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Imaging Natural Occlusal Caries Lesions with Optical Coherence Tomography

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Abstract

Several studies have demonstrated that polarization-sensitive optical coherence tomography (PS-OCT) can be used to nondestructively measure the severity of demineralization in the important occlusal surfaces. The purpose of this study was to assess the potential of PS-OCT and OCT methods for the measurement of the depth of natural occlusal carious lesions. Teeth were screened for potential occlusal lesions using near infrared imaging (NIR). A PS-OCT system operating at 1310-nm was used to acquire polarization resolved images of the area of interest on the occlusal surface. The teeth were serial sectioned to 200 μm thickness and examined with polarized light microscopy (PLM) and Transverse Microradiography (TMR) for comparison. The lesion depth measured nondestructively with PS-OCT was compared to the lesion depth measured with PLM and TMR to assess the performance of these methods and determine if polarization sensitivity is required. The lesion depth measured using OCT correlated well with the lesion depths measured with TMR and PLM. Although polarization sensitivity provided better contrast it was not necessary to have polarization sensitivity to identify deep occlusal lesions.

Keywords

near-IR imaging; optical coherence tomography; dental enamel; dental caries; polarization; microradiography; occlusal lesions

1. INTRODUCTION

Caries lesions are routinely detected in the United States using visual/tactile (explorer) methods coupled with radiography. Unfortunately, these methods have numerous shortcomings and are inadequate for the detection of the early stages of the caries process. Radiographic methods do not have the sensitivity for early lesions, particularly occlusal lesions, and by the time the lesions are radiolucent they have often progressed well into the dentin at which point surgical intervention is necessary (1–3). At that stage in the decay process, it is far too late for preventive and conservative intervention and a large portion of carious and healthy tissue will need to be removed, often compromising the mechanical integrity of the tooth. If left untreated the decay will eventually infect the pulp, leading to loss of tooth vitality and possible extraction. The caries process is potentially preventable

and curable. If carious lesions are detected early enough, it is likely that they can be arrested/reversed by non-surgical means through fluoride therapy, anti-bacterial therapy, dietary changes, or by low intensity laser irradiation(4, 5).

Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structure (6). Several groups have used OCT and PS-OCT to image dental caries (7–15) on both smooth surfaces and occlusal surfaces. We have demonstrated that polarization-sensitivity is invaluable for providing depth-resolved measurements of the severity of demineralization both *in vitro* and *in vivo* (16–20). The strong reflectivity of the tooth surface produces a signal much more intense than the scattering from the lesion itself which prevents direct integration of the reflectivity from the lesion area to quantify the lesion severity. Therefore, with a conventional OCT system, the loss of penetration depth must be used as a measure of lesion severity (21). If the incident light is linearly polarized, surface reflections do not depolarize light so the surface reflection does not interfere with the signal in the orthogonal ($\perp$) polarization state. This can also be called the cross polarization OCT image. Caries lesions depolarize incident linearly polarized light and the reflectivity in the cross polarization image can be directly integrated to provide a measure of the lesion severity. By exploiting polarization sensitivity, the reflectivity from lesion areas can be directly integrated—including the very important surface zones—thus overcoming the interference of strong surface reflections at tooth surfaces, a serious limitation of conventional OCT systems.

Quantitative depth resolved measurements are useful for clinical studies and for monitoring the state of early lesions and our studies indicate that polarization sensitivity provides considerable advantages for these types of measurement particularly for early demineralization near tooth surfaces(16–20). However, many clinicians are only interested in knowing how deep the occlusal lesions have actually penetrated into the tooth so that they can decide whether a restoration is necessary. The Diagnodent which employs fluorescence from bacteria porphyrin molecules was developed for this purpose, however the reading only reflects the amount of fluorescence and does not indicate the depth (22–25). Previous studies have explored the use of PS-OCT to measure demineralization and remineralization in the occlusal surfaces. Jones et al. (26) showed that the reflectivity of artificially demineralized enamel could be measured nondestructively and that the integrated reflectivity correlated with the mineral loss. Ngaotheppitak et al. (27) measured the integrated reflectivity of shallow natural lesions (depth < 500- μ m) with PS-OCT which correlated well with the mineral loss. Jones et al. (17) also demonstrated that the demineralization could be measured under sealants and that better images could be acquired in occlusal surfaces by use of index matching agents applied to the fissure areas(19). