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Abstract. Exposed root surfaces due to gingival recession are subject to biofilm stagnation that can result in caries formation. Cervical enamel and dentin demineralization induced by a cariogenic biofilm was evaluated using swept-source optical coherence tomography (SS-OCT). The cemento-enamel junction (CEJ) sections of extracted human teeth were subjected to demineralization for 1, 2, or 3 weeks. A suspension of *Streptococcus mutans* was applied to form a cariogenic biofilm using an oral biofilm reactor. After incubation, demineralization was observed by SS-OCT. For the analysis of SS-OCT signal, the value of the area under the curve (AUC) of the signal profile was measured. Statistical analyses were performed with 95% level of confidence. Cervical demineralization was displayed as a bright zone in SS-OCT. The demineralization depth of dentin was significantly deeper than that of enamel ($p < 0.05$). Enamel near the CEJ demonstrated a significant increase of AUC over the other enamel region after the demineralization. The gaps along the dentino-enamel junction were additionally observed in SS-OCT. SS-OCT was capable of monitoring the cervical demineralization induced by a cariogenic biofilm and is considered to be a promising modality for the diagnosis of cervical demineralization. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JMI.3.1.014504]

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1 Introduction

Due to the advancement of dentistry and an increase in life expectancy, there has recently been a rapid increase in the dentate elderly population.¹ Root caries is an issue of clinical concern in this population. Exposed root surfaces due to gingival recession can result in the accumulation of plaque and an increased risk of caries. The lesion depth of root caries is commonly underestimated because of the difficulty in distinguishing between the surface zone and demineralized dentin in advanced lesions.² Unlike coronal caries, root caries does not typically present any symptoms of pain or discomfort.³ Therefore, there is a possibility that caries-related pulpitis develop due to the delay in detection and management.

Dental caries is a biofilm-dependent oral disease, mainly caused by certain oral pathogens.⁴ Acid-producing bacteria from a dental biofilm can demineralize dental enamel and dentin on the surface of the tooth.⁵ Several species of bacteria have been reported to be isolated from plaque associated with caries lesions. *Streptococcus mutans* is one bacterial species most frequently implicated in dental caries.⁶ Successful management of root caries requires the investigation of the mechanism of cervical demineralization due to cariogenic bacteria.

Artificial mouth models, such as the oral biofilm reactor (OBR), can be used *in vitro* to study oral biofilm formation on the human tooth by simulating the human oral environment. Using OBR, artificial caries lesions were produced on enamel and dentin surfaces by forming cariogenic biofilms.^{4,6,7}

Optical coherence tomography (OCT) has seen broad applications in medicine and biology in the past decades. This imaging technique has also been used to image hard and soft dental tissues.⁵ The detection of carious lesions remains diagnostically challenging; therefore, dentists require an imaging technology that can noninvasively and reliably quantify the extent of caries. OCT is an emerging diagnostic method for obtaining cross-sectional images revealing the internal biological structure.⁸ Swept-source OCT (SS-OCT) is one of the most recent implementations of spectral discrimination, uses a wavelength-tuned near-infrared laser as the light source, and provides improved imaging resolution and scanning speed.^{9,10} In dentistry, several studies have reported the characterization of caries under OCT. However, few studies have reported the method development and validation for quantitative measurements of the demineralization depth or restoration defects using OCT.^{11,12}

The aim of this study was to evaluate the effectiveness of SS-OCT in the diagnosis of cervical caries *in vitro*. The results of

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OCT were compared with those of confocal laser scanning microscopy (CLSM).

2 Materials and Methods

2.1 Specimen Preparation

The experimental design of this study and the use of extracted human teeth were in accordance with the guidelines of the Ethics Committee of Tokyo Medical and Dental University. A total of 30 intact third molar teeth that were free of caries or obvious cracks were used in this study. Cubical specimens ($5 \times 5 \times 2 \text{ mm}^2$) were prepared from the buccal portion of the cementoenamel junction (CEJ) of sound human teeth with a diamond bur (102R, GC, Tokyo, Japan) attached to an air turbine headpiece under copious cooling water. The specimens were divided into three groups and used in the following experiment detailed below for 1, 2, or 3 weeks of demineralization.

