Observation of white spot lesions using swept source optical coherence tomography (SS-OCT): *in vitro* and *in vivo* study

Takahide IBUSUKI¹, Yuichi KITASAKO¹, Alireza SADR^{2,3}, Yasushi SHIMADA¹, Yasunori SUMI⁴ and Junji TAGAMI¹

¹ Cariology and Operative Dentistry, Department of Restorative Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan

² University of Washington, School of Dentistry, 2959 NE Pacific Street, Seattle, WA 98195, USA.

³ Tokyo Medical and Dental University, International Exchange Center, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan

⁴ Department for Advanced Dental Research Center of Advanced Medicine for Dental and Oral Disease, National Center for Geriatrics and Gerontology, 35 Gengo, Morioka-cho, Obu, Aichi 474-8511, Japan

Corresponding author, Yuichi KITASAKO; E-mail: kitasako.ope@tmd.ac.jp

This study aimed to assess swept source optical coherence tomography (SS-OCT) for *in vitro* and *in vivo* detection of enamel white spot lesion (WSL). WSLs without surface breakdown on 33 extracted human posterior teeth were non-invasively scanned using SS-OCT. The teeth were then cross-sectioned and imaged under confocal laser scanning microscope (CLSM) and light microscopy (LM). SS-OCT cross-sectional images were compared with CLSM and LM. WSL shapes in SS-OCT images closely corresponded to those of LM. There were significant correlations ($p < 0.001$) in WSLs depth between SS-OCT and LM ($r = 0.92$), SS-OCT and CLSM ($r = 0.80$) and CLSM and LM ($r = 0.85$). Six WSLs were also evaluated clinically using SS-OCT; clear in-depth images of these natural WSLs were obtained *in vivo*. SS-OCT appears to be an effective tool for observation of the internal structure of WSLs, enabling quantitative assessment of WSL depth. Such data can be considered in the clinical management of WSLs.

Keywords: White spot lesion, Enamel, OCT, *In vitro*, *In vivo*

INTRODUCTION

Initial carious lesion results from an imbalance between the processes of demineralization and remineralization. White spot lesion (WSL) is a noncavitated carious lesion that has reached the stage where the subsurface mineral loss has produced changes in the optical properties of enamel such that these are visibly detectable as a loss of translucency, resulted in a white appearance of the enamel surface¹. Fontana *et al.*¹ also stated that although initial lesions appear white, and opaque to the naked eye, not all of these visible changes are either initial or incipient, as they may be present for many years.

The detection of carious lesions at an early stage is necessary in order to implement preventive and interceptive treatment strategies. In recent years, methods of detection of early carious lesions have developed considerably, moving firstly towards the identification of the earliest mineral changes and then to controlling the demineralization process using non-operative procedures. However, high evidence-level studies are in agreement that the ideal tool for detection of the initial lesion, the 'gold standard' has not yet been identified. The conventional and validated tools for detecting early carious lesions include visual and tactile examination and bitewing radiographs. Although it is well accepted that bitewing radiography provides additional benefits in the detection of non-

cavitated proximal lesions, evidence for their value in epidemiological studies is still controversial^{2,3}. Some optical technologies are developed and it is of interest to link the new method to conventional methods with a view to improving caries detection and diagnosis.

Optical coherence tomography (OCT) is a rapidly growing non-invasive imaging technique which enables cross-sectional imaging of internal biological structures without the use of X-ray irradiation in a short time^{4,5}. Swept source optical coherence tomography (SS-OCT) is a variant of OCT^{6,7}, wherein the light source is a tunable laser that sweeps the wavelength over a certain range. SS-OCT time-encodes the wave number by rapidly tuning the narrowband and source through a broad optical bandwidth. The resolution of SS-OCT images, which is around 10–15 μm , is higher than that of radiographic or current clinical computed tomography (CT) images^{8,9}. When reflections from the reference mirror and a backscattered light from the tissue are recombined, an interference signal is detected within the coherence length of the source. A series of those optical signals can then be interpreted to create two-dimensional (2-D) images, and serial 2-D scans can create three-dimensional images. As for clinical dental applications so far, OCT has been used to image structures of caries, crack, fracture, and so on¹⁰. The principle of evaluation of enamel demineralization by OCT shown as increased signal intensity is induced by multiple scattering of the incident light in the demineralized enamel because of expanded porosity, and light depolarization by the demineralized enamel.

Color figures can be viewed in the online issue, which is available at J-STAGE.

Received Feb 10, 2015; Accepted May 2, 2015

doi:10.4012/dmj.2015-058 JOI JST.JSTAGE/dmj/2015-058

The latter necessitates a polarization-sensitive OCT (PS-OCT) or cross-polarization OCT setup, but the former phenomenon can be observed as increased signal intensity by both conventional and polarization-sensitive OCT systems^{11–13}. Image analysis techniques in correlative OCT studies have been mainly based on the increased signal intensity values to quantify parameters such as depth (as a cut-off point) and mineral loss (dB values integrated over depth) in demineralized lesions; however, there are fewer studies on the assessment of remineralization by conventional OCT¹⁴.

Nakagawa *et al.*¹⁵ demonstrated the effectiveness of SS-OCT in *in vitro* evaluating the extent of caries on smooth enamel surfaces. The results of SS-OCT were compared with those of Visual Inspection (VI) on sensitivity and specificity, and validated by direct observation of the sectional samples under a confocal laser scanning microscope (CLSM). Cross-sectional imaging of the natural caries lesions on smooth enamel surface by OCT enables efficient diagnosis of the caries lesion type, including extent of dentinal lesions with a sensitivity and specificity superior to those of VI, and good correlation to CLSM results. On the other hand, clinical OCT systems are under development based on the conventional SS-OCT technology. Shimada *et al.*¹⁶ demonstrated the diagnostic accuracy of SS-OCT in detecting and estimating the depth of proximal caries in posterior teeth *in vivo*. In their study, SS-OCT images and bitewing radiographs were evaluated by six dentists and results were compared with clinical observation obtained after the clinical treatment. SS-OCT could detect the presence of proximal cavitated enamel lesions in tomograms that were synthesized based on the backscatter signal obtained from the proximal carious lesion through occlusal enamel. Lesion depth is an important attribute of early enamel lesions extent; however, only few studies have systematically investigated the power of conventional SS-OCT for the objective assessment of depth on a wide range of non-cavitated natural enamel WSL on human teeth.

The aim of this study was to assess SS-OCT as a diagnostic tool for the *in vitro* and *in vivo* detection of WSL in posterior teeth. The *in vitro* results of cross-sectional SS-OCT images were compared with the direct observation of sectioned samples under confocal laser scanning microscope (CLSM) and light microscopy (LM).

MATERIALS AND METHODS

Thirty-three extracted human posterior teeth with visual localized enamel WSLs without surface breakdown were stored in 4°C in saline containing a few crystals of thymol, the usage of which was approved by the Institutional Review Board of Tokyo Medical and Dental University.

ICDAS classification

Thirty-three extracted human posterior teeth were cleaned by using dental hand-piece (INTRA matic LUX

2 20LM, Kavo, Germany) with polishing brush. Smooth surface of the teeth were assessed using ICDAS criteria by two examiners (YK, AS) with the aid of a three-in-one air syringe. First, the teeth were assessed moist and then dried after 5 s. The visual examination was coded as follows: (code 0) sound tooth surfaces, no evidence of caries after prolonged air drying (5 s); (code 1) first visual change in enamel, opacity or discoloration (white or brown) is visible at the surface of WSL after prolonged air drying, which is not or hardly seen on a wet surface; (code 2) distinct visual change in enamel, opacity, or discoloration distinctly visible at the surface of WSL under dry condition, and lesion is still visible when wet. In the case of disagreement between the scores from the two examiners, the consensus was made through discussion. WSLs with visible enamel surface defects were excluded and WSLs which correspond to ICDAS code 1 and 2 were selected for this study. In this manner, 40 WSLs were chosen for investigation.

SS-OCT imaging of WSL

The SS-OCT system used in the current work (Prototype 2, Panasonic Healthcare, Ehime, Japan) has the center wavelength is 1,330 nm (bandwidth 110 nm) with a

Fig. 1 Photographic image of the SS-OCT system used in this study.

(a) the complete system with screen, scanning laser source, interferometer, and PC to process data; (b) The equipped hand-held probe of clinical SS-OCT. Near-infrared light is emitted from the device.

