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The effects of single application of pastes containing ion-releasing particles on enamel demineralization

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We investigated single application of pastes containing a surface reaction-type pre-reacted glass-ionomer (S-PRG) filler on enamel demineralization. Human enamel blocks were polished using pastes containing S-PRG filler (0, 5, and 30%) and immersed in demineralizing solution for 5 days with daily change of solutions. The pH measurement and nanoindentation testing was carried out during the immersion period, and the enamel surfaces were examined using scanning electron microscopy and atomic force microscopy. A non-fluoride paste and a hydroxyapatite-containing paste were used for comparison. The specimens polished with the S-PRG filler-containing paste exhibited acid-neutralizing properties, which became stronger with an increasing S-PRG filler content. Following immersion in the demineralizing solution, specimens polished with the S-PRG filler-containing paste exhibited significantly greater hardness and elastic modulus values than those polished with the other pastes and exhibited a smoother surface than did the other specimens. Pastes containing S-PRG filler inhibits the demineralization of enamel.

Keywords: Enamel demineralization buffering, Nanoindentation, Professional cleaning tooth polishing

INTRODUCTION

In recent years, the incidence of dental caries has decreased; however, dental caries continues to be a major health problem throughout the world, affecting both adults and children[1,2]. Acids produced by oral bacteria lead to a loss of calcium and phosphate from dental enamel. This is termed demineralization, and it leads to caries following prolonged exposure to an acidic environment (pH<5.5)[3]. Remineralization is the process whereby calcium and phosphate ions are supplied to dental enamel, and apatite-like crystals form on the enamel surface[4]. Dental caries is caused by an imbalance between demineralization and remineralization.

Fluoride plays an important role in preventing demineralization[5], and fluoride-containing products such as toothpastes, mouth rinses, fluoride-releasing adhesives, and sealants are widely used in dentistry[6-8]. Although the benefits of fluoride in terms of reducing the incidence of dental caries are well established, fluoride cannot prevent caries in all patients[1]; thus, the development of novel anti-caries and remineralization agents is desirable. In addition to daily brushing, professional cleaning processes such as scaling and polishing are important to prevent caries[9,10].

Recently, nano-hydroxyapatite (nano-HA), which is similar to the apatite crystal of dental enamel, has been proposed for the remineralization of early caries lesions[11,12]. 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[13-15], and it can release Al, B, F, Na, Si, and Sr ions. Supplying Si and Na ions into the surrounding environment leads to a buffering effect[16,17], further inhibiting demineralization. In addition, Si and F ions are known to improve the acid resistance of teeth by converting hydroxyapatite to strontiumapatite[18,19] and fluoroapatite, respectively[19-21].

The purpose of this *in vitro* study was to investigate the effect of a single application of paste containing S-PRG filler. Non-fluoride and nano-HA-containing pastes were also used for comparison. The null hypothesis was that polishing samples of dental enamel using an S-PRG filler-containing paste do not inhibit demineralization.

MATERIALS AND METHODS

Materials

Thirty-eight human noncarious premolars were obtained by extraction from patients who underwent orthodontic treatment. The buccal enamel surfaces were subjected to nanoindentation tests to investigate demineralization following exposure to an acidic environment. Following the nanoindentation tests, the surfaces were observed by scanning electron microscopy (SEM) and examined by atomic force microscopy (AFM). Figure 1 shows a schematic diagram of the specimen preparation procedure. The premolars were cut with a

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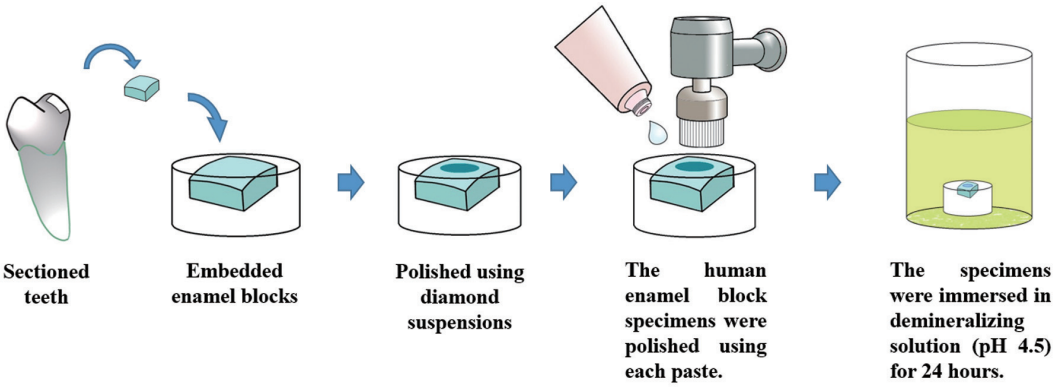

Fig. 1 A schematic illustration of the specimen preparation process.

low-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) following removal of the roots, dividing them into mesial and distal half-crowns. The resulting specimens were then encapsulated in epoxy resin (Epofix, Struers, Copenhagen, Denmark). After 24 h, the specimens were ground lightly using 600-grit sandpaper and polished using a diamond suspension of 0.05 μm grit (Buehler). This polishing procedure removed approximately 200 μm from the surface of the teeth. The surface area of the polished samples was approximately 4×4 mm.

We created a paste containing hydrated silica (Evonik Industries, Essen, Germany; Mean particle size: 6.4 μm), carboxymethylcellulose sodium (Nacalai tesque, Kyoto, Japan; Molecular weight: 725,000), glycerol (Nacalai tesque; Purity: 84–87%), sorbitol (Nacalai tesque; Purity: 97%), sodium dodecyl sulfate (Nacalai tesque; Purity: 93.5%), flavoring mint (Yamamoto perfumery, Osaka, Japan), and an SPR-G filler (0, 5, or 30 wt%). The S-PRG filler (Mean particle size: 1.0 μm) was formed as described elsewhere[14]. The non-fluoride paste Merssage Plus (Shofu, Kyoto, Japan) and nano-HA-containing paste Renamel (Sangi, Tokyo, Japan) were used for comparison. This study was approved by the ethics committee of the Health Sciences University of Hokkaido.

Demineralization

The polished buccal enamel surfaces (n=15; approximate area: 4×4 mm) were polished for 10 s using a low-speed handpiece with a rotating brush and with the various pastes. The samples were then rinsed lightly and dried using a moisture-free air source. The specimens were immersed in individual 2-mL plastic vials of demineralizing solution (*i.e.*, 1.5 mmol/L of $CaCl_2$, 0.9 mmol/L of NaH_2PO_4, and 50 mmol/L of CH_3COOH; pH=4.55) for 5 days at 37ºC. The solution was changed daily, and the pH was measured using a pH meter (SI600, Sentron, Roden, The Netherlands) with a micro-pH electrode (9070-008, Sentron).

Hardness and elastic modulus measurements by nanoindentation testing

Nanoindentation testing was carried out at 28ºC with loads of 2, 10, and 100 mN using a Berkovich indenter (n=15) (ENT-1100a, Elionix, Tokyo, Japan). Linear extrapolation (ISO standard 14577) was used to obtain unloading curves between 95 and 70% of the maximum force, which were used to calculate the elastic modulus[22]. The hardness and elastic modulus of the buccal enamel surfaces were calculated using the software provided with the nanoindentation apparatus.

Enamel surface observation with SEM and measurement of the surface roughness with AFM

Following the nanoindentation tests, representative specimens from each group were observed by SEM (SSX-550, Shimadzu, Kyoto, Japan). The specimens were sputter-coated with gold (SC-701 AT, Sanyu Electron, Tokyo, Japan) and examined at 15 kV. The surfaces of the control specimens that were not immersed in the demineralization solution were also observed for comparison.

The specimens were then examined by AFM (SPM-9500J2, Shimadzu). The mean surface roughness (Ra) was calculated at seven different areas for each specimen using the software supplied with the AFM.

Statistical analysis

The 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 the hardness, elastic modulus and Ra were calculated for the five groups and compared using a one-way analysis of variance with Tukey's test. Statistical significance was considered as p<0.05.

RESULTS

Figure 2 shows the changes in pH of the solutions in which the enamel specimens were immersed. All solutions exhibited an increase in pH up to 3 days. The specimens polished with the S-PRG filler-containing

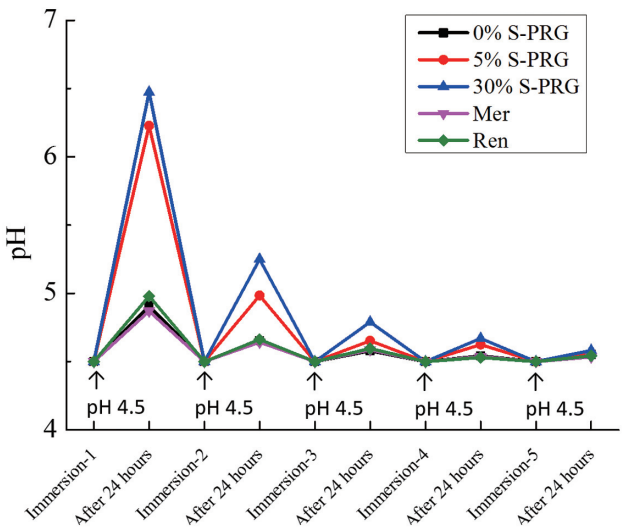

Fig. 2 Changes in the pH of the solutions in which the polished enamel specimens were immersed.

pastes exhibited acid-neutralizing properties, which increased with increasing S-PRG filler content.

Figure 3 shows the mean hardness values. Figure 4 shows the mean elastic modulus of the enamel specimens before immersion and at various times during the immersion period. Tables 1 and 2 present statistical comparisons of the five specimen groups. There were no significant differences in hardness or elastic modulus in any group prior to immersion in the demineralization solution. After 1 day of immersion, the specimens polished with the S-PRG filler-containing paste exhibited significantly greater hardness and elastic modulus values than those polished with the other pastes. The hardness and elastic modulus of all specimens decreased as the immersion time increased.

After 5 days of immersion, the specimens polished with the 0% S-PRG filler-containing paste and Merssage Plus exhibited porous surfaces with numerous enamel prisms and a honeycomb-like structure (Fig. 5). The specimens polished with the 5 and 30% S-PRG filler-containing pastes, as well as those polished with

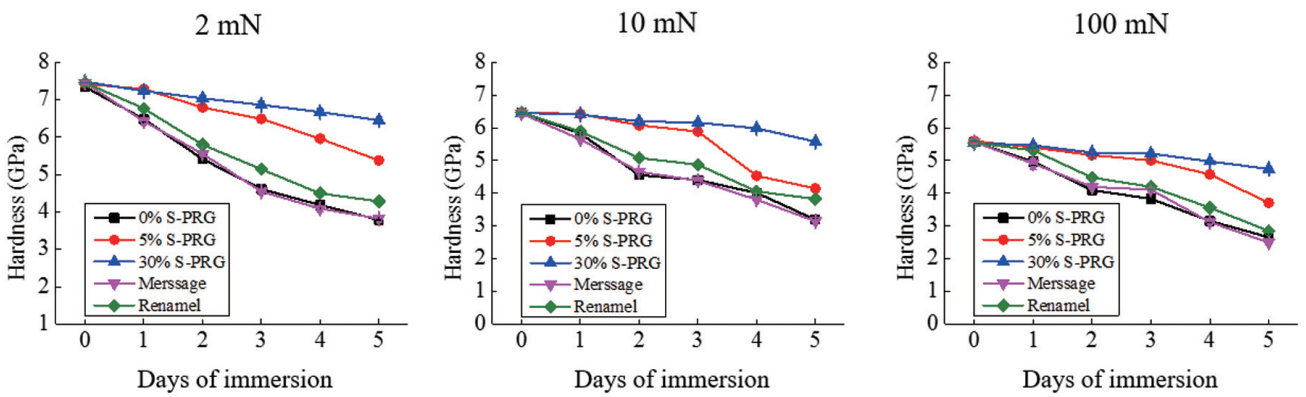

Fig. 3 Mean hardness of the buccal enamel surface prior to immersion and during the immersion period.

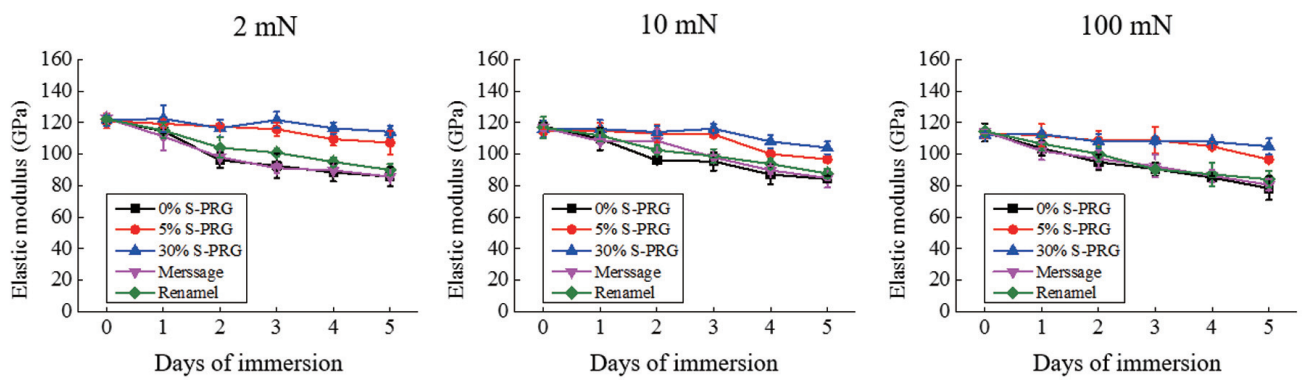

Fig. 4 Mean elastic modulus of the buccal enamel surface prior to immersion and during the immersion period.

Table 1 Mean values for hardness of the enamel specimens before immersion and at various times during the immersion (GPa)

2 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	7.35	0.64	7.42	0.46	7.48	0.17	7.46	0.29	7.46	0.20	0.893
1	6.48[a]	0.51	7.29[b]	0.34	7.24[b]	0.36	6.42[a]	0.36	6.77[a]	0.55	0.000
2	5.41[a]	0.49	6.80[b]	0.96	7.04[b]	0.42	5.55[a]	0.31	5.80[a]	0.26	0.000
3	4.61[a]	0.85	6.49[b]	0.65	6.87[b]	0.45	4.55[a]	0.25	5.14[a]	0.60	0.000
4	4.19[a]	0.94	5.96[b]	0.55	6.67[c]	0.27	4.09[a]	0.24	4.50[a]	0.35	0.000
5	3.79[a]	0.65	5.38[b]	0.94	6.45[b]	0.57	3.83[a]	0.24	4.28[a]	0.56	0.000

10 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	6.46	0.26	6.47	0.12	6.46	0.18	6.44	0.27	6.47	0.31	0.995
1	5.83[a]	0.27	6.42[b]	0.32	6.40[b]	0.20	5.65[a]	0.44	5.89[a]	0.55	0.000
2	4.55[a]	0.68	6.08[b]	0.32	6.20[b]	0.64	4.66[a]	0.75	5.08[a]	0.44	0.000
3	4.41[a]	0.52	5.89[b]	0.49	6.16[b]	0.21	4.39[a]	1.53	4.87[a]	0.95	0.000
4	4.01[a]	0.51	4.53[a]	1.14	5.99[b]	0.37	3.81[a]	1.09	4.06[a]	0.69	0.000
5	3.19[a]	0.73	4.15[b]	0.43	5.58[c]	0.66	3.14[a]	0.39	3.82[b]	0.46	0.000

100 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	5.57	0.09	5.60	0.16	5.54	0.18	5.57	0.19	5.54	0.19	0.872
1	4.96[a]	0.38	5.40[b]	0.31	5.46[b]	0.17	4.91[a]	0.42	5.31[b]	0.30	0.000
2	4.10[a]	0.57	5.17[b]	0.17	5.25[b]	0.35	4.19[ac]	0.36	4.48[c]	0.27	0.000
3	3.83[a]	0.27	5.01[b]	0.27	5.21[b]	0.39	4.11[ac]	0.42	4.20[c]	0.28	0.000
4	3.16[a]	0.24	4.58[b]	0.26	4.98[c]	0.49	3.12[a]	0.45	3.56[d]	0.22	0.000
5	2.65[a]	0.38	3.71[b]	0.54	4.74[c]	0.64	2.50[a]	0.24	2.85[a]	0.37	0.000

One-way ANOVA followed by the Tukey test. Identical letters indicate that mean values were not significantly different.