2.2 Biofilm Formation and Demineralization

S. mutans MT8148 was used in this study. A suspension of *S. mutans* in phosphate-buffered saline (PBS) was prepared using a 16-h freshly cultured bacteria in brain heart infusion

broth (Becton Dickinson, Sparks, Maryland) after washing three times with PBS and was then stored at 4°C with gentle stirring. For growing of biofilms, a solution of heart infusion broth (HI, Becton Dickinson, Sparks, Maryland) with sucrose (1% final concentrations) was used.^{1,13}

Artificial biofilms were grown on the dentin surfaces inside two identical water jacket-encircled chambers of an OBR (Fig. 1).

After 20 h of incubation of the biofilm in the OBR chamber, each specimen containing artificial biofilms was removed from the Teflon holder.

Specimens were transferred to 24-well tissue culture plates (Corning Inc. New York, New York) and were incubated at 37°C in the HI medium containing 1% sucrose for each demineralization period (1, 2, or 3 weeks), with the media replenished every other day. After the demineralization process, each specimen was transferred into 1 ml of 0.25-mol/l sodium hydroxide solution to remove the biofilm.

2.3 Cross-Sectional Imaging of Bacterial Demineralization

The SS-OCT system (Prototype 2, Panasonic Healthcare Co. Ltd. Ehime, Japan) was used in this study. A schematic

Fig. 1 Specimen preparation and biofilm formation. Samples were placed on a Teflon holder around a flat bulb pH electrode of OBR using red utility wax (GC, Tokyo, Japan) such that only the experimental surface remained open for biofilm attachment. The open surfaces of the samples were maintained at the horizontal level to the bulb surface. The Teflon holder bearing the samples was set at the bottom opening of the chamber using a silicon plug. The chamber encircled by a water jacket was sealed with another silicon plug fitted with five stainless steel tubes (21 gauge) so that the chamber itself served as an incubator with a 37°C inner temperature. The other ends of the four-stainless steel tubes were connected to silicon tubes passing through peristaltic pumps that were regulated by a computer-operated controller (EYELA EPC-2000, Tokyo Rika, Tokyo, Japan). One of the tubes was used to collect the *S. mutans* suspension, two to collect HI, and the other one to collect PBS from the prepared stock. All of these liquids were pumped into the chambers at a rate of 6 ml/h/tube so that the liquids could continuously drop onto the center of the specimen holder. The liquids collectively formed water domes that are mixed by gravity exerted from the falling liquid drops on the holder and are diffusely distributed over all the samples. When the liquid domes reached their maximum height, the mixture of excess liquids dropped off from the edges of the holders. Both of the chambers were simultaneously operated, and the pH on the flat bulb electrode was continuously recorded.

Fig. 2 SS-OCT system. (a) Schematic representation of SS-OCT. SS-OCT uses an interferometer with a narrow-band, frequency-swept laser, and detectors. The output from the swept light source was divided into signal and reference beams. Reference and backscattered lights from the sample were recombined with a second fiber coupler to create the interferogram in time. Fringe response was detected with a balanced detector, converted to an electrical signal, and digitized by an Analog/Digital board. (b) The SS-OCT System (Prototype2, Panasonic Healthcare Co. Ltd. Ehime, Japan) was used in this study. A high-speed frequency-swept laser light with a center wavelength of 1330 nm was projected onto the sample, and the sample was scanned in 2-D using a probe. 2-D cross-sectional images were created from serial backscatter (reflectivity) profiles (A-scans) along the surface. The axial and lateral resolutions of the system in air were 12 and 20 μm , respectively.

representation of the SS-OCT system is shown in Fig. 2. A high-speed frequency swept laser light with a center wavelength of 1330 nm was projected onto the samples and scanned cross-sectional image in two-dimensions (2-D) using a hand-held probe. The hand-held scanning probe connected to the SS-OCT system was set at a 5-cm distance from the specimen surface with the scanning beam oriented $\sim 90^\circ$ deg to the surface. The sample was mounted on a stage. For each specimen, the cross-sectional images were acquired before and after demineralization. To ensure the repeatability of the OCT scan before and after demineralization, the specimens were placed at the same orientation as accurately as possible, and the B scan was performed along a line between the two points marked by a marker pen on the

specimen surface. OCT images were scanned in a controlled hydrated condition after blot drying of the surface so that no water droplets were visible.

2.4 Cross-Sectional Viewing of Specimens Using Confocal Laser Scanning Microscopy

After the SS-OCT imaging, the specimens were longitudinally sectioned with a low-speed diamond cutting machine (Isomet, Buehler) under running water at the center of the specimen that corresponded with the OCT image location. The specimens were polished with a diamond paste down to a particle size of 0.25 μm in a circular motion under copious cooling water.