30-kHz sweep rate. The system is equipped with a hand-held probe with power of <10.0 mW, specifically designed for intraoral imaging. The probe also includes a complementary metal-oxide semiconductor (CMOS) camera that enables real-time photographic observation of the surface being scanned by the OCT beam. A schematic presentation of the system is shown in Fig. 1a. The focused light beam is projected onto the subject and scans the plane of interest. The light is reflected from the sample and coupled back into the system. Since the wavelength is encoded with time, the backscattered light can be digitized according to time, followed by the Fourier transform to determine distance travelled by light or depth information, along with light intensity. The data scanned along a plane can be used to create 2-D image 2,000×1,019 pixels within 0.3 s at lateral resolution of 20 μm and axial resolution of 12 μm in vacuum or 8 μm , if a refractive index of 1.5 is assumed.

The scanning probe was set at a certain distance over the tooth surface to obtain SS-OCT images of WSL, where the visual inspection had been performed¹⁴. The cross-sectional scans were obtained along the long axis of tooth. Lesion shapes on the resulting SS-OCT images were then classified as follows;

Bowl type: Remarkable increase in backscattering from superficial enamel mainly following a round or bowl-shaped pattern (Fig. 2).

Layered type: Remarkable increase in backscattering from superficial enamel

mainly as a band of a certain depth and following a homogenous layer pattern (Fig. 3).

Complex type: The increase in backscattering appeared as a combination of both layer and bowl types.

2D OCT scans of the lesions were compared according to two parameters derived from the images using specially developed code in ImageJ (ImageJ version 1.45S, Wayne Rasband, NIH, Bethesda, MD, USA). OCT lesion depth is the depth of the boundary that is observable between bright, highly-scattering area of the lesion and the darker region beneath the lesion^{17,18}.

Using Image J, the obtained OCT image was rotated to compensate for the tilting and reach a horizontal surface. A noise reducing median filter (size 2) was applied to the data. For optical lesion depth calculation (not considering refractive index), the region of interest (ROI) was set as the whole lesion extent visible on the cross section. An experimental threshold determination algorithm was used to estimate the threshold intensity for lesion depth, or dark-bright boundary in the ROI. The intensity value was then checked and adjusted by the operator to ensure that it matched visible boundary of the lesions. The cut off value was then applied to the ROI to calculate an average optical lesion depth (LD_{OCT}) by the software subsequently¹⁸. The measurements were repeated 4 times for each OCT image and the average value was reported.

Fig. 2 Representative case of bowl type WSL *in vitro*.

The left-upper second premolar tooth was extracted for orthodontic reason from a 14 years old female; the WSL was located at distal surface. (a) In the digital photograph, WSL is located on the smooth surface of enamel around the contact area; (b) On the *en face* LM photograph ($\times 8$ magnification), WSL is verified on the smooth surface of enamel around the contact area clearly; (c) On the SS-OCT image, WSL corresponding to the red dotted line in *en face* LM image (b) was observed. The bowl type lesion is distinct at the lower half of the image with an enhanced brightness; (d) The CLSM image ($\times 125$ magnification) is obtained from the same physical cross-section as the red dotted line in (b) and OCT image in (c). Structural changes are seen at the subsurface of enamel as following a bowl pattern; (e) On the cross-sectional LM image ($\times 8$ magnification), the shape of WSL is similar to the images of SS-OCT (c) and CLSM (d).

Fig. 3 Representative case of layer type WSL *in vitro*.

The lower right third molar tooth was extracted for periodontal reason from a 20 years old female; the WSL was located at mesial surface. (a) In the digital photograph, WSL is located on the smooth surface of enamel around the contact area; (b) On the *en face* LM photograph ($\times 8$ magnification), WSL is verified on the smooth surface of enamel around the contact area clearly; (c) On the SS-OCT image, WSL corresponding to the red dotted line in *en face* LM image (b) was observed. The WSL distinguished with its enhanced brightness following a layer pattern lesion is detected; (d) The CLSM image ($\times 125$ magnification) is obtained from the same physical cross-section as the red dotted line in (b) and OCT image in (c). The less reflective, dark band on CLSM is thought to represent structures with lower mineral content at the subsurface of enamel; (e) On the cross-sectional LM image ($\times 8$ magnification), WSL corresponding to the one presented in (b), (c) and (d) was observed. The shape of WSL is similar to the images of SS-OCT (c) and CLSM (d).

CLSM and LM observation

The *in vitro* results of cross-sectional SS-OCT images were compared with the direct observation of sectioned samples under CLSM (1LM15W, Lasertec, Yokohama, Japan) and LM (SMZ1000, Nikon, Tokyo, Japan) attached to a true-color charge-coupled device (CCD) camera (DS-Fi1; Nikon, Tokyo, Japan). In order to achieve this aim, two points were marked on the surface of the tooth along the plane imaged by CMOS camera using a marker pen. To ensure that cross cutting plane closely resembled OCT beam orientation, a silicone putty (Exafine, GC) impression of each tooth was obtained to register its orientation while taking the SS-OCT scan of each WSL. The tooth was set on the equipped metal block (11 $\times$ 28 $\times$ 49 mm) of low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) to fix specimen using an adhesive for dental technique (MODEL REPAIR II blue and MODEL REPAIR HARDNER, Dentsply Sankin K.K., Japan), then cross-sectioned along the OCT imaging plane using the above low-speed diamond saw, and the cross section was further trimmed using silicon carbide papers (#800, #1000, #1200, #1500, #2000) to reach a smooth section in the appropriate orientation through the marked points on the enamel surface. Finally, the surface was polished with diamond paste down to 3 μ m in particle size under running water. The polished specimens were then put in a beaker with distilled water and applied

to ultrasonic cleaning (ultrasonic cleaning machine US-102, SND, Japan) in order to remove their polishing particles for 10 min. Totally 40 polished surfaces were investigated for direct viewing at magnification of 125 $\times$ under CLSM and 8 $\times$ under CLSM and LM. Their microscopic shapes were also classified according to the progress pattern of demineralization to bowl shape and layer shape.

In order to calculate the average lesion depth at the observed cross section, the outline of the lesion was traced in the image analysis software, and then whole surface area of the lesion was calculated. Then the horizontal length of lesion was measured on x axis, average depth was calculated by dividing the surface area value to the length. The measurements were repeated 4 times for each CLSM and LM image and the average values were recorded as LD_{CLSM} and LD_{LM}, respectively. All the analyses were performed by the same operator who was blind to the OCT assessment results.

Clinical examination of WSL using SS-OCT

Besides the *in vitro* study, non-cavitated buccal caries of clinical cases were also evaluated using SS-OCT on six posterior teeth. The *in vivo* investigation was also approved by the Ethics Committee of the Tokyo Medical and Dental University and was performed followed by the Declaration of Helsinki. Five healthy

subjects (median age: 37 year) were recruited following obtaining ethical approval for the study from the Ethics Committee of the Tokyo Medical and Dental University and signing the informed consent forms. Each of the volunteers completed the medical history forms and underwent intraoral examination for caries experience. For inclusion in the study, the subjects were required to have at least one WSL on the anterior teeth, with an ICDAS visual score of 1 or 2¹⁹⁾. Exclusion criteria included smoking and major systemic health condition. Each subject had between one and two WSL with a total of 6 WSL on the buccal surfaces of posterior teeth examined throughout the course of this study.

The teeth were cleaned using a brush cone attached to a low-speed handpiece. Photographs of the white spot lesion were taken using a digital camera. After taking photographs, cross-sectional imaging of the lesion was performed in 2-D using the SS-OCT hand-held probe.

Intraoral digital photographs were taken at baseline with a digital camera (D5000, Nikon, Tokyo, Japan). The camera was approximately 45 cm from the subject perpendicular to the buccal surface of the teeth and the photo was taken under fine image quality; ISO 200 and Speedlight white balance settings.

Statistical analysis

A statistical software package (SPSS, Chicago, IL, USA) was used to perform statistical analysis. The results of WSL depths obtained from OCT, CLSM and LM image analysis (LD_{OCT} , LD_{CLSM} and LD_{LM} , respectively) were subjected to Pearson's correlation coefficient (r) to elucidate the relationship among three analyses, followed by regression analysis with 95% confidence interval.

RESULTS

The *in vitro* WSLs were visually scored as ICDAS 2 in this study. OCT images of the WSLs appeared as areas with increased reflectivity; representative cases of bowl and layered type are shown in Figs. 2 and 3. Using the morphological assessment of SS-OCT images revealed that 25 lesions showed bowl shape while 9 lesions appeared as layer type and 6 lesions showed a complex of both layer and bowl. The lesion shapes recorded by LM were in agreement with SS-OCT. On the other hand, 2 lesions recorded as bowl type under OCT and LM appeared as layer and 2 others as complex type.