Renamel, exhibited significantly smoother surfaces, which was confirmed by the mean values of Ra (Table 3). Following immersion in the demineralization solution, the decrease in hardness and elastic modulus [*i.e.*, (original value —value after immersion)/original value×100] as measured using a nanoindenter with a 10-mN load were as follows: the samples treated with the 30 wt% S-PRG filler-containing paste exhibited a 14% decrease in hardness and a 7% decrease in elastic modulus; the samples treated with the 5 wt% S-PRG filler-containing paste exhibited a 34% decrease in hardness and a 15% decrease in elastic modulus; the samples treated with the 0 wt% S-PRG filler-containing paste exhibited a 52% decrease in hardness and a 31% decrease in elastic modulus; the samples treated with Merssage exhibited a 55% decrease in hardness and a 30% decrease in elastic modulus; and the samples treated with Renamel exhibited a 48% decrease in hardness and a 27% decrease in elastic modulus. These results show that the S-PRG filler-containing pastes inhibited enamel demineralization. The protective effect against demineralization was significantly greater

Table 2 Mean values for elastic modulus of the enamel specimens before immersion and at various times during the immersion period (GPa)

2 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	121.84	4.72	120.99	8.39	121.41	7.16	122.82	6.10	122.08	4.02	0.947
1	114.63 [ab]	8.47	119.29 [ab]	6.43	122.54 [b]	5.98	111.18 [a]	10.86	115.01 [ab]	6.96	0.002
2	96.13 [a]	13.77	117.45 [b]	7.48	121.81 [b]	7.16	98.06 [a]	8.30	104.10 [a]	8.53	0.000
3	92.60 [a]	9.55	115.75 [b]	7.41	121.58 [b]	8.55	93.13 [a]	7.66	100.89 [a]	9.83	0.000
4	88.38 [a]	10.29	109.47 [b]	6.91	116.45 [b]	5.52	89.70 [a]	5.77	95.05 [a]	7.30	0.000
5	85.77 [a]	11.61	107.28 [b]	11.75	114.13 [b]	7.88	85.71 [a]	7.64	90.01 [a]	13.96	0.000

10 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	117.49	4.89	114.46	2.54	115.87	4.13	116.69	3.77	116.83	4.32	0.292
1	109.63 [ab]	7.19	114.83 [ab]	5.98	115.87 [b]	5.85	108.21 [a]	6.82	111.82 [ab]	6.90	0.008
2	96.01 [a]	12.80	113.01 [b]	3.77	114.00 [b]	6.87	108.45 [bc]	10.91	102.49 [ac]	4.93	0.000
3	95.35 [a]	15.70	112.47 [b]	4.14	116.11 [b]	7.25	97.89 [a]	12.95	98.75 [a]	11.46	0.000
4	87.03 [a]	10.41	100.04 [b]	9.03	108.00 [bc]	9.78	89.73 [ab]	15.89	93.74 [ab]	5.59	0.000
5	84.27 [a]	4.52	96.74 [b]	16.00	104.02 [bc]	9.68	84.80 [a]	8.12	87.71 [ab]	6.57	0.000

100 mN

Days of immersion	0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	113.80	3.17	113.27	3.10	112.27	4.22	114.40	3.25	114.47	4.02	0.430
1	103.71 [ab]	14.70	111.94 [b]	4.66	112.67 [b]	6.40	102.00 [a]	5.86	106.64 [ab]	5.28	0.001
2	94.95 [a]	17.60	108.53 [b]	4.34	107.95 [b]	10.68	97.00 [a]	9.36	100.15 [ab]	6.35	0.001
3	90.51 [a]	12.05	108.87 [b]	3.89	108.61 [b]	5.97	92.34 [a]	7.37	90.36 [a]	11.97	0.000
4	85.14 [a]	9.15	104.86 [b]	4.63	107.84 [b]	5.07	86.14 [a]	6.41	87.18 [a]	10.83	0.000
5	78.30 [a]	7.02	96.63 [b]	8.70	104.85 [c]	6.95	80.53 [a]	4.99	84.03 [a]	6.77	0.000

One-way ANOVA followed by the Tukey test. Identical letters indicate that mean values were not significantly different.

Table 3 Mean values for surface roughness of the original enamel surface and enamel surfaces following immersion in the acidic environment (Ra)

	Original		0% S-PRG		5% S-PRG		30% S-PRG		Merssage		Renamel		*p*
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Surface roughness	9.4	1.0	64.2	7.8	18.7	1.8	11.7	1.5	62.3	5.2	14.2	2.5	0.000

One-way ANOVA followed by the Tukey test. Identical letters indicate that mean values were not significantly different.

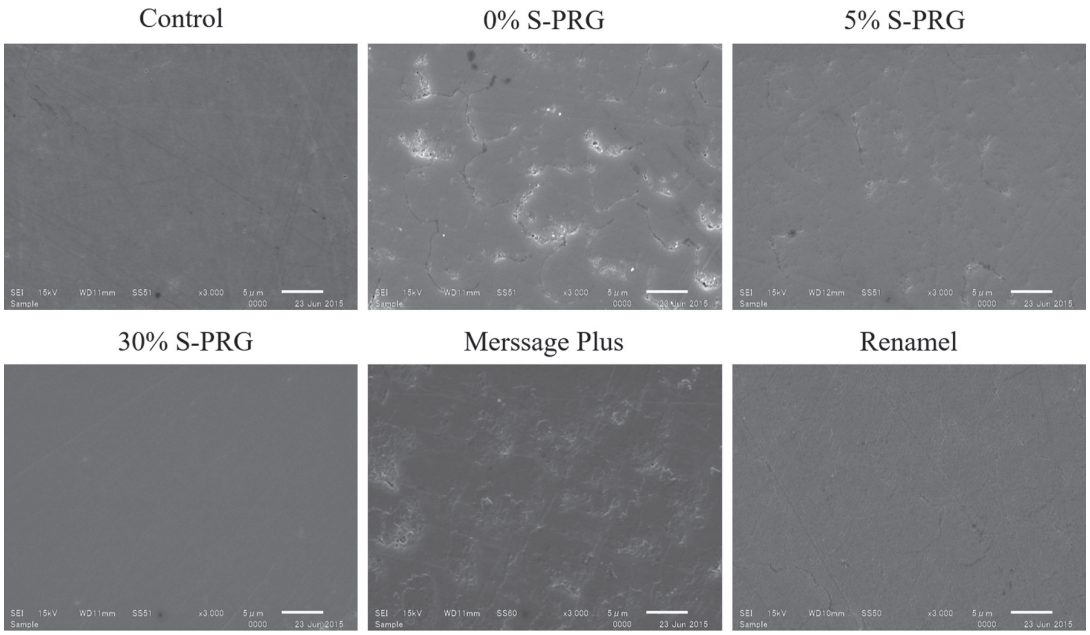

Fig. 5 Scanning electron micrographs of the original enamel surface (control) and enamel surfaces following immersion in the acidic environment.

for the 30% S-PRG filler-containing paste than for the 5% S-PRG filler-containing paste. Thus, the null hypothesis that polishing the enamel samples using an S-PRG filler-containing paste would not inhibit demineralization was rejected.

DISCUSSION

We examined the effects of polishing enamel samples with a paste containing 5 and 30 wt% S-PRG filler on resistance to acid attack. Nanoindentation tests following immersion in demineralization solution showed that the specimens polished with the S-PRG filler-containing pastes exhibited significantly greater hardness and a higher elastic modulus than specimens polished with the other pastes. The results indicate that S-PRG filler-containing pastes exhibited inhibition of enamel demineralization due to the acid-neutralizing properties. The composition except for S-PRG fillers may have little influence to inhibition of the enamel demineralization, because 0 wt% S-PRG filler-containing paste exhibited a little inhibition of enamel demineralization.

