Optical coherence tomography examination of the effect of S-PRG filler extraction solution on the demineralization of bovine enamel

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The purpose of this study was to determine the effect of PRG filler extraction solution on the demineralization of enamel using optical coherence tomography (OCT). Bovine enamel was treated with lactic acid buffer solution and then placed in artificial saliva (De group). In the second group, specimens were stored in PRG filler extraction solution followed by immersion in lactic acid buffer solution (PRG group). In the control group, specimens were simply stored in artificial saliva. From the OCT image, the peak intensity (dB) and width at (1/e²) were obtained, and the integrated value was calculated. The data were analyzed using Tukey-Kramer tests (α=0.05). There was a slight but significant increase in the integrated value observed for the control group, and a slight but significant decrease in the value observed for the De group. For the PRG group, integrated values were doubled after seven days from the start of the experiment.

Keywords: Enamel, Demineralization, OCT, S-PRG filler, Released ions

INTRODUCTION

In the normal oral environment, there is continuous demineralization and remineralization of the tooth structure. When this balance is disrupted, demineralization can lead to a carious lesion. During the initial stages, decayed lesions are characterized by dissolution of the hydroxyapatite, which leads to a non-uniform mineral loss and an increase in the porosity of the subsurface area of the enamel. Estimations of the amount of enamel subsurface porosity can be used to detect and quantify the early stages of carious lesion. By being able to detect and diagnose carious lesions at an early stage, dentists can prevent progression of the decay, and avert the need for invasive removal of sound tooth structures. However, an accurate diagnosis of the initial stages of the decayed lesion including an assessment of the affected depth can be particularly challenging due to hypermineralization of the outer enamel surface that masks the invisible underlying lesion.

Pre-reacted glass-ionomer (PRG) filler is prepared by an acid-base reaction between fluoroaluminosilicate glass and polyacrylic acid in the presence of water, which preliminarily forms a stable glass-ionomer phase. It has been reported that this single-step adhesive containing PRG fillers has an ion-releasing ability that results in the uptake of fluoride by the tooth substrate that is adjacent to the adhesive, along with a decreased demineralization in the corresponding areas after an acidic attack. The use of surface-reacted (S)-PRG fillers has been shown to promote rapid fluoride release through a ligand exchange within the pre-reacted hydrogel. In addition to the F ion, the S-PRG fillers also release Al, B, Na, Si and Sr ions. Si is known as a strong inducer of the remineralization of the dentin matrix. F and Sr also improve the acid resistance of teeth by acting on hydroxyapatite to convert it to fluoroapatite and strontium-apatite. The S-PRG filler also has a modulation capacity and when it comes in contact with water or acidic solutions, it will change the pH of the surrounding environment to a weak alkaline range.

Recently, many diagnostic techniques have been developed for detecting early carious lesions that are the result of the demineralization process. Optical coherence tomography (OCT) can be used for non-destructive imaging of the demineralization of the tooth without having to remove the surface or expose patients to radiation. Time-domain (TD) OCT is a form of OCT that utilizes low coherence interferometry to determine the echo time delay, along with the magnitude of backscattered light that is reflected from a sample. TD-OCT combines light from a low coherence light source with a Michelson interferometer to produce cross-sectional images of the tissue structures. The images are generated as a result of an interaction between a partially coherent beam of optical radiation and components of the sample.
S-PRG filler was obtained using the same method described in a previously published report on the extraction solution of the filler\(^9\). Briefly, distilled water was mixed with S-PRG filler at a 1:1 ratio (1 L:1,000 g) by weight. A chromatodisk (25A Hydrophilic Type; diameter, 25 mm; pore size, 0.2 μm; GL Sciences Inc., Tokyo, Japan) was used to filter the supernatant solution and obtain the test liquid. Elemental analysis of ions released from the S-PRG filler was performed according to a previously described method\(^9\). The amount (mg/g) of ions released from the S-PRG filler were 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\(^9\).

Bovine incisors from 2–3-year old cattle were used as substitutes for human teeth. After separating root with a low-speed diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA), the pulps were removed. After slicing the lingual surfaces of bovine incisors with the diamond saw, a super-fine diamond point (ISO #021, Shofu Inc., Kyoto, Japan) was used to carefully shape each slab into a rectangular form that was 4 mm×4 mm×2 mm in size. Each surface of the specimen was ground on wet silicon carbide paper through the use of successive grit sizes (range #600 to #2,000). The thickness and size of the specimens were measured using a dial gauge micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan). After the preparation, a total of six specimens in each group were treated as follows:

1) DE group: Specimens were treated with undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM CaCl\(_2\)·H\(_2\)O; 0.45 mM KH\(_2\)PO\(_4\)) for 10 min and then placed in artificial saliva (pH 7.0, 14.4 mM NaCl; 16.1 mM KCl; 0.3 mM MgCl\(_2\)·6H\(_2\)O; 2.0 mM KH\(_2\)PO\(_4\); 1.0 mM CaCl\(_2\)·2H\(_2\)O; 0.10 g/100 mL sodium carboxymethyl cellulose CMC-Na). These procedures were performed twice a day throughout the 4-week test period. All specimens were maintained in artificial saliva at 37°C between treatments.

2) PRG group: Specimens were stored in PRG extraction solution for 10 min prior to storage in demineralizing undersaturated 0.1 M lactic acid buffer solution for 10 min, and then placed in artificial saliva. These procedures were performed twice a day throughout the 4-week test period. All specimens were maintained in artificial saliva at 37°C between treatments.

3) Control group: All control specimens were stored in only artificial saliva for the same period of time.

The TD-OCT imaging system used in this study is shown schematically in Fig. 1. The focused light beam was projected on the selected locations and scanned across the area of interest in two dimensions using a probe attached to a mounting device. Superluminescent diodes with a central wavelength of 1,310 nm, a spectral bandwidth of 40 nm, and an optical output power of 7.5 mW (DL-CS3184B, DensLight Semiconductors, Singapore) were used as the light source. The emission light was coupled to a single-mode fiber-optic Michelson interferometer and delivered to both the reference mirror and the sample. The reference mirror was mounted on a linearly translating galvanometer, which is driven with a triangular voltage waveform with a fringe modulation frequency of 1 kHz. The light was reflected off the mirror and back onto the retroreflector and re-imaged on the reference arm fiber.
Fig. 2 Prove and specimen stage of the TD-OCT. The scanning probe connected to the TD-OCT was set at a fixed distance from the enamel surface. The scanning beam was set at a right angle with respect to the surface of the tooth.

system comparisons were considered to be significant at a level of 0.05. All statistical analyses were carried out using the Sigma Stat software system (Ver. 3.1, SPSS Inc., Chicago, IL, USA).

RESULTS

The TD-OCT images (B-scans) of the specimens are shown in Fig. 3. The abscissa of the tomograms corresponds to the scan depth, while the ordinate corresponds to the vertical measurement position at the tooth surface. A weak and narrow signal without any back-scattered intensity was observed for the control group, with no other changes in the OCT images observed during the actual test period. For the DE group on day 1, there was a weak signal that was visible from the enamel surface and the back-scattered light was well above the noise level. Thus, this led to a grainy appearance of the OCT image. After 28 days, although an area of strong scattering on the enamel surface was visible, the back-scattered grainy appearance was very weak. For the PGR group, the signal from the enamel surface was visible and became broader after 28 days of treatment, with a slight back-scattered intensity observed.

Average signal intensities (dB), widths at the \(1/e^2\) (μm), and integrated values are shown in Tables 1–3. Representative A-scan modes for each condition are shown in Fig. 4. Though there were no significant changes in the signal intensities and the widths at the \(1/e^2\) for the control (−32.1–−34.1 dB) and the De groups (−27.6–−33.8 dB) during test period, significant changes were observed for the PRG group during the first test period, which occurred during days 0–7 (−33.6–−46.1 dB). Different changes were observed in each of the groups for the integrated values. The control group exhibited a slight but significant increase in the integrated value (from 2,889 to 3,351), while a significant decrease in the value was observed for the De group (from 3,042 to 1,932). At 7 days after the start of the experiment in the PRG group, the integrated values were doubled, after which there was a slight increase in the value (from 3,024 to 6,915).
### Table 1: Influence of different treatment procedures on signal intensity (dB)

