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Effects of immersion in solution of an experimental toothpaste containing S-PRG filler on like-remineralizing ability of etched enamel

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This study investigated the like-remineralizing ability of experimental toothpaste containing surface reaction-type pre-reacted glass-ionomer (S-PRG) filler on etched enamel. Human enamel blocks were etched with 35% phosphoric acid and immersed in 5-mL distilled water, fourfold diluted solution of NaF-containing toothpaste, or S-PRG filler-containing experimental toothpaste. Nanoindentation testing was carried out during immersion and the enamel surfaces were observed by scanning electron microscopy. Elemental analysis of the ions in each solution was performed using inductively coupled plasma atomic emission spectroscopy and fluoride electrode. After 1 month of immersion, the hardness and elastic modulus of the specimen immersed in S-PRG filler-containing toothpaste showed significantly greater values than those of the specimen immersed in NaF-containing toothpaste. Considerable amounts of Al, B, Na, Si, Sr, F ions were detected in the solution of S-PRG filler-containing toothpaste. Experimental S-PRG filler-containing toothpaste may enhance the like-remineralizing ability of etched enamel surfaces due to its ion-releasing ability.

Keywords: Remineralization, Enamel, Nanoindentation, Ion-releasing, S-PRG

INTRODUCTION

An acid-etching technique was introduced in the 1950s by Buonocore[1)]. Since the direct bonding of orthodontic brackets to enamel was introduced by Newman[2)] in the mid 1960s, acid-etching of enamel surfaces for bracket bonding procedures has been accepted in clinical orthodontics[3)]. Also, pit-and-fissure sealant using resin-based materials that require an acid-etching process has been accepted to prevent occlusal caries in pediatric dentistry[4)]. A previous *in vitro* study[5)] showed that the mechanical properties of the enamel surface region were decreased by etching-based bracket bonding, and irreversible alteration of the enamel might increase the risk of enamel micro cracks during debonding procedures. Therefore, the enhancement of remineralization in etched enamel regions is ideally required after bracket bonding and resin-based sealant application.

To enhance the remineralization of etched enamel surfaces, increased calcium or fluoride concentrations in oral fluids would seem reasonable[6,7)]. Because fluoride plays an important role in the prevention of demineralization, fluoride-containing products, such as toothpaste, mouth rinse, fluoride-releasing adhesives and sealants, have been widely introduced in dentistry[8-10)]. Surface pre-reacted glass-ionomer (S-PRG) technology, which forms a stable glass-ionomer phase in fillers by pre-reacting acid-reactive glass-containing fluoride with polycarboxylic acid in the presence of water, was introduced recently[11,12)], and the S-PRG fillers have the ability to release Al, B, F, Na, Si, and Sr ions. Si and F ions are known to be strong inducers of remineralization of the dentin matrix[13)]. Sr and F ions also improve the acid resistance of teeth by acting on hydroxyapatite to convert it to strontiumapatite[14,15)] and fluoroapatite, respectively[14,16,17)].

The purpose of this *in vitro* study was to investigate the like-remineralizing ability of a new experimental toothpaste containing S-PRG filler on a human etched enamel surface. Conventional fluoride-containing toothpaste was used as a reference for comparative purposes. We hypothesized that application of S-PRG filler-containing toothpaste would result in like-remineralizing ability equal to that of fluoride-containing toothpaste.

MATERIALS AND METHODS

Materials

Eighteen human noncarious premolars, obtained by extraction from patients, who were undergoing orthodontic treatment, were subjected to nanoindentation tests of the buccal enamel surface. Surfaces before and after nanoindentation testing were observed using a scanning electron microscope (SEM). Figure 1 shows a schematic illustration of the specimen preparation sequence for the *in vitro* like-mineralization of etched enamel surfaces. All premolars were cut with a slow-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL) so that they were divided into