Fig. 3 The analysis of SS-OCT images. (a) Enamel and dentin regions selected for SS-OCT image analysis. For SS-OCT signal analysis after demineralization, two rectangular zones of 200 μm length and 100 μm depth were individually chosen from enamel and dentin, where the SS-OCT signal was analyzed as the area of interest using imaging software. The demineralized lesion depth was additionally measured at the center of the selected region. E, enamel; D, dentin; CEJ, cemento-enamel junction; E1, cervical enamel zone between 100 and 300 μm from CEJ; E2, cervical enamel zone between 1000 and 1200 μm from CEJ; D1, cervical dentin zone between 0 and 200 μm from CEJ; D2, cervical dentin zone between 1000 and 1200 μm from CEJ. (b) The signal obtained from the area of interest. The signal intensities were integrated through the 100 μm depth as the AUC from the A scan signal.

The cervical caries lesion on each cross-section of the specimens was then directly observed using CLSM (1LM21H/W, Lasertec Co., Yokohama, Japan) at 50× magnifications.

2.5 Swept-Source Optical Coherence Tomography Image Analysis

2.5.1 Cervical demineralization

For image analysis, a custom code in the image analysis software (Image-J version 1.47t; Wayne Rasband, NIH, Bethesda, Maryland) was used. We evaluated the signal intensity area under the curves (AUC) to analyze the OCT signal after demineralization. Two cervical regions were individually chosen from enamel and dentin, and AUC were calculated from the A scan signal (Fig. 3).

Enamel area 1: cervical enamel zone between 100 and 300 μm from CEJ (E1).

Enamel area 2: cervical enamel zone between 1000 and 1200 μm from CEJ (E2).

Dentin area 1: cervical dentin zone between 0 and 200 μm from CEJ (D1).

Dentin area 2: cervical dentin zone between 1000 and 1200 μm from CEJ (D2).

Using Image-J, the obtained SS-OCT image was rotated to compensate for the tilting and to obtain a horizontal surface. SS-OCT signal intensities were averaged over the 200 μm width of enamel and dentin on each B-scan image from the first pixel beneath the surface to exclude the Fresnel reflection of the surface. AUC was obtained from the plot against a 100- μm depth.^{14–16} The demineralized lesion depths in E1, E2, D1, and D2 were measured at the center region of the selected region, and the refractive index was corrected to represent the lesion depth from the SS-OCT images.

2.5.2 Gaps along dentinoenamel junction

The gaps of dentinoenamel junction (DEJ) at the demineralized tooth surface were detected as distinct bright lines along DEJ at the cervical zone using SS-OCT.¹⁷

The length of each gap at DEJ was measured using the Image-J software, and the number of specimens obtained at the gaps at the DEJ in each experimental period was calculated.

2.6 Statistical Analyses

Statistical analyses were performed using a statistical software package (SPSS for Windows; SPSS Inc., Chicago, Illinois). The data of SS-OCT signal and demineralized lesion depth were initially analyzed using a three-way analysis of variance (ANOVA) (demineralization period, region, and intact or demineralized). As significant interactions between all three factors were present, the data were analyzed by two-way ANOVA with a demineralization period (1, 2, or 3 weeks) and region (E1, E2 or D1, or D2, respectively). Student's *t*-tests with Bonferroni corrections were performed to compare between the groups. All statistical analyses were performed with 95% level of confidence. Chi-squared test with Bonferroni corrections was performed for statistical analysis of the frequency of the gap along DEJ.

3 Result

Cervical demineralization induced by a cariogenic biofilm was displayed as a bright zone with a highlighted backscattered

Fig. 4 SS-OCT and CLSM images after the demineralization induced by cariogenic biofilm and the gaps along DEJ. (a) SS-OCT image before the demineralization. (b) CLSM image before the demineralization. (c) SS-OCT image after 1 week demineralization. (d) CLSM image after 1 week demineralization. (e) SS-OCT image after 2 week demineralization. (f) CLSM image after 2 week demineralization. (g) SS-OCT image after 3 week demineralization. (h) CLSM image after 3 week demineralization. In SS-OCT, highlighted zone due to the bacterial demineralization was observed on the enamel and dentin with a boundary distinguished from the intact zone (white arrow). CLSM cross-sectional observation revealed that these bright zones showed demineralized enamel and dentin lesion (white arrow). In SS-OCT, the brightness at CEJ was increased (black arrow). The white line was penetrated along DEJ after the demineralization (finger point). In CLSM, the white line was found as a gap caused by bacterial demineralization. E, enamel; D, dentin; CEJ, cemento-enamel junction.

