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Effect of Pre-reacted Glass-ionomer Filler Extraction Solution on Demineralization of Bovine Enamel

K Asano • R Kawamoto • M Iino
T Fruichi • K Nojiri • T Takamizawa
M Miyazaki

Clinical Relevance

S-PRG filler extraction solution has an ability to protect the surface of enamel from demineralization.

SUMMARY

Objective: To determine the effect of pre-reacted glass-ionomer (PRG) filler extraction solution on the demineralization of bovine enamel by measuring changes in the ultrasound transmission velocity.

Methods: The specimens were prepared by cutting bovine teeth into enamel blocks. The specimens were immersed in buffered lactic acid solution for 10 minutes twice a day, and then stored in artificial saliva. Other specimens were stored in PRG filler extraction solution for 10 minutes, followed by 10-minute immersion in the buffered lactic acid solution twice a day. The propagation time of longitudinal ultrasonic waves was measured by a pulser receiver. Six specimens were used for each condition, and analyses of variance followed by Tukey tests (α=0.05) were done.

Results: No changes in sonic velocity were found for specimens stored in the PRG filler extraction solution, indicating that the PRG extraction solution had an effect on inhibiting the demineralization of bovine enamel.

Conclusions: The results obtained with the use of an ultrasound measurement technique suggested that PRG filler extraction solution has the ability to prevent demineralization of enamel.

Kazumasa Asano, DDS, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

Ryo Kawamoto, DDS, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

Masayoshi Iino, DDS, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

Tetsuya Fruichi, DDS, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

Kie Nojiri, DDS, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

Toshiki Takamizawa, DDS, PhD, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

*Masashi Miyazaki, DDS, PhD, Operative Dentistry, Nihon University School of Dentistry, Chiyoda-Ku, Tokyo, Japan

*Corresponding author: 1-8-13 Kanda-Surugadai, Chiyoda-Ku, Tokyo 101-8310, Japan; e-mail: iyazaki.masashi@nihon-u.ac.jp

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INTRODUCTION

The development of erosion involves a chemical process in which the inorganic phase of the tooth is demineralized, thereby reducing the hardness of the

tooth substrates.[1] Subsequent abrasive challenges through brushing increase the loss of the tooth substrates.[2] Dietary changes and inadequate oral hygiene have led to erosion becoming more frequent among young people. This phenomenon is largely due to physical and chemical factors that act in the area of the tooth neck, resulting in enamel loss and dentin exposure. Even in populations with a decreased prevalence of caries, the relative importance of occlusal wear has significantly increased. The inclusion of active ingredients in oral-care products to help prevent enamel loss may contribute greatly to the improvement and maintenance of oral health.

Pre-reacted glass-ionomer (PRG) filler is prepared by an acid-base (glass ionomer) reaction between fluoroaluminosilicate glass and polyacrylic acid in the presence of water, preliminarily forming a stable glass-ionomer phase within the glass particles.[3,4] Upon freeze-drying, the desiccated xerogel is further milled and silane-treated to form PRG fillers of a specific size range. Full reaction-type and surface reaction-type (S-PRG) PRG fillers can be prepared, and this technology is used in the formulation of "giomer" products.[5]

Both types of PRG fillers promote rapid fluoride release through ligand exchange within the pre-reacted hydrogel.[6] The gel phase of the glass core acts as a source of released ions, as the reaction between polyacid and glass powders is thought to produce soluble ions, many of which are released from PRG fillers into the surrounding solution. A previous study showed that PRG filler extraction solution had a modulation effect on an acidic environment as a result of ion release.[7] The release of considerable levels of aluminum (Al), boron (B), fluorine (F), sodium (Na), silicon (Si), and strontium (Sr) from S-PRG filler into surrounding distilled water was detected.[7] Furthermore, although minor amounts of Na are present in S-PRG filler compared with F and Si, more Na was released than other ions. As Na is the only cation eluted to preserve electroneutrality, an equivalent amount of cations is expected to be released.[8] During previous acidic attachment to the glass powder, F and Na were liberated into the matrix, and together formed soluble salt.[9] This might contribute to a rapid release of F into the PRG filler extraction solution, preventing demineralization of the enamel substrate.

Ultrasonic imaging is a noninvasive technique that shows considerable diagnostic potential as well as being a valuable research tool.[10,11] Ultrasonic devices can be used to detect carious lesions[12] and to measure the dentin thickness between the tooth surface and the pulp chamber.[13] Assuming that the enamel substrate is mainly composed of hydroxyapatite, differences in ultrasonic velocity can be related to differences in the degree of mineralization and histologic structures, as the ultrasonic velocity increases proportionally with the volumetric concentration of minerals.[14] Ultrasonic velocity has also been shown to be related to the mineral content of the enamel lesion body, and so is an index of the degree of mineralization.[15] When the tooth substrate suffers demineralization, the mineral volume concentration at the tooth surface, as well as the specific ultrasonic velocity, decreases.

The present study evaluated the effect of PRG filler extraction solution on enamel demineralization by the measurement of changes in ultrasonic velocity using an ultrasonic device, and by observation using scanning electron microscopy (SEM). The null hypothesis was that PRG ion leaching prevented demineralization of enamel substrate.

MATERIALS AND METHODS

Fluoroboroaluminosilicate glass was prepared by fusing 14.0 wt% silica (SiO_2), 27.0 wt% mullite ($3Al_2O_3 \cdot 2SiO_2$), 19.0 wt% boric oxide (B_2O_3), 5.0 wt% cryolite (Na_3AlF_6), 29.0 wt% strontium fluoride (SrF_2), and 6.0 wt% strontium carbonate ($SrCO_3$). The mixture was heated and melted in an arc furnace at 1400°C for two hours. The melted mixture in liquid form was removed from the furnace and quenched in running water, then dried for 12 hours at 150°C in an air oven to obtain glass frit. Analysis of the glass frit with an X-ray fluorescence spectrometer (ZSX100e, Rigaku Corp, Tokyo, Japan) and an X-ray diffractometer (Multiflex 2 kW, Rigaku Corp) showed that the structure was amorphous and consisted of 21.6 wt% SiO_2, 21.6 wt% Al_2O_3, 16.6 wt% B_2O_3, 27.2 wt% SrO, 2.6 wt% Na_2O, and 10.4 wt% F.

The glass frit was coarsely ground with a ball mill (BM-10, Seiwa Giken Co, Hiroshima, Japan) and then wet-ground with an agitator bead mill in the presence of water to obtain irregular-shaped filler particles. The resulting glass slurry was agitated with the addition of polysiloxane (Mitsubishi Chemical Co, Tokyo, Japan) solution (SiO_2 content, 16 wt%), then aged at 50°C for 40 hours, and heat-treated at 120°C for six hours in a heat dryer. The heat-treated solidified material was then disintegrated in a high-speed mixer (FS-GC-20JE, Fukae Powtec Co, Osaka, Japan) to obtain surface-treated glass filler. During stirring in the cutter mixer, the glass filler was subjected to spray treatment with

polyacrylic acid aqueous solution (polymer content, 13.0 wt%) and then treated in a heat dryer at 150°C for three hours to obtain S-PRG filler. The mean particle size of the S-PRG filler was 3.0 μm, as measured using a laser-diffraction particle-size analyzer (Microtrac HRA 9320-X100, Nikkiso Co, Tokyo, Japan).

Distilled water (pH 5.9) was mixed with S-PRG filler at a 1:1 ratio (1 L:1000 g) by weight. The mixture was stirred for 24 hours and centrifuged to precipitate S-PRG filler. The supernatant solution was filtrated using a chromato disk (25A hydrophilic type, diameter 25 mm, pore size 0.2 μm, GL Sciences Inc, Tokyo, Japan) to obtain the test liquid. Elemental analysis of ions (Al, B, Na, Si, and Sr) released from S-PRG filler was performed using inductively coupled plasma atomic emission spectroscopy (ICPS-8000, Shimadzu Co, Kyoto, Japan). Analysis was conducted after preparing calibration curves corresponding to each element. Concentration of F was also analyzed using a fluoride electrode (9609BN, Orion Research Inc, Jacksonville, FL, USA) connected to a pH/ion meter (720A, Orion Research Inc) after preparing calibration curves. The amount (mg/g) of ions released from S-PRG filler was 0.04 for Al, 2.07 for B, 0.09 for F, 0.51 for Na, 0.03 for Si, and 0.25 for Sr.[7]

