_4 ratio of 1.67 was prepared, and its pH was adjusted to 4.3 and 6.8 using CH_3COOH and NaOH . The specimens were immersed in individual 2-mL plastic vials of artificial saliva (pH 4.3) as demineralizing solution for 3 days at 37°C (Fig. 1). The demineralized specimens were polished with the various pastes (0, 5, and 30% S-PRG, Renamel) for 10 s using a low-speed handpiece with a rotating brush. The specimens were rinsed lightly and dried using a moisture-free air source. They were then immersed in remineralization solution (artificial saliva, pH 6.8) at 37°C for 1 month. The specimens were polished once a week during the 1-month period, and the solution was changed weekly. Nanoindentation testing of the dentin surfaces (ENT-1100a, ELIONIX, Tokyo, Japan) was

carried out at 28°C using 10- and 100-mN loads before and after demineralization and during the remineralization period. The hardness and elastic modulus of the dentin surfaces were calculated using the software provided with the nanoindentation apparatus.

After 1 month of immersion, the specimens were sectioned vertically using a low-speed water-cooled diamond saw (IsoMet); one of the sectioned halves of each specimen was then encapsulated in epoxy resin (EpoFix). After 24 h, the specimens were ground using 600-grit sandpaper and polished using diamond suspensions with particle sizes of 3, 1, and 0.25 μm (Buehler) to obtain surfaces suitable for studying the depth-dependent mechanical dentin properties. Nanoindentation testing was carried out at 28°C with a peak load of 2 mN ($n=15$). The indentations were made at depths of 1–98.5 μm (40 locations spaced 2.5 μm apart).

Scanning electron microscope (SEM) observations of enamel surfaces

Representative surfaces of dentin specimens were examined under a SEM (JSM-6610LA, JEOL, Tokyo, Japan) before demineralization, after 3 days of demineralization, and after 1 month of remineralization by immersion in artificial saliva. The specimens were sputter coated with gold (SC-701 AT, Sanyu Electron, Tokyo, Japan) and examined at 15 kV. The surfaces of control specimens, which were not immersed in the demineralization solution, were also observed for comparison.

Statistical analysis

Experimental data were analyzed using the PASW Statistics software package (ver. 18.0J for Windows, IBM, Armonk, NY, USA). The means and standard deviations of hardness and elastic modulus values were calculated for the five groups and compared using one-way analysis of variance with Tukey's test. The significance level was set at $p<0.05$.

RESULTS

Figures 2 and 3 show the mean hardnesses and elastic modulus of the dentin specimens before and after demineralization and during the immersion period. Hardness and elastic modulus values measured before and immediately after immersion in the demineralization solution did not differ significantly in any group. Demineralization by immersion in acidic artificial saliva solution markedly decreased the hardness and elastic modulus of the dentin surfaces. Following immersion in remineralization solution, the hardness and elastic modulus of dentin specimens polished with the S-PRG filler-containing pastes (5 and 30%) and nano-HA-containing paste increased gradually with the immersion time. In nanoindentation tests conducted with both loading conditions (10 and 100 mN), specimens polished with the S-PRG filler-containing pastes (5 and 30%) exhibited significantly greater hardness and elastic modulus than did unpolished specimens and those polished with the other pastes after 1 month of immersion in remineralization solution.

Figure 4 shows the mean hardness and elastic modulus at different depths of the dentin specimens after 1 month of immersion in remineralization solution and statistical comparisons of the values from the dentin surface to 11 μm depths of five specimen groups. No significant difference in hardness at depths of 11–98.5 μm or elastic modulus at depths of 3.5–98.5 μm from the dentin surface was observed among the five specimen groups. Hardness values at depths ≤ 8.5 from the dentin surface and elastic modulus values at depths ≤ 1 μm from the dentin surface were greater for specimens polished with S-PRG filler-containing pastes than for specimens in the other groups.