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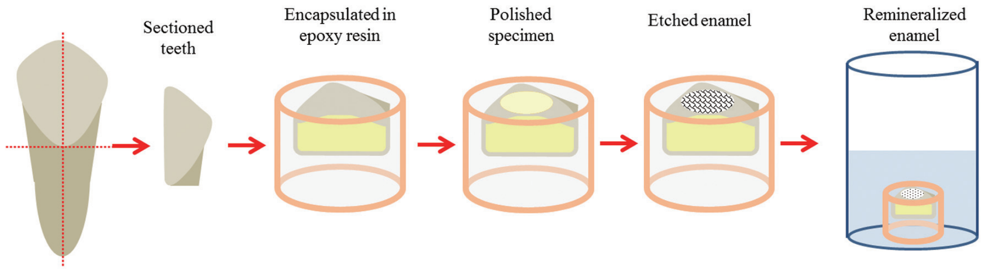

Fig. 1 Schematic illustration of the specimen preparation sequence for *in vitro* mineralization of an etched enamel surface.

mesial and distal halves after their roots were cut off; the sectioned specimens were then encapsulated in epoxy resin (Epofix, Struers, Copenhagen, Denmark). After 24 h, the specimens were ground slightly (600-grit sandpaper) and polished using diamond suspensions. This polishing procedure removed approximately 200 µm of the tooth surface; polished-surface enamels with an approximate area of 4×4 mm were finally obtained.

S-PRG filler was used to create an experimental toothpaste, which contained hydrated silica, carboxy methylcellulose, sodium, glycerol, sorbitol, sodium lauryl sulfate, flavor and SPR-G filler (5 and 30 wt%). The technique of S-PRG filler manufacture is shown in a previous publication that described the effects of S-PRG fillers on mineral induction by phosphoproteins[12].

Etching by phosphoric acid and remineralization by immersion in each solution

The specimens (polished buccal enamel surfaces with approximate areas of 4×4 mm) were etched with 35% phosphoric acid gel (Transbond XT Etching Gel, 3M Unitek, Monrovia, CA) for 15 s, washed for 20 s, and dried with an air stream (Fig. 1). The etched specimens were then immersed in 5 mL of distilled water in a plastic vial, fourfold-diluted solution of NaF (950 ppm)-containing toothpaste (Check-Up Standard, Lion Dental Products), or fourfold-diluted solution of S-PRG filler-containing (5 or 30 wt%) experimental toothpaste at 37°C for 3 months (n=7).

Measurements of hardness and elastic modulus by nanoindentation tests

Nanoindentation testing of buccal enamel surfaces, involving production of five indentations in different regions of each specimen, was carried out at 28°C (ENT-1100a, ELIONIX, Tokyo, Japan) using a 10 mN load before etching, after etching, and during immersion periods (1 or 3 days, 1 week, 1 or 3 months). Linear extrapolation methods (ISO Standard 14577) were used for the unloading curve between 95% and 70% of the maximum test force to calculate the elastic modulus[18]. The hardness and elastic modulus of the buccal enamel surfaces were calculated using the software provided with the nanoindentation apparatus.

SEM observations and energy dispersive spectroscopy (EDS)

After nanoindentation testing of the buccal enamel surface (after immersion for 3 months), 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 using an SEM operating at 15 kV. Surfaces of non-etched and etched specimens were observed for comparison. The composition of the specimen surfaces (Ca, P, Sr, Al, Na, F) for representative specimens was determined by EDS analysis with a working distance of 15 mm. Five locations for each specimen were chosen for analysis.

Analysis of released ions

Fourfold-diluted NaF-containing toothpaste and S-PRG filler-containing toothpastes (toothpaste: 12 g; distilled water: 36 g) were prepared. After 24 h, elemental analysis of the ions in supernatant liquid of each solution after centrifugal separation was performed using ICP-AES (inductively coupled plasma atomic emission spectroscopy) (ICPS-8000, Shimadzu, Kyoto, Japan) after preparing calibration curves with ionic concentrations from 0 to 20 ppm corresponding to each element (n=4). The ionic fluoride concentration in the solution was also quantified using fluoride ion-selective electrodes (Model 720A, Orion Research, Beverly, MA, USA) connected to an ion analyzer (F-53, Horiba). To stabilize the pH, 0.1 mL of TISAB III (Orion Research) was added to the solution. The electrodes were calibrated with a series of standard fluoride solutions with ionic concentrations from 0.02 to 20 ppm of fluoride. A calibration graph was prepared and used to calculate the concentration of fluoride in each sample solution.