In total, 18 freshly extracted bovine incisors, without cracks or erosion, were cleaned and stored in physiologic saline for up to two weeks. The teeth were sliced longitudinally at a 1-mm thickness and then cut in the buccolingual direction with a low-speed diamond saw (Buehler Ltd, Lake Bluff, IL, USA). Each slab was carefully shaped into a rectangular form ($4 \times 4 \times 1$ mm) using a super-fine diamond finishing point (ISO #021, Shofu Inc, Kyoto, Japan). Specimen surfaces were ground successively on wet silicon carbide paper with a grit size of 600, 1200, and 2000. The thickness and size of the specimens were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan).

The specimens in the demineralization group (n=6) were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM $CaCl_2 \cdot 2H_2O$, and 0.45 mM KH_2PO_4) for 10 minutes and then placed in artificial saliva (pH 7.0, 14.4 mM NaCl, 16.1 mM KCl, 0.3 mM $MgCl_2 \cdot 6H_2O$, 2.0 mM K_2HPO_4, 1.0 mM $CaCl_2 \cdot 2H_2O$, and 0.10 g/100 mL sodium carboxymethyl cellulose). These procedures were performed twice daily (interval time 10 hours) over the four-week test period, and the specimens were stored between treatments in artificial saliva at

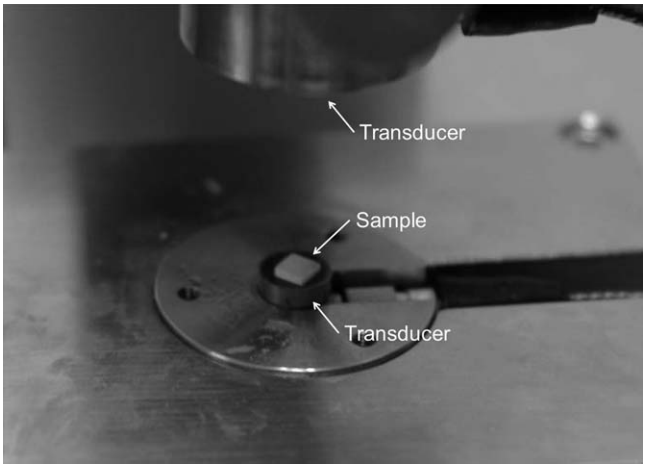

Figure 1. *Image of testing setup.*

37°C. The specimens in the PRG group (n=6) were stored in PRG extraction solution for 10 minutes prior to storage in demineralizing undersaturated 0.1 M lactic acid buffer solution. The specimens in the control group (n=6) were stored in artificial saliva for the same period of time.

The ultrasonic velocity was measured using a pulser receiver (Model 5900PR, Panametrics, Waltham, MA, USA), a transducer for longitudinal waves (V112, Panametrics), and an oscilloscope (Wave Runner LT584, LeCroy Corp, Chestnut Ridge, NY, USA).[16] Measurements were taken before the test, and then on days 1-7, 14, 21, and 28. The equipment was initially calibrated using a standard procedure with 304 stainless steel calibration blocks (2211M, Panametrics) with thicknesses of 2.5, 5.0, 7.5, 10.0, and 12.5 mm.

The transducer was oriented perpendicularly to the contact surface of each specimen, to obtain the echo signal (Figure 1). The ultrasonic waves propagated from the transducer to the tooth were transmitted through the tooth and were detected by the transmitter on the opposite side. Each measurement was conducted at 23 ± 1°C and 50 ± 5% relative humidity.