Figure 5 shows representative SEM photomicrographs of the original dentin surface, demineralized dentin surface, and dentin surface after immersion in remineralization solution for 1 month.

Fig. 2 Mean hardness values of dentin surfaces before and after demineralization, and after immersion in remineralization solution (artificial saliva). BD, before demineralization; AD, after demineralization; 1W, 1-week immersion; 1M, 1-month immersion. * $p<0.05$ (Tukey test).

Fig. 3 Mean elastic modulus values of dentin surfaces before and after demineralization, and after immersion in remineralization solution (artificial saliva). BD, before demineralization; AD, after demineralization; 1W, 1-week immersion; 1M, 1-month immersion. * $p < 0.05$ (Tukey test).

Fig. 4 Mean hardness and elastic modulus values of dentin specimens at different depths after 1-month immersion (a, Hardness; b, Elastic modulus). Statistical comparisons of the values from the dentin surface to 11 μm depths of five specimen groups (c, Hardness; d, Elastic modulus). * $p < 0.05$ (Tukey test).

Numerous open dentinal tubules were observed in the specimens after demineralization. After polishing and

immersion for 1 month, these tubules were completely filled with a remineralization layer in specimens treated

Fig. 5 Representative SEM photomicrographs of the original dentin surface (BD, before demineralization), demineralized dentin surface (AD, after demineralization), and dentin surface after immersion in remineralization solution for 1 month.

with 30% S-PRG filler-containing paste, and partly filled with a remineralization layer in specimens treated with 5% S-PRG filler-containing paste and nano-HA-containing paste. Open dentinal tubules were observed in unpolished specimens and those polished with 0% S-PRG filler-containing paste.

DISCUSSION

This study examined the effects of polishing of demineralized dentin samples using pastes containing 5 and 30 wt% S-PRG filler on remineralization ability in artificial saliva solution. In the nanoindentation test, the specimen surface was ground and then polished because with the nanoindentation test, the mechanical properties are influenced by the surface roughness of the specimen. In addition, the polished surface might simulate dentin surface exposed by daily brushing. Nanoindentation tests followed by immersion in remineralization solution showed that specimens polished with the S-PRG filler-containing pastes (5 and 30%) had significantly greater hardnesses and elastic modulus than did unpolished specimens and those polished with the other pastes (0% S-PRG and nano-HA). The recovery of hardness and elastic modulus [*i.e.*, (post-immersion value/original value)×100], as measured using a nanoindenter with a 10-mN load, was characterized as follows. Samples treated with the 30 wt% S-PRG filler-containing paste exhibited 66% recovery of hardness and 98% recovery of the elastic modulus, samples treated with the 5 wt% S-PRG filler-containing paste exhibited 67% recovery of hardness and 89% recovery of the elastic modulus, samples treated with the 0 wt% S-PRG filler-containing paste exhibited 53% recovery of hardness and 80% recovery of the elastic modulus, and samples treated with nano-HA-containing paste exhibited 53% recovery of hardness and 80% recovery of the elastic modulus. Thus, the

null hypothesis that polishing of dentin samples using an S-PRG filler-containing paste would not enhance remineralization was rejected. The remineralization ability did not differ significantly between specimens treated with the 5 and 30% S-PRG filler-containing pastes. Paste components other than the S-PRG filler may have little influence on dentin remineralization, as specimens treated with the 0 wt% S-PRG filler-containing paste exhibited little enhancement of remineralization following demineralization. Although artificial saliva contains the inorganic ions required for remineralization²⁰, the recovery of unpolished specimens was inferior to that of specimens polished with the 5 and 30% S-PRG filler-containing pastes and nano-HA-containing paste. Remineralized layers may differ qualitatively. Dental plaque accumulation on enamel and dentin, without mechanical or chemical disturbance, leads to demineralization. Although daily plaque control with toothbrushing and mouthwash use is important, additional professional tooth cleaning with pastes may help to avoid tooth demineralization and enhance remineralization.