Table 1 Demineralized lesion depth (μm , mean $\pm$ SD).

	E1	E2	D1	D2
1w	60.6 $\pm$ 17.2 ^{A*}	45.5 $\pm$ 8.1 ^{B*}	163 $\pm$ 11.0 ^C	160 $\pm$ 11.8 ^D
2w	98.3 $\pm$ 26.9 ^{A*}	72.6 $\pm$ 23.6 ^{B*}	238 $\pm$ 39.4 ^C	245 $\pm$ 41.3 ^D
3w	124 $\pm$ 16.0 ^{A*}	101 $\pm$ 15.3 ^{B*}	280 $\pm$ 22.7 ^C	281 $\pm$ 13.3 ^D

Same superscript letter indicates significant difference among the experimental periods (*t*-test, $p < 0.05$).

Asterisk indicates significant difference between E1 and E2, or D1 and D2.

SD, standard deviation.

signal on the SS-OCT gray-scan B-scan images. Representative images of SS-OCT and CLSM are shown in Fig. 4.

In all SS-OCT images after the incubation of cariogenic biofilm, the boundary of the bright zone was shown below the

Table 2 AUC value before and after the bacterial demineralization (Mean $\pm$ SD).

	E1		E2	
	Before	After	before	After
1w	146 $\pm$ 16.7	192 $\pm$ 34.7 ^{AA*}	139 $\pm$ 12.8	160 $\pm$ 23.8 ^{BB*}
2w	144 $\pm$ 28.8	230 $\pm$ 27.5 ^{A*}	150 $\pm$ 20.6	198 $\pm$ 33.1 ^{B*}
3w	149 $\pm$ 11.4	231 $\pm$ 23.3 ^{A*}	141 $\pm$ 16.5	213 $\pm$ 20.0 ^{B*}
	D1		D2	
	Before	After	before	After
1w	129 $\pm$ 7.57	140 $\pm$ 17.9 ^{C,C'}	125 $\pm$ 21.1	144 $\pm$ 15.8 ^{D,D'}
2w	134 $\pm$ 26.8	165 $\pm$ 30.4 ^C	133 $\pm$ 29.1	166 $\pm$ 17.4 ^D
3w	136 $\pm$ 11.7	168 $\pm$ 18.7 ^{C'}	130 $\pm$ 15.2	168 $\pm$ 24.3 ^{D'}

All the groups showed significant increase of AUC value after the demineralization (t -test, $p < 0.05$). Same superscript letter indicates significant difference among the experimental periods (t -test, $p < 0.05$). Asterisk indicates significant difference between E1 and E2, or D1 and D2.
SD, standard deviation.

lesion surface, thereby providing an indication of the demineralized lesion depth. Confirmatory CLSM cross-sectional observation demonstrated that these bright zones represented demineralized enamel and dentin lesions (Table 1).

The SS-OCT images revealed that the penetration depth of cervical demineralization significantly increased with a longer incubation period ($P < 0.05$). The dentin demineralization progressed deeper than that in enamel in each demineralization period. After the bacterial demineralization, CEJ in many specimens revealed intensified brightness compared with the base line in SS-OCT.

The increase of SS-OCT backscattered signal after the demineralization was additionally detected from the shift of AUC values; the values were gradually increased in both enamel and dentin after the extension of the demineralization period (Table 2). Enamel near the CEJ (E1) showed a significant increase in AUC over that in E2 after the demineralization.

In contrast, although dentin AUC values were increased after the extension of demineralization, there was no significant difference between D1 and D2 at each experimental period.

In this study, the gaps that were penetrated along DEJ and were formed after the bacterial demineralization displayed as

distinct white lines in SS-OCT (Table 3). The frequency of gap formation along DEJ was 5 in 1 week demineralization, 6 in 2 week demineralization, and 8 in 3 week demineralization. There was no statistically significant difference in the frequency of gap formation between the experimental periods.

4 Discussion

In this study, SS-OCT was used to monitor the cervical lesions after demineralization induced by a cariogenic biofilm. The B-scan images obtained from each demineralization stage displayed an intensified brightness in the lesion body, of which depth was increased by the extension of the experimental period. In general, demineralization in tooth tissue results in an increased porosity because of the mineral loss in the lesion body. A cariogenic biofilm of *S. mutans* forms acid in response to a sufficient sucrose challenge, resulting in enamel and dentin demineralization. It is known that higher porosity results in higher reflectivity due to an enormous number of microinterfaces between water and demineralized mineral crystals or collagen fibers in the porosity. Therefore, the increased porosity is associated with an increase in the backscattering of light.^{10,18,19}

In enamel, the value of AUC was significantly higher in E1 than in E2 after the demineralization. This finding suggested that enamel demineralization can rapidly take place closer to the CEJ in the cervical region compared to the coronal region apart from CEJ.