Figure 4a showed the average depth of WSLs obtained by three devices. Significant correlations ($p < 0.001$) between LD_{OCT} and LD_{LM} ($r = 0.92$), LD_{OCT} and

Fig. 4 The depth distribution of WSLs obtained from three different methods *in vitro*. WSLs on 40 specimens obtained from 33 extracted teeth were evaluated. Significant correlations ($p < 0.001$) were observed between OCT and LM ($r = 0.92$), OCT and CLSM ($r = 0.80$) and CLSM and LM ($r = 0.85$). (a) An overview of WSL depth distribution among 40 lesions with three methods (SS-OCT, CLSM, LM). Note that OCT depths are optical values (before refractive index correction); (b) Scatter plots with 95% confidence intervals of linear regression in OCT versus LM ($R^2 = 0.85$); (c) Scatter plots with 95% confidence intervals of linear regression in OCT versus CLSM ($R^2 = 0.64$); (d) Scatter plots with 95% confidence intervals of linear regression in CLSM versus LM ($R^2 = 0.73$).

LD_{CLSM} ($r=0.80$) and LD_{LM} and LD_{CLSM} ($r=0.85$). Scatter plots with 95% confidence intervals of linear regression are presented in Figs. 4 (b, c, d). A summary of all values is graphically presented in Fig. 4a. Considering the data size and distribution a depth cut-off depth value of 200 μm was selected to examine correlations for shallows *vs.* deep lesions. For shallow lesions ($<200 \mu m$, $n=19$), significant ($p<0.005$) correlation coefficients were noted (0.86, 0.68, 0.62 for LD_{OCT} *vs.* LD_{LM} , LD_{OCT} *vs.* LD_{CLSM} and LD_{LM} *vs.* LD_{CLSM} , respectively). On the other hand

for deeper ($>200 \mu m$, $n=21$), a significant correlation was not found for LD_{OCT} *vs.* LD_{CLSM} ($r=0.40$, $p>0.05$) while the correlation was significant ($p<0.005$) for LD_{OCT} *vs.* LD_{LM} ($r=0.63$) and LD_{LM} *vs.* LD_{CLSM} ($r=0.61$).

As for clinical imaging, 6 WSLs were evaluated on the buccal surfaces of posterior teeth were visually scored as ICDAS 2. Among these lesions 4 were assessed as primarily caries-related and 2 as complex caries and developmental hypoplasia according to the clinical observation and patient history. Figure 5 shows representative photographs and SS-OCT images of distinct WSL on the smooth surface of the lower right second premolar in a 37 years old male subject (Fig. 5a). Considering caries-related involvement (*e.g.* located at caries-susceptible areas in a high-risk subject), this lesion was thought to be caries lesion and showed bowl type under SS-OCT image.

In another subject (38 years old female), the layer shaped lesion appearing on upper right first molar was thought to be enamel hypoplasia (Fig. 5c).

DISCUSSION

In previous studies, validation of cross-sectional caries observation by microscopy methods has been performed^{15,20}. Direct observing method using CLSM enabled confirming any desired site of interest corresponding to image of SS-OCT without specimen processing such as coating or desiccating¹⁰. High-resolution conventional LM is another alternative for such cross-sectional observation. Light stereo microscopes in are the most basic types of microscopes that in combination with newly developed high-resolution C-mount CDD cameras can provide interesting information about biological tissues at low-intermediate magnification levels. With extensive field of view and improved depth of focus and resolution, LM enabled inspection of the large number of specimens without the use of flash photography.

SS-OCT could detect the enamel demineralization as bright lesion with intensified backscattered signals. The area with increased brightness on SS-OCT images appeared to correspond well with the chalky white lesion in the cross-sectional LM images. While depth has been considered as an important attribute of the lesion, it is noteworthy that the parameter obtained in the 2D ROI essentially considers only a limited area of the lesion and does not indicate lateral extent of the lesion, which would require 3D imaging.

The optical principle governing appearance of demineralization under the two methods (OCT and LM) is thought to be somewhat similar. The increase is attributed to scattering at the numerous microinterfaces created in the hard tissue between media of different refractive indices, due to increased porosity by such causes as the dissolution of minerals. The chalky white appearance of enamel surface has been a well-known sign for detection of lesions by visual inspection clinically. For OCT, the visible boundary between bright and dark areas on the grayscale OCT image was

Fig. 5 Clinical representative case of WSL imaging with digital photography and SS-OCT.