| Group | Treatment time (d) |          |          |          |          |
|-------|--------------------|----------|----------|----------|----------|
|       | 0                  | 7        | 14       | 21       | 28       |
| Control | $-32.1 (6.4)^*$ | $-32.7 (5.6)^*$ | $-32.1 (4.0)^*$ | $-33.7 (4.9)^*$ | $-34.1 (4.2)^*$ |
| De     | $-33.8 (6.2)^b$  | $-29.6 (7.6)^b$  | $-29.7 (3.8)^b$  | $-28.1 (4.9)^b$  | $-27.6 (3.6)^b$  |
| PRG    | $-33.6 (6.2)^c$  | $-44.3 (6.9)^d$  | $-45.6 (8.3)^d$  | $-45.9 (5.6)^d$  | $-46.1 (4.5)^d$  |

De, Demineralization group; PRG, PRG filler extraction solution. Data are shown as mean (standard deviation). $n=6$ per group. Values in the same group with the same superscript letters indicate no significant different ($p>0.05$).

### Table 2: Influence of different treatment procedures on width at 1/e$^2$ ($\mu$m)

| Group | Treatment time (d) |          |          |          |          |
|-------|--------------------|----------|----------|----------|----------|
|       | 0                  | 7        | 14       | 21       | 28       |
| Control | 90 (12)^a          | 110 (11)^a | 100 (10)^a | 100 (13)^a | 110 (12)^a |
| De     | 90 (10)^b          | 80 (11)^b  | 80 (10)^b  | 70 (11)^b  | 70 (11)^b  |
| PRG    | 90 (11)^d          | 150 (12)^e | 150 (13)^e | 150 (12)^e | 150 (14)^e |

De, Demineralization group; PRG, PRG filler extraction solution. Data are shown as mean (standard deviation). $n=6$ per group. Values in the same group with the same superscript letters indicate no significant different ($p>0.05$).

### Table 3: Influence of different treatment procedures on integrated values (dB•μm)

| Group | Treatment time (d) |          |          |          |          |
|-------|--------------------|----------|----------|----------|----------|
|       | 0                  | 7        | 14       | 21       | 28       |
| Control | 2,889 (74)^a       | 3,297 (65)^b | 3,210 (65)^b | 3,370 (65)^b | 3,351 (56)^b |
| De     | 3,042 (77)^c       | 2,368 (90)^d  | 2,376 (46)^d  | 1,967 (49)^e  | 1,932 (48)^e  |
| PRG    | 3,024 (80)^f       | 6,645 (80)^g  | 6,684 (75)^h  | 6,885 (76)^h  | 6,915 (68)^h  |

De, Demineralization group; PRG, PRG filler extraction solution. Data are shown as mean (standard deviation). $n=6$ per group. Values in the same group with the same superscript letters indicate no significant different ($p>0.05$).

**DISCUSSION**

In TD-OCT, depth scans (A-scan) are generated by mechanically scanning the coherence gate along the depth range, while in spectral domain OCT (SD-OCT) either a Fourier-domain OCT or a swept source-OCT is used to recover the A-scan in a single shot\(^{14}\). SD-OCT offers several benefits over TD-OCT, including a higher sensitivity and exponentially faster scan rates\(^{15}\). It should be noted, however, there are some disadvantages when using SD-OCT, such as the limited depth range that is related to the finite depth of field of the imaging optics. While the TD-OCT is compatible with dynamic focus schemes that can simultaneously scan the coherence and confocal gates, the inherently single-shot nature of the A-scan acquisition in SD-OCT precludes this. Therefore, TD-OCT continues to be useful when a large scanning range is required, for example, when performing subsurface carious lesion imaging\(^{16}\).

A previous study has reported finding four phenomena that occur during interactions between a tooth and light flux. These include specular transmission of the light flux through the tooth, specular reflection at the surface, diffuse light reflection at the surface, and then absorption and scattering of the flux within the tooth\(^{17}\). Thus, accurate determination of
Though there were no significant changes in the signal intensities and the widths at the (1/e^2) for the control and the De groups during test period, significant changes were observed for the PRG group during the first test period, which occurred during days 0–7.

In our sound enamel samples (controls), there was an intense surface reflection at the tooth surface, with the rest of the signal that propagated into the tooth structure decaying in conjunction with the scan depth. These images remained unchanged during the test period. For the De group on day 1, there was a relatively similar intense reflection rise at the enamel surface, with the signal exhibiting back-scattering in line with the depth beyond the surface. For the De group on day 28, there was no significant difference observed for the signal due to the intense depolarization caused by the light scattering on the etched and roughened enamel surface. It was thought that this was responsible for masking the subsurface scattering and thereby creating speckles in the image. Since we found incident light was strongly scattered by the sound and demineralized enamel surfaces, this suggests that light propagation is affected by convoluted structures on the enamel surface. It is important to note that the tooth structure produces a strong light reflection at the surface of the tooth. As such, a strong surface reflection could very well dominate the reflectively at greater depths from the tooth surface. Therefore, definition of the light penetration depth can be difficult, especially on complicated sound and demineralized tooth surfaces. Overall, these findings suggest that in order to successfully quantify the demineralization status from OCT images, another approach will also be needed.

When attempting to measure lesion depth and lesion severity, definition of the cut-off point is difficult because of the high dynamic range of the reflectivity. Moreover, determination of the signal fall off is very complicated because the light is exponentially attenuated during its propagation into the tooth substrate. Since it was previously proposed that the depth at which there is a (1/e^2) decrease in intensity can be used to determine the cut-off intensity values, we used this determination in our current study. In addition, to define the changes in quantity of the tooth substrate, we used the area of the peak intensity to calculate the integrated value, signal intensity and band width at the (1/e^2). As a result, the lower signal intensity with the wider (1/e^2) width that was seen for the PRG group on day 28 indicates that the OCT signal was generated by light that traveled a longer pathway than was observed for the light in the De group samples. Due to the presence of ions from the PRG extraction solution, the porosities of the enamel surface will accumulate minerals, which ultimately can lead to changes in the optical properties.

Drastic changes in integrated value after 7-day storage was observed for PRG group (Table 3). A previous study reported finding a release of considerable levels of Al, B, F, Na, Si, and Sr from the S-PRG filler into the surrounding distilled water. Among the ions released from the SPR filler, Sr was thought to play an important role in the mineralization of the tooth substrate. The influence of Sr on the remineralization of tooth has also been previously investigated, and it appears to have a capacity to enhance the tooth remineralization in conjunction with F. Si is additionally thought to promote hydroxyapatite formation, as hydroxyapatite nucleation has been shown to be triggered in the presence of Si. A previous study examined the release of Si from bioactive glass particles and determined it was absorbed onto the hard tissue, thereby providing sites for heterogeneous CaP nucleation and subsequent creation of a bone-like apatite layer. This suggests that the S-PRG fillers might be able to release ions involved in the mineralization of tooth substrates and thus, promote
a modulation effect on the acidic conditions produced by cariogenic oral organisms.

Our current study demonstrated that the TD-OCT system can be used to nondestructively measure the inhibition of artificial demineralization on the enamel surface. TD-OCT was successfully used to track the development of demineralization and test the efficacy of a liquid formulation of the PRG filler extraction solution in inhibiting demineralization. The inclusion of active ingredients in oral care products as a way of preventing dental disease has been shown to contribute greatly to the improvement and maintenance of oral health. Further research into whether PRG filler extract solutions can act as oral hygiene materials in clinical situations we need to be undertaken.