S-PRG filler features a glass ionomer phase around the glass core, which slowly releases multiple ions such as Al, B, Na, Si, Sr, and F[13-15]. We would speculate that these ions were released from the S-PRG filler-containing paste, inhibiting demineralization of the enamel. The elements Si and Al formed the structure of the glass, and Sr and F were added as a modifier. Boron was also included, which is highly soluble; it exhibits antibacterial and anti-inflammatory properties[23]. Supplying Si and Na ions into the surrounding environment leads to a buffering effect, further inhibiting demineralization. We expect that larger quantities of B, Na, Si, and Sr ions were released from the 30 wt% S-PRG filler-containing paste than from the 5 wt% S-PRG filler-containing paste, although the elution rate into an equivalent amount of the solution differed for each element.

Recently, various novel remineralization agents, including nano-HA and casein phosphopeptide amorphous calcium phosphate nanocomplexes, have been developed[12,13]. Nano-HA is biocompatible and bioactive, and its nano-sized particles are similar to the apatite crystal of tooth enamel[11,12,24]. It has been shown that nano-HA has the ability to remineralize caries legions in enamel and dentin[11,12]. In remineralization process of the porous etched enamel surface, the nano-HA may be served as a template for remineralization, which may control the nucleation and growth of mineral crystal to form enamel-like structure. The precipitation process may attracts Ca^{2+} and PO_4^{3-} from the environment to the enamel surface and fill vacant positions in the crystal structure[25]. However, enamel polishing using Renamel (which contains nano-HA) did not result in improved buffering capability and did not inhibit demineralization of the enamel surface. A possible reason for this is the nano-HA may have low solubility due to the high degree of crystallinity, and it is well known that amorphous or smaller imperfect crystals have a higher dissolution rate compared with crystalline compounds[26]. However, further research is needed to confirm the crystal structure of the nano-HA.

To investigate the remineralization and demineralization of enamel and dentin *in vitro*, several studies have used pH cycling experiments

(demineralizing and remineralizing periods) to simulate the oral environment. We used a demineralization-only model to facilitate investigation of the buffering capability of S-PRG filler-containing pastes. The test protocol used here differs from *in vivo* conditions, making it difficult to compare our results with published data due to the inconsistent test configuration and inconsistent assumptions and approximations[27].

In general, the enamel surface is often aprismatic and more highly mineralized than the enamel subsurface[28]. However, here the enamel surface was removed completely by the polishing process to obtain flat specimens in an attempt to standardize the nanoindentation tests. Therefore, the mechanical properties of the polished (bulk) enamel might differ slightly from those of the tooth surface. Microhardness measurements using a Knoop indenter have been used to quantitatively investigate enamel demineralization[19,21,22]. There is a strong correlation ($0.84<r^2<0.92$) between the microhardness of enamel and the mineral content, as determined by transverse microradiography of carious lesions[19,21]. Although conventional microhardness testing generally results in large indentations (*i.e.*, an indentation length equivalent to a width of 5–20 enamel prisms) and is influenced by the substrate[19], recent advances in nanoindentation techniques have enabled measurement of the mechanical properties of extremely small volumes of material, and both the hardness and elastic modulus can be determined simultaneously based on load-displacement data at the submicron scale[28-30]. Changes in the elastic modulus of enamel may be associated with the brittleness of the structure; thus, investigating the elastic modulus of enamel provides valuable data.

We examined the enamel surface using SEM. The surface morphology following immersion in an acidic environment is expected to vary with the buffering capability of the paste. The specimens polished with 0% S-PRG filler-containing paste and Merssage Plus exhibited very porous surfaces with numerous visible prisms, consistent with demineralization of the enamel surface. The specimens polished with the 5 and 30% S-PRG filler-containing pastes and Renamel exhibited significantly smoother surfaces, suggesting that the multiple ions released from the pastes improved the buffering capability of the enamel surfaces.

CONCLUSIONS

Under the condition of this study, the following conclusions can be drawn:

1. The single application of a paste containing an S-PRG filler may raise the pH of the environment surrounding the tooth and inhibit demineralization of the enamel, as well as assist in remineralization.
2. Further investigation is required to identify effective application methods and specific protocols.

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