Statistical analysis

The experimental results were analyzed using PASW Statistics software (ver. 18.0J for Windows, IBM, Armonk, NY). The mean hardness and elastic modulus values, along with the standard deviations, for the five groups were compared by one-way ANOVA, followed by Tukey's test. For all statistical tests, significance was predetermined at p<0.05.

RESULTS

Figure 2 shows mean values of hardness and elastic modulus of the buccal enamel surface before etching, after etching, and during the 3-month immersion period. The results of the statistical comparisons of the four specimen groups are summarized in Tables 1 and 2. There was no significant difference in the hardness or elastic modulus in any group before etching and immediately after etching. Although the hardness and elastic modulus values of specimens immersed in distilled water after etching were constant during the 3-month immersion, the values of the other three specimens increased gradually. After 1 and 3 months' immersion, the hardness and elastic modulus of the specimen immersed in S-PRG filler-containing toothpaste showed significantly greater values than that of the specimen immersed in NaF (950 ppm)-containing toothpaste.

The polished buccal enamel surface after etching with 35% phosphoric acid for 15 s showed a very porous surface and numerous enamel prisms could be observed, reflecting a typical honeycomb pattern (Fig. 3). After immersion for 3 months in the solutions, the honeycomb-like structure caused by acid etching was partly filled by a like-remineralized layer. In particular, S-PRG filler-containing toothpaste (30%) showed progressive micro-particle deposition. The elements detected by EDS on the surface of the enamel are summarized in Table 3. Similar amounts of calcium, phosphate, fluorine and strontium were detected in all specimens.

Table 4 summarizes the amounts of ions detected in each solution after 24 h. Several ions, such as Al, B, Na, Si, Sr, F, were detected in the solutions containing NaF-containing toothpaste and S-PRG filler-containing toothpaste. Similar amounts of fluoride ion were detected in the NaF-containing toothpaste specimen and S-PRG filler-containing toothpaste specimen. Strontium ion was detected only in the S-PRG filler-

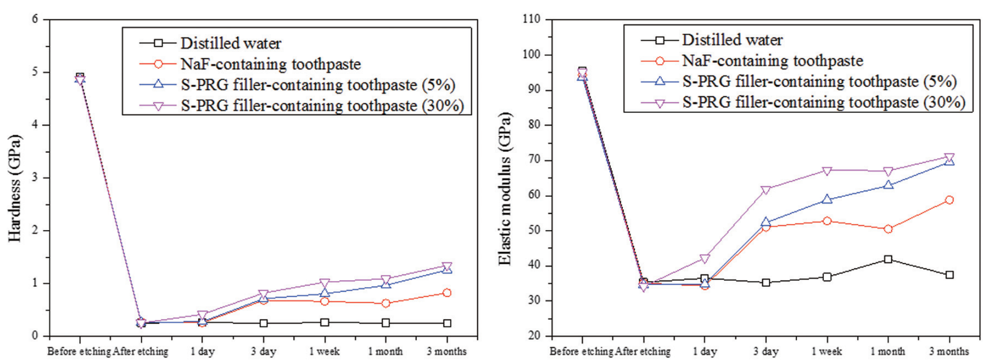

Fig. 2 Mean hardness and elastic modulus values of the buccal enamel surface before etching, after etching and during a 3-month immersion period.

Table 1 Mean values for hardness of the buccal enamel surface before etching, after etching and during 3 months of immersion periods (GPa)

	Distilled Water		NaF-containing toothpaste		S-PRG filler-containing toothpaste (5%)		S-PRG filler-containing toothpaste (30%)		1-way ANOVA *p* value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Before etching	4.91	0.56	4.88	0.5	4.87	0.46	4.87	0.32	0.978
After etching	0.25	0.09	0.27	0.15	0.26	0.09	0.25	0.08	0.895
1 day	0.26^{a}	0.09	0.26^{a}	0.1	0.28^{a}	0.15	0.42^{b}	0.18	0.0001
3 days	0.25^{a}	0.09	0.69^{b}	0.31	0.71^{b}	0.29	0.82^{b}	0.22	0.0001
1 week	0.26^{a}	0.44	0.66^{b}	0.2	0.81^{b}	0.33	1.03^{c}	0.34	0.0001
1 month	0.25^{a}	0.09	0.63^{b}	0.21	0.97^{c}	0.34	1.09^{c}	0.41	0.0001
3 months	0.25^{a}	0.07	0.83^{b}	0.22	1.26^{c}	0.44	1.35^{c}	0.42	0.0001

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

Table 2 Mean values for elastic modulus of the buccal enamel surface before etching, after etching and during 3 months of immersion periods (GPa)

	Distilled Water		NaF-containing toothpaste		S-PRG filler-containing toothpaste (5%)		S-PRG filler-containing toothpaste (30%)		1-way ANOVA *p* value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Before etching	95.5	6.68	94.25	5.6	93.65	5.52	95.11	5.33	0.535
After etching	35.35	10.36	35.16	9.24	34.71	11.78	34.21	10.2	0.969
1 day	36.56^{a,b}	8.7	34.4^{a}	8.8	34.85^{a}	9.92	42.29^{b}	10.78	0.003
3 days	35.29^{a}	7.53	51.07^{b}	15.4	52.35^{b}	14.57	61.87^{c}	14.9	0.0001
1 week	36.86^{a}	25.3	52.81^{b}	11.6	58.83^{b,c}	14.67	67.27^{c}	12.02	0.0001
1 month	41.89^{a}	10.95	50.51^{a}	11.18	62.85^{b}	13.24	67.09^{b}	20.19	0.0001
3 months	37.42^{a}	10.4	58.79^{b}	9.7	69.55^{c}	16.2	71.2^{c}	15.2	0.0001

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

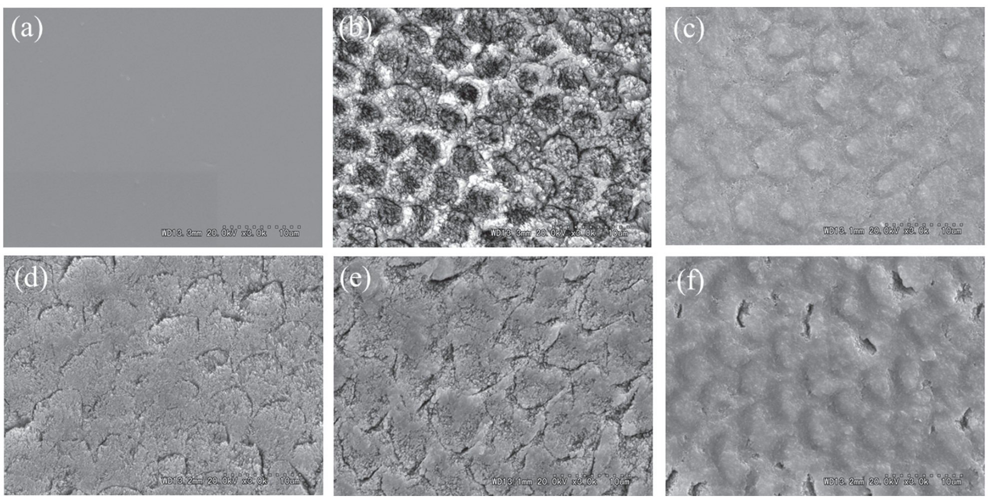

Fig. 3 SEM photomicrographs of (a) the original enamel surface, (b) an etched enamel surface, (c) a enamel surface after immersion in distilled water for 3 months, (d) a enamel surface after immersion in solution of NaF-containing toothpaste for 3 months, (e) and (f) enamel surface after immersion in solutions of S-PRG filler-containing experimental toothpastes [(e) 5%; (f) 30%] for 3 months.

Table 3 Composition of the enamel surfaces (wt. %)*

Elements	Ca	P	F	Na	Sr	Al
Original enamel surface	74.3	16.1	3.1	1.1	4.8	0.4
Etched enamel surface	70.3	19.2	5.6	1.4	2.8	0.6
Distilled water	76.4	12.4	4.3	1.3	4.8	0.8
NaF-containing toothpaste	73.1	17.2	5.5	1.5	2.2	0.6
S-PRG filler-containing toothpaste (5%)	69.2	19.4	5.8	1.5	3	1.1
S-PRG filler-containing toothpaste (30%)	67.9	20.8	5.5	1.3	3.3	1.2

* Determined by EDS.

Table 4 Mean amounts of released ions (ppm)

Elements	Al	B	Na	Si	Sr	F
NaF-containing toothpaste	0.1	0.6	555.6	42.0	—	325.5
S-PRG filler-containing toothpaste (5%)	353.9	348.8	1259.2	7.1	824.2	383.0
S-PRG filler-containing toothpaste (30%)	322.8	798.8	1489.0	13.4	1282.8	234.5

containing toothpaste specimens. Greater amounts of Al, B and Na ions were detected in the S-PRG filler-containing toothpaste specimens compared to the NaF-containing toothpaste specimen.

DISCUSSION

Etched enamel adjacent to brackets is susceptible to further demineralization. Additionally, because the enamel surface after bracket bonding using the conventional etch-and-rinse method has poor mechanical properties[5], the deteriorated enamel may lead to micro cracks within the enamel structure as a result of stress during orthodontic treatment and may also cause enamel fracture during debonding procedures[2,19]. Also, pit-and-fissure sealant with resin-based materials requires an acid etching process. The acid etching destroys the enamel surface integrity, causing deep-seated caries and reducing the caries-preventive effect of the sealant[4]. Thus, etched enamel after bracket bonding and sealant should ideally be remineralized as soon as possible. Because fluoride improves the acid resistance of enamel by acting on hydroxyapatite to convert it to fluoroapatite[14,16,17] and enhances mineral uptake during remineralization[20], fluoride-containing products, such as toothpaste, mouth rinse, and fluoride-releasing adhesives, have been used widely in clinical orthodontics[8-10]. The present *in vitro* study showed that the like-remineralization efficacy of S-PRG filler-containing toothpaste for etched enamel was superior to that of NaF-containing toothpaste. Compared with the etched enamel surface, the mechanical properties of the specimens immersed in the solution of S-PRG filler-containing toothpaste after 3-month period were significantly enhanced by *in vitro* like-remineralization effects. However, the loss of hardness and elastic modulus of the enamel surface after acid etching was not totally recovered after the *in vitro* like-remineralizing behavior. The decreased hardness recovered by 24% and the elastic modulus by 61% after a 3-month immersion in S-PRG filler-containing (30 wt%) toothpaste; these values were higher than those for NaF-containing toothpaste (12% for hardness, 40% for elastic modulus). According to the data for periods of less than 1 month, the solution of S-PRG filler-containing (30 wt%) toothpaste showed earlier recovery of hardness and elastic modulus than the solution of NaF-containing toothpaste; this may be an advantage of S-PRG filler-containing toothpaste. Thus, the null hypothesis (S-PRG filler-containing toothpaste would result in like-remineralizing effects equal to those of fluoride-containing toothpaste) was partially rejected.

Although the mechanism of ion release from S-PRG filler is not completely understood, it was believed to have been due to the presence of a glass-ionomer phase around the glass core of the filler. In the present study, a considerable amount of ions, such as Al, B, Na, Si, Sr, F, were detected in the solution of S-PRG filler-containing toothpaste; this is in agreement with a previous finding[11]. These ions released from the S-PRG toothpaste may have contributed to the improvement of the mechanical properties of the etched enamel surface. However, the elemental analysis by EDS on the surface of the enamel after 3 months *in vitro* like-remineralization showed that similar amounts of calcium, phosphate, fluorine and strontium were detected in all specimens. A likely reason is that the depth from the enamel surface for like-remineralization behavior by released ions might be smaller than the detectable depth of the EDS analysis (approximately 1 μm). Further studies will be needed for depth-profiling using more surface-sensitive analytical methods such as X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometer (TOF-SIMS). Si and Al are elements that form the structure of glass, while Sr and F are added into glass as a modifier. B plays both roles and is highly soluble. Regarding S-PRG filler-containing toothpaste with different concentrations, greater amounts of B, Na, Si and Sr ions were released from 30 wt% S-PRG filler-containing toothpaste than from 5 wt% S-PRG filler-containing toothpaste. However, the lower levels of Al and F ions were released from 30 wt% S-PRG filler-containing toothpaste than from 5 wt% S-PRG filler-containing toothpaste. A likely reason is that the elution rates with balance related to an equivalent amount of distilled water were different for each element. Strontiumapatite and fluoroapatite might have formed on the etched enamel surface by the released Sr and F ions. Because Sr is structurally similar to Ca, it might have replaced the calcium released from the enamel surface[21]. In the present study, a detectable amount of strontium was released from the enamel surfaces immersed in S-PRG filler-containing toothpaste and this might confirm the hypothesis that strontiumapatite was formed on the etched enamel surface. Previous studies indicated that Al (aluminum lactate) is effective for dental hypersensitivity[22] and B (boron-containing compound) has antibacterial and anti-inflammatory properties[23]. The Al and B ions released from S-PRG particles

in the present study might not contribute to like-remineralization behavior of enamel surfaces.

The enamel surface is often aprismatic and more highly mineralized than the enamel subsurface[24]. However, the enamel surface was removed completely by the polishing process in the present study to obtain flat and polished specimens in an attempt to standardize specimens for nanoindentation testing. Therefore, the mechanical properties of the polished (bulk) enamel might differ slightly from those of the top surface region. On the other hand, to investigate remineralization and demineralization of enamel and dentin *in vitro*, many recent studies used pH-cycling experiments (demineralizing and remineralizing periods) to simulate the real oral environment. However, we used a remineralization-only model because it facilitated determination of the remineralization ability of S-PRG filler-containing toothpaste. The test protocol in the present study differs from *in vivo* conditions, and various methods have been used to assess deminerization and remineralization of teeth. It is difficult to compare our results with values in the literature, due to the multiple test configurations, assumptions and approximations integrated into the experimental methodologies[25]. Because we did not assess the effects of combinations of saliva and S-PRG filler-containing toothpaste on the remineralization of etched enamel surfaces, further research is needed to assess the effects of this combination. In addition, we used distilled water for immersion to investigate the like-remineralizing ability of NaF-containing toothpaste; thus the remineralization ability of NaF-containing toothpaste could be improved using a Ca-containing environment, such as artificial saliva.

After *in vitro* remineralization for 3 months, the honeycomb-like structure caused by acid etching was partially filled by deposited layers, which were thicker for specimens immersed in the solution of S-PRG filler-containing (30 wt%) toothpaste than those of other solutions. Although the specimens immersed in distilled water, the solution of the NaF-containing toothpaste, and the solution of S-PRG filler-containing (5 wt%) toothpaste showed similarly thin deposited layers, their mechanical properties were different and there may be differences in the quality of the layers.

The use of microhardness measurements with a Knoop indenter has been a popular method for quantitatively investigating the demineralization of enamel[26,27]. Recent advances in the nanoindentation test allow the measurement of mechanical properties with extremely small volumes of material compared with the Knoop indenter. The hardness and elastic modulus can be determined simultaneously[28-30], and the elastic modulus of enamel is an important mechanical factor. Because enamel deteriorated by etching may develop cracks within its structure as a result of stress during orthodontic treatment and bracket debonding, nanoindentation testing for determining the fracture toughness should be investigated in future research to understand the changes in brittleness during demineralization and remineralization of the enamel structure.

From the findings of the present study, the daily application of toothpaste containing S-PRG may raise the pH in the surrounding environment, and inhibit demineralization of enamel, in addition to assisting remineralization of etched enamel. Furthermore, application of S-PRG toothpaste using a custom tray before sleep may also facilitate like-remineralization of the enamel surface. Further investigation is required to identify more effective application methods and specific protocols.

CONCLUSIONS

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

1. The experimental S-PRG filler-containing toothpaste may enhance like-remineralizing ability of an etched enamel surface due to its ability to release various ions; the efficacy was found to be superior to that of NaF (950 ppm)-containing toothpaste.
2. The mechanical properties of the specimens immersed in the solution of S-PRG filler-containing toothpaste after a 3-month period were significantly enhanced due to the like-remineralizing ability, although the decreased hardness and elastic modulus of the enamel surface due to acid etching was not totally recovered.

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