The ultrastructural observation of enamel surfaces was carried out using field-emission (FE) SEM. Specimens were dehydrated in ascending concentrations of tert-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for two hours) and then transferred to a critical-point dryer for 30 minutes. The surfaces were coated in a vacuum evaporator (Quick Coater Type SC-701, Sanyu Denshi Inc, Tokyo, Japan) with a thin film of Au. Specimens were observed by FE-SEM (ERA

Table 1: *Average Ultrasonic Velocities (m/s) of Bovine Enamel Specimens by Treatment*[a]

Group	Treatment Time, d				
	0	7	14	21	28
Control	6110 (80)	6114 (99)	6112 (101)	6113 (105)	6114 (108)
De	6050 (165)	5663 (140)[bc]	5598 (143)[bc]	5580 (146)[bc]	5523 (150)[c]
PRG	6129 (80)	6251 (98)	6267 (92)	6281 (94)	6290 (94)

Abbreviations: De, demineralization group; PRG, prereacted glass-ionomer filler extraction solution.
[a] *Data are shown as mean (standard deviation). n=6 per group.*
[b] *Significant within-group difference from mean at days 0 and 28.*
[c] *Significant between-group difference.*

8800FE, Elionix Ltd, Tokyo, Japan) at an accelerating voltage of 10 kV.

The ultrasonic velocity data were analyzed by two-way analysis of variance (ANOVA), with time and treatment as factors; time was treated as a repeated measure. *Post hoc* pairwise tests among groups were performed using the Tukey test. The level of significance (p-value) was 0.05. Calculations were performed using Sigma Stat software version 3.1 (SPSS Inc, Chicago, IL, USA).

RESULTS

The average ultrasonic velocities of the enamel specimens are shown in Table 1 and Figure 2. The differences between storage periods were greater than expected by chance after allowing for the effects of storage conditions, so multiple comparisons were conducted on the data. The average ultrasonic velocity in intact bovine enamel (control group) ranged from 6090 to 6119 m/s and did not vary significantly with treatment time. The ultrasonic velocities in the demineralization group decreased and were significantly lower than those in the control group after seven days. There was no significant change in the ultrasonic velocity with treatment time in the PRG group, and no significant difference from the control group was detected up to 28 days.

Representative SEM images of enamel specimens are shown in Figure 3. SEM images of the enamel specimens revealed morphologic differences in treatment effects. Pronounced demineralization of enamel surfaces was observed over the test period in the demineralization group, whereas the PRG group showed relatively minor or no morphologic change.

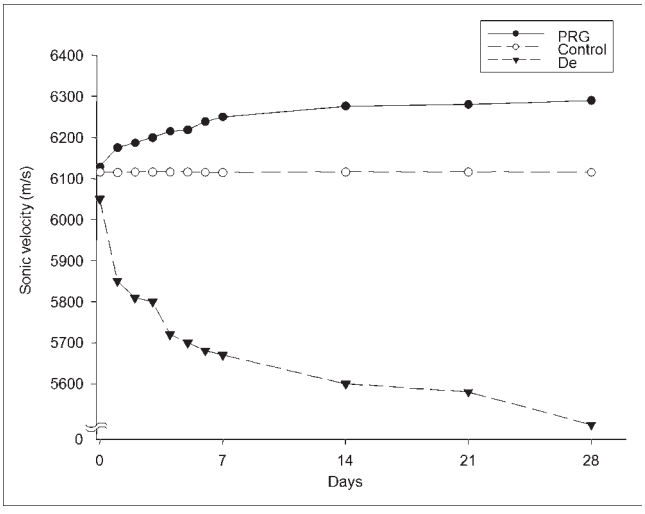

Figure 2. *Influence of storage conditions on changes in ultrasonic velocities of enamel specimens.*

DISCUSSION

Comparative data on the properties of human and bovine hard dental tissue are scarce; however, bovine enamel is widely used as a substitute for human enamel.[17] Human teeth are thought to be most relevant for conducting *in vitro* studies.[18] However, bovine teeth were used in the present study because they are easy to obtain in large quantities, are in good condition, and have fewer composition variables. Bovine teeth have large flat surfaces and have not undergone prior caries challenges that might affect test results.[19] Moreover, structural changes and the mineral distribution of