The left column (a, b) are representative of a lesion in a 37 years old male subject. The right column (c, d) are representative of lesion in a 38 years old female subject. (a) Arrow on the digital photograph indicates a WSL on buccal cervical enamel of the right lower second premolar; (b) SS-OCT image of the same WSL indicated by arrow in digital photograph (a). E: enamel, D: dentin. Dentin-enamel junction (DEJ) was clearly detected on SS-OCT image. The WSL is observed as a bowl shaped lesion (arrow), average LD_{OCT} : 105.3 μm ; (c) Arrow on digital photograph indicates a WSL on buccal smooth enamel of the right upper first molar; (d) SS-OCT image of the same WSL indicated by arrow in digital photograph (c). E: enamel, D: dentin. Dentin-enamel junction (DEJ) was partially detected on SS-OCT image. The WSL is observed as a layer shaped lesion (arrow), average LD_{OCT} : 92.1 μm .

reported to be associated with depth of lesion¹⁷). These similarities may explain the fairly strong agreement between OCT and LM results.

The effective imaging depth of OCT is a few millimeters, but depends on the structure being imaged. In the current study, for deeper lesions, the correlation between LM and OCT was somewhat weaker than shallower lesions (Figs. 4a and b). For OCT, sound enamel is reported to be almost transparent at near infrared region, while demineralization increases signal attenuation through the lesion^{15,21}); in fact, the attenuation coefficient is thought to be a distinctive parameter for detection of demineralization^{22,23}). Therefore, it is likely that the depth boundary detection will be less accurate for deep lesions due to lower signal intensity levels. Despite a weaker nominal correlation in deeper lesions, Nakagawa *et al.*¹⁵) reported that OCT was still a powerful diagnostic tool for a general estimation of lesion progress based on a four-level scoring system. The results of this study with regard to the appearance of enamel carious lesions are in general agreement with those of previous studies that used PS-OCT or SS-OCT for detection caries^{13–15,17}). On the other hand, with the CLSM used at 632 nm single wavelength reflection mode and $\times 125$ magnification in this study, very early changes of enamel at the prism level could not be observed, making it difficult to outline the demineralization front in deep lesions. It appears that the limitation of OCT for accurate positioning of deeper lesion boundary in combination with the limitation of CLSM to detect the very front of these lesions, resulted in increased scatter of data and loss of correlation when only lesions deeper than 200 μm were concerned (Figs. 4a and c). It is noteworthy that the current approaches available for remineralization and/or monitoring are thought to be less effective for deeper lesions, and a strong emphasis has been put on detection and monitoring of the lesions at an earlier, shallower stage.

In this study, WSL which were assessed to be either of caries nature or complex nature (*i.e.* a developmental lesion at caries susceptible area) based on the clinical judgment of the case were employed. It is notable that near infrared light of SS-OCT is subjected to attenuate at superficial enamel. In the case of progressed enamel caries, it might include the extensive loss of internal enamel structure creating a large hollow space within the crystals; strong scattering and attenuation of light occurs at the upper border of the hollow space so that the axial extent of the lesion would be difficult to estimate over 200 μm in this study^{10,20,24,25}). It is believed that WSLs may respond to remineralization treatment; in a previous *in vitro* study about remineralization after artificial demineralizing of bovine enamel, it was confirmed that it was difficult to remineralize beyond 200 μm of the optical depth in OCT image²⁶). Other examples involve remineralization efforts by fluoride application or newly introduced agents such as bioavailable calcium releasing chewing gums. It was reported that the combination of surface layer removal (50–75 μm from the enamel surface) and NaOCl

treatment could improve mineral augmentation down to 200 μm from the surface in hypomineralized lesions. However, mineral augmentation deeper than this depth was not achieved stably²⁷).

In this study, WSLs shapes on enamel were classified to either bowl or layer or complex type of bowl and layer. The near infrared light from SS-OCT reflects and backscatters at the border of lesion and intact area; resulting in such appearances that were confirmed by microscopy. From the *in vitro* results, the nature and pathology of lesions were based merely on estimation. However, from *in vivo* observation of teeth with a clear case history, it was confirmed that carious lesions appeared to have curved lesion front resulting in a bowl shape under OCT, while hypomineralized developmental lesions appeared rather like a horizontal layer. It was reported that WSLs clinically diagnosed as a carious lesion appeared as bowl type by OCT, and a histological section in the same plane after tooth extraction confirmed that shape²⁸). On the other hand, hypomineralized developmental lesions visually appear as hypoplastic bands in real clinical situation; their in-depth appearance as layers under OCT is due to their developmental nature. Further investigation with regard to the validity of this morphological classification is necessary. A narrow surface layer with reduced porosity and increased hardness and mineral content has been reported from histological observations for hypermineralized enamel lesions and arrested lesions²⁷). Interestingly, when such a surface layer is thick, it could also be observed on the cross-sectional OCT images, which can help in diagnosis and clinical decision making regarding these lesions^{18,29}).

Differentiating WSL with hypomineralization of developmental origins (localized pre-eruption hypoplasia or hypocalcification) from those enamel lesions associated with plaque accumulation and cariogenic diet can be difficult^{30–33}). A critical distinction should be based on pattern differences and low socioeconomic status. On the other hand, it has been shown that teeth with hypoplasia are more likely to develop caries^{30,31,34–36}). Clinical monitoring of enamel hypoplasia with individual caries risk assessment might be a strategy to manage such lesions.

CONCLUSION

In conclusion, SS-OCT appears to be an effective tool for observation of the internal structure of WSLs. Images of natural WSLs with different shape patterns that showed increased reflectivity were also clinically obtained by SS-OCT. Data analysis enabled quantitative assessment of the lesions; this modality can be a valid adjunct tool to assist visual assessment of WSLs.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Scientific Research (No.25462946) from the Japan Society for the Promotion of Science (JSPS); a Research

Grant for Longevity Sciences (21A-8) from the Ministry of Health, Labor and Welfare. The author(s) declare no potential conflicts of interest with respect to the authorship and/ or publication of this article.

REFERENCES

- Fontana M, Young DA, Wolff MS, Pitts NB, Longbottom C. Defining dental caries for 2010 and beyond. *Dent Clin North Am* 2010; 54: 423-440.
- Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: part 4. Detection and diagnosis of initial caries lesions. *Br Dent J* 2012; 213: 551-557.
- Newman B, Seow WK, Kazoullis S, Ford D, Holecombe T. Clinical detection of caries in the primary dentition with and without bitewing radiography. *Aust Dent J* 2009; 54: 23-30.
- Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, et al. Optical coherence tomography. *Science* 1991; 254: 1178-1181.
- Drexler W, Fujimoto JG. State-of-the-art retinal optical coherence tomography. *Prog Retin Eye Res* 2008; 27: 45-88.
- Chinn SR, Swanson EA, Fujimoto JG. Optical coherence tomography using a frequency-tunable optical source. *Opt Lett* 1997; 22: 340-342.
- Yun S, Tearney G, Bouma B, Park B, de Boer J. High-speed spectral-domain optical coherence tomography at 1.3 μm wavelength. *Opt Express* 2003; 11: 3598-3604.
- Fujimoto JG. Optical coherence tomography for ultrahigh resolution *in vivo* imaging. *Nat Biotechnol* 2003; 21: 1361-1367.
- Xiang X, Sowa MG, Iacopino AM, Maev RG, Hewko MD, Man A, Liu KZ. An update on novel non-invasive approaches for periodontal diagnosis. *J Periodontol* 2010; 81: 186-198.
- Nakajima Y, Shimada Y, Sadr A, Wada I, Miyashin M, Takagi Y, Tagami J, Sumi Y. Detection of occlusal caries in primary teeth using swept source optical coherence tomography. *J Biomed Opt* 2014; 19: 16020.
- 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.
- 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.
- Jones RS, Darling CL, Featherstone JD, Fried D. Remineralization of *in vitro* dental caries assessed with polarization-sensitive optical coherence tomography. *J Biomed Opt* 2006; 11: 014016.
- Natsume Y, Nakashima S, Sadr A, Shimada Y, Tagami J, Sumi Y. Estimation of lesion progress in artificial root caries by swept source optical coherence tomography in comparison to transverse microradiography. *J Biomed Opt* 2011; 16: 071408.
- Nakagawa H, Sadr A, Shimada Y, Tagami J, Sumi Y. Validation of swept source optical coherence tomography (SS-OCT) for the diagnosis of smooth surface caries *in vitro*. *J Dent* 2013; 41: 80-89.
- Shimada Y, Nakagawa H, Sadr A, Wada I, Nakajima M, Nikaido T, Otsuki M, Tagami J, Sumi Y. Noninvasive cross-sectional imaging of proximal caries using swept-source optical coherence tomography (SS-OCT) *in vivo*. *J Biophotonics* 2014; 7: 506-513.