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REFERENCES
1) Featherstone JDB. The science and practice of caries prevention. J Am Dent Assoc 2000; 131: 887-899.
2) Featherstone JDB. The continuum of dental caries—evidence for a dynamic disease process. J Dent Res 2004; 83 (Special Issue C): C39-C42.
3) Angmar-Mansson B, Al-Khateeb S, Tranaeus S. Monitoring the caries process. Optical methods for clinical diagnosis and quantification of enamel caries. Eur J Oral Sci 1996; 104: 480-485.
4) White JM, Eakle WS. Rationale and treatment approach in minimally invasive dentistry. J Am Dent Assoc 2000; 131 (Suppl): 138-198.
5) Tam LE, Mccomb D. Diagnosis of occlusal caries: Part II. Recent diagnostic technologies. J Can Dent Assoc 2001; 67: 459-463.
6) Ikemura K, Tay FR, Endo T, Pashley DH. Review of chemical approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers. Dent Mater J 2008; 27: 315-339.
7) Han L, Okamoto A, Fukushima M, Okiji T. Evaluation of a new fluoride-releasing one-step adhesive. Dent Mater J 2006; 25: 509-515.
8) Kamijo K, Mukai Y, Tominaga T, Iwaya I, Fujino F, Hirata Y, Terasaka T. Fluoride release and recharge characteristics of denture base resins containing surface pre-reacted glass-ionomer filler. Dent Mater J 2009; 28: 227-233.
9) Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. Dent Mater J 2010; 29: 392-397.
10) Saito T, Toyooka H, Ito S, Crenshaw MA. In vitro study of remineralization of dentin: effects of ions on mineral induction by decalcified dentin matrix. Caries Res 2003; 37: 445-449.
11) Thuy TT, Nakagaki H, Kato K, Hung PA, Inukai J, Tsuibo S, Nakagaki H, Hirose MN, Igarashi S, Robinson C. Effect of strontium in combination with fluoride on enamel remineralization in vitro. Arch Oral Biol 2008; 53: 1017-1022.
12) Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG. Optical coherence tomography. Science 1991; 254: 1178-1181.
13) Chinn SR, Swanson EA, Fujimoto JG. Optical coherence tomography using a frequency-tunable optical source. Opt Lett 1997; 22: 340-342.
14) Colston B, Sathyam U, Dasilva L, Everett M, stroeeve P, Otis L. Dental OCT. Opt Express 1998; 3: 230-238.
15) Fried D, Featherstone JD, Darling CL, Jones RS. Ngaoteppitak P, Bühler CM. Early caries imaging and monitoring with near-infrared light. Dent Clin North Am 2005; 49: 771-793.
16) Sousa FB, Vianna SS, Santos-S-Magakhões NS. Dental enamel birefringence for a wide mineral content range and for different immersion media’s refractive indexes: an improved mathematical interpretation. J Microsc 2009; 235: 69-75.
17) Karlsson L. Caries detection methods based on changes in optical properties between healthy and carious tissue. Int J Dent 2010; 2010: 270-279.
18) Colston B, Sathyam U, Dasilva L, Everett M, stroeeve P, Otis L. Dental OCT. Opt Express 1998; 3: 230-238.
19) Jones RS, Darling CL, Featherstone JDB, Fried D. Imaging artificial caries on the occlusal surfaces with polarization-sensitive optical coherence tomography. Caries Res 2006; 40: 81-89.
20) Jones RS, Fried D. Remineralization of enamel caries can decrease optical reflectivity. J Dent Res 2006; 85: 804-808.
21) Ngaoteppitak P, Darling CL, Fried D. Measurement of the severity of natural smooth surface (interproximal) caries lesions with polarization sensitive optical coherence tomography. Lasers Surg Med 2005; 37: 78-88.
22) Le MH, Darling CL, Fried D. Automated analysis of lesion depth and integrated reflectivity in PS-OCT scans of tooth demineralization. Lasers Surg Med 2010; 42: 62-68.
23) Can AM, Darling CL, Ho C, Fried D. Non-destructive assessment of inhibition of demineralization in dental enamel irradiated by a λ=9.3-μm CO2 laser at ablative irradiation intensities with PS-OCT. Lasers Surg Med 2008; 40: 342-349.
24) Kang H, Darling CL, Fried D. Nondestructive monitoring of the repair of enamel artificial lesions by an acidic remineralization model using polarization-sensitive optical coherence tomography. Dent Mater 2012; 28: 488-494.
25) Mishima H, Kozawa Y. SEM and EDS analysis of the repair of enamel artificial lesions by an acidic remineralization model using polarization-sensitive optical coherence tomography. Dent Mater 2012; 28: 488-494.
26) Colston B, Sathyam U, Dasilva L, Everett M, stroeeve P, Otis L. Dental OCT. Opt Express 1998; 3: 230-238.