According to the results of the depth-dependent nanoindentation test, the hardness and elastic modulus of the non-polished specimens decreased with depth up to 20 μm , suggesting that these regions of the dentin structure were least demineralized by immersion in demineralizing solution for 3 days. For the nanoindentation test, we used peak loads of 10 and 100 mN. The indentation depths were approximately 600 nm (0.6 μm) for the 10 mN peak load and 2,000 nm (2 μm) for the 100 mN peak load. Considering the demineralization depth (20 μm) of the dentin surfaces, the mechanical properties obtained with peak loads of 10 and 100 mN were meaningful, and the peak loads for nanoindentation testing were reasonable. However, the indentations produced by 100 mN affected approximately

16 μm of the width, and the area contained the dentinal tubules, although we tried to minimize the overlap of the indentation with the dentinal tubules, as mechanical properties would be influenced by overlap between the indentation and the dentinal tubules.

S-PRG filler features a glass-ionomer phase around the glass core, which slowly releases multiple ions, such as Al^{3+} , BO_3^{3-} , F^- , Na^+ , SiO_3^{2-} , and Sr^{2+} (11–13). We speculate that these ions were released from the S-PRG filler-containing pastes, inhibiting demineralization and enhancing remineralization of the dentin and enamel. Si and Al form the structure of the glass, and Sr and F were added as modifiers. Boron, which is highly soluble and has antibacterial and anti-inflammatory properties, was also included (21). The release of Na^+ and SiO_3^{2-} into the surrounding environment has a buffering effect, inhibiting demineralization. The present study revealed no significant difference in remineralization ability between specimens treated with the 5 and 30% S-PRG filler-containing pastes, although the amount of ion release was influenced by the mixing ratio of solution to S-PRG filler in a previous study (11).

Recently, various novel remineralization agents, including nano-HA and casein phosphopeptide amorphous calcium phosphate nanocomplexes, have been developed (9,10). Nano-HA is biocompatible and bioactive, and its nano-sized particles are similar to the apatite crystal of tooth enamel (9,10,22). Nano-HA has been shown to remineralize carious lesions in enamel and dentin (9,10). In the remineralization of a demineralized dentin surface, nano-HA may serve as a template, controlling the nucleation and growth of mineral crystals to form a dentin-like structure. The precipitation process may attract Ca^{2+} and PO_4^{3-} from the environment to the dentin surface and fill vacant positions in the crystal structure (23). However, dentin polishing with nano-HA-containing paste did not enhance the remineralization ability relative to polishing with the S-PRG filler-containing pastes in the present study. This result may be explained by the low solubility of nano-HA due to its high degree of crystallinity; in addition, amorphous and small imperfect crystals are known to have higher dissolution rates than crystalline compounds (24). However, further research is needed to confirm the crystal structure of nano-HA.

We examined demineralized dentin surfaces during remineralization using SEM. The surface morphology following immersion in an artificial saliva solution after polishing is expected to vary among paste treatments. The surfaces of unpolished specimens and those polished with the 0% S-PRG filler-containing paste showed numerous open dentinal tubules, consistent with a demineralized dentin surface. By contrast, the dentinal tubules were completely filled by a remineralization layer in specimens polished with the 30% S-PRG filler-containing paste, and partially filled in specimens polished with the 5% S-PRG filler-containing paste and nano-HA-containing paste. The coverage of exposed dentin and sealing of dentinal tubules with a remineralization layer may reduce hypersensitivity and

inhibit root caries (2).

CONCLUSIONS

Under the study conditions, the following conclusions were drawn:

1. Polishing with an S-PRG filler-containing paste enhances the remineralization ability of demineralized dentin surfaces.
2. Dentin polishing with nano-HA-containing paste did not enhance remineralization ability compared with polishing with the S-PRG filler-containing pastes.
3. Further investigation is warranted to identify optimal application methods and specific protocols.

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