Several studies demonstrate morphological distinctions in cervical enamel. In this region, aprismatic enamel and transition enamel present near CEJ, which contain randomly oriented hydroxyapatite crystals and atypical enamel prisms.^{20,21} These morphological differences appear to influence the progress of enamel demineralization. Furthermore, carbonate is an important factor that affects the mechanical properties of enamel. As previously reported, an increase in carbonate substitutions reflects a decrease in crystallinity of hydroxyapatite and contributes to the mechanical property.²² The high carbonate distribution results in an increase of sensitivity to acids. Enamel near CEJ contains high carbonate substitutions, and the tooth surface is more soluble and less resistant to attack from acidic by-products produced in dental plaque that result in dental decay.²³

Meanwhile, dentin demineralization in both D1 and D2 penetrated significantly deeper than those in enamel E1 and E2. Enamel is a highly mineralized crystalline structure containing on an average 96% mineral and 1% organic material by weight. In contrast, dentin contains 70% mineral and 10% organic material by weight and possesses microscopic tubules that provide a pathway for the ingress of bacterial acid and the egress of minerals.^{24,25} It is highly probable that these structural differences contribute to the progression of dentin demineralization.² The deeper demineralization in dentin observed in this study is consistent with clinical aspects observed in previous studies; root caries is commonly located on exposed root surfaces as a cavitation below the CEJ.^{2,3,26}

It is noteworthy that SS-OCT could detect the gap that developed due to the demineralization, and penetrated deep along DEJ as a white line with intensified brightness.²⁷ In the current study, the gaps along DEJ were first found after 1 week of demineralization and slightly increased after the extension of the experimental period. According to these findings, the integrity of DEJ was considered to be vulnerable to the carious demineralization. The region of DEJ has been demonstrated to be rich in organic material, has less mineral content in the

Table 3 Gap length and frequency of gap along DEJ

	1w	2w	3w
length (μ m), mean $\pm$ SD	85.7 $\pm$ 98.3	134 $\pm$ 129	198 $\pm$ 113
Frequency (number)	5	6	8

No significant difference was found by Chi-squared test with Bonferroni correction ($p < 0.016$).
 $N = 10$.

presence of parallel-oriented coarse collagen bundles, and contains predominant branches of dentinal tubules. DEJ has reduced hardness and is less mineralized than the rest of the coronal dentin. These structural factors appear to contribute to the higher susceptibility of DEJ to cariogenic acid attack and separation.^{22,23,28,29}

The OBR used in this study facilitates *S. mutans*-biofilm formation, both under aerobic and anaerobic conditions with a constant temperature of 37 °C. The reactor allows the continual recording of pH, and the reduction curve for pH was the same over all experiments; pH began to fall from 7.35 within 2 h and was reduced to below 4.0 by 20-h because of the bacterial acid forming demineralization in both enamel and dentin.

Although OBR demineralization may not accurately reflect caries *in vivo*, the bacterial demineralization in this system appears to be a more clinically relevant approach over the conventional demineralization using acidic solutions. *S. mutans*, which comprises a significant proportion of the oral *Streptococci* in caries lesions, is one of the main pathogens responsible for the development of dental caries. Using *S. mutans* to form bacterial demineralization in OBR, dentin demonstrated a significantly deeper lesion depth over enamel.^{1,6}

Similarly in this study, the demineralized lesion depth induced by a cariogenic biofilm was deeper in dentin than in enamel, for which the process was clearly monitored in SS-OCT. The result from our experiment appears in accordance with clinical aspects; root caries is frequently observed in exposed dentin after gingival recession.^{2,3,30} Compared to other smooth enamel surfaces, cervical enamel was more vulnerable to the cariogenic bacteria, resulted in more severe demineralization, and DEJ separation. These phenomena may accelerate the carious progress to form cavities. Clearly, early diagnosis is very important in the cervical region to prevent the progress of cervical caries.

Within the limitation of this *in vitro* study, SS-OCT was capable of detecting the extent of cervical caries and the gaps along DEJ in the early stage. Consequently, SS-OCT is considered to be a promising modality to diagnose cervical demineralization in a clinic.

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