- Nazari A, Sadr A, Campillo-Funollet M, Nakashima S, Shimada Y, Tagami J, Sumi Y. Effect of hydration on assessment of early enamel lesion using swept-source optical coherence tomography. *J Biophotonics* 2013; 6: 171-177.
- Espigares J, Sadr A, Hamba H, Shimada Y, Otsuki M, Tagami J, Sumi Y. Assessment of natural enamel lesions with optical coherence tomography in comparison to microfocus x-ray computed tomography. *J Med Imag* 2015; 2: 014001.
- Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol* 2007; 35: 170-178.
- Shimada Y, Sadr A, Burrow MF, Tagami J, Ozawa N, Sumi Y. Validation of swept-source optical coherence tomography (SS-OCT) for the diagnosis of occlusal caries. *J Dent* 2010; 38: 655-665.
- Hariri I, Sadr A, Shimada Y, Tagami J, Sumi Y. Effect of structural orientation of enamel and dentine on light attenuation and local refractive index: An optical coherence tomography study. *J Dent* 2012; 40: 387-396.
- Mandurah MM, Sadr A, Shimada Y, Kitasako Y, Nakashima S, Bakhsh TA, Tagami J, Sumi Y. Monitoring remineralization of enamel subsurface lesions by optical coherence tomography. *J Biomed Opt* 2013; 18: 046006.
- Manesh SK, Darling CL, Fried D. Polarization-sensitive optical coherence tomography for the nondestructive assessment of the remineralization of dentin. *J Biomed Opt* 2009; 14: 044002.
- Hariri I, Sadr A, Nakashima S, Shimada Y, Tagami J, Sumi Y. Estimation of the enamel and dentin mineral content from the refractive index. *Caries Res* 2013; 47: 18-26.
- Darling CL, Huynh GD, Fried D. Light scattering properties of natural and artificially demineralized dental enamel at 1310 nm. *J Biomed Opt* 2006; 11: 034023.
- Sadr A, Mandurah MM, Nakashima S, Shimada Y, Kitasako Y, Tagami J, Sumi Y. Monitoring of enamel lesion remineralization by optical coherence tomography: an alternative approach towards signal analysis. *Proc SPIE* 2013; 8566: 856602.
- Crombie FA, Cochrane NJ, Manton DJ, Palamara JE, Reynolds EC. Mineralisation of developmentally hypomineralised human enamel *in vitro*. *Caries Res* 2013; 47: 259-263.
- Popescu DP, Sowa MG, Hewko MD, Choo-Smith LP. Assessment of early demineralization in teeth using the signal attenuation in optical coherence tomography images. *J Biomed Opt* 2008; 13: 054053.
- Lee RC, Kang H, Darling CL, Fried D. Automated assessment of the remineralization of artificial enamel lesions with polarization-sensitive optical coherence tomography. *Biomed Opt Express* 2014; 5: 2950-2962.
- Caufield PW, Li Y, Bromage TG. Hypoplasia-associated severe early childhood caries —a proposed definition. *J Dent Res* 2012; 91: 544-550.
- Oliveira AF, Chaves AM, Rosenblatt A. The influence of enamel defects on the development of early childhood caries in a population with low socioeconomic status: a longitudinal study. *Caries Res* 2006; 40: 296-302.
- Needleman HL, Allred E, Bellinger D, Leviton A, Rabinowitz M, Iverson K. Antecedents and correlates of hypoplastic enamel defects of primary incisors. *Pediatr Dent* 1992; 14: 158-166.
- Seow WK. Clinical diagnosis of enamel defects: pitfalls and practical guidelines. *Int Dent J* 1997; 47: 173-182.
- Milgrom P, Riedy CA, Weinstein P, Tanner AC, Manibusan L, Bruss J. Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. *Community Dent Oral Epidemiol* 2000; 28: 295-306.
- Targino AG, Rosenblatt A, Oliveira AF, Chaves AM, Santos VE. The relationship of enamel defects and caries: a cohort study. *Oral Dis* 2011; 17: 420-426.
- Lai PY, Seow WK, Tudehope DI, Rogers Y. Enamel hypoplasia and dental caries in very-low birthweight children: a case-controlled, longitudinal study. *Pediatr Dent* 1997; 19: 42-49.