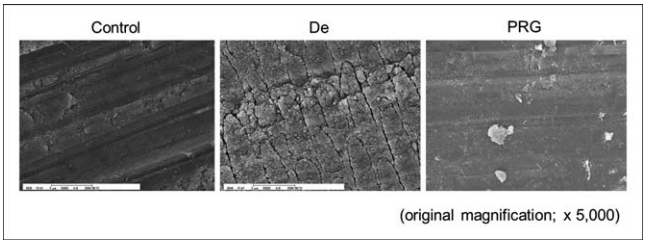

Figure 3. *Representative FE-SEM images of dentin surfaces (scale bar, 5 μm). SEM observations revealed differences in morphologic features among storage conditions. Demineralization of the enamel surfaces was more pronounced in the demineralization group, whereas the PRG group showed only slight morphologic changes compared to those of the control group.*

carious lesions are reported to be similar in human and bovine teeth.[20]

Enamel is a mineralized material with a highly complex hierarchical structure that is composed mainly of aligned rods arranged almost perpendicular to the tooth surface. Enamel should be modeled as an anisotropic material,[21] with both the longitudinal section and the buccal surface shown to be elastically anisotropic, so the orientation of the enamel prism might modify the ultrasonic velocity.[22] In a longitudinal section, the anisotropy appears to be closely related to the prismatic orientation.[23] The enamel prisms are at different angles to the direction of the ultrasonic beam according to the tooth section plane. Therefore, to avoid any effect on ultrasonic velocity, we obtained enamel specimens from the labial surfaces of bovine teeth.

Our results demonstrated changes in the ultrasonic velocity of enamel in the control group as a function of demineralization time, although they were not linear (Figure 2). The ultrasonic velocities of specimens in the PRG group did not change significantly with time. Twice-daily application of PRG filler extraction solution resulted in the maintenance of normal ultrasonic velocity for the enamel, contrary to the reduced velocity observed in the demineralization group. These results are consistent with our previous study using casein phosphopeptide-amorphous calcium phosphate to prevent demineralization.[24] The PRG filler extraction solution therefore appeared to prevent enamel demineralization. These results were supported by the absence of signs of demineralization in the PRG group according to SEM (Figure 3). The S-PRG fillers released Al, B, F, Na, Si, and Sr ions.[7] Silicate and fluoride are known as strong inducers of remineralization of the dentin matrix.[25] Strontium and fluoride also improve the acid resistance of teeth by acting on hydroxyapatite to convert it to strontium apatite and fluoroapatite, respectively.[26]

Although the role of F in forming acid-resistant tooth substrate is well documented,[27] the influence of other ions is less well defined. Among the ions released from S-PRG filler, Sr is thought to play a role in tooth mineralization.[28] The effect of Sr on enamel remineralization was previously investigated, and it appeared to have the capacity to enhance enamel remineralization in conjunction with F.[29] Si is thought to promote hydroxyapatite formation, as hydroxyapatite nucleation has been shown to be triggered in the presence of silica gel.[30] Hydrated silica gel has sufficient silanol groups to induce apatite nucleation on its own surface; nucleation then proceeds by taking Ca and P from the surrounding environment. Another report suggested that Si released from bioactive glass particles is absorbed onto the substance, thus providing sites for heterogeneous CaP nucleation. Once nucleated, it spontaneously grows in solution to form a bone-like apatite layer.[31] The effects observed in the present study are likely to provide clinical benefits after long-term usage in the oral environment.

Oral hygiene products, such as toothpaste with antiplaque chemicals or functionally designed toothbrushes, have been shown to provide benefits in terms of plaque removal.[32] However, the consequences of abrasion from both toothbrushes and toothpaste are not fully understood.[33] Here we tested a liquid formulation of PRG filler extraction solution, which can be used similar to mouth rinse or applied with cotton swabs. The use of this solution might reduce sensitivity caused by home whitening procedures through the prevention of demineralization. The inclusion of active ingredients in oral-care products to help prevent dental disease has been shown to contribute greatly to the improvement and maintenance of oral health. Further research is needed into whether PRG-filler extract solutions can act as an oral hygiene material in clinical situations.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the S-PRG filler extraction solution has an ability to prevent demineralization of the enamel. Released ions such as Al, B, F, Na, Si, and Sr from the S-PRG fillers might improve the acid resistance of teeth.

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Conflict of Interest

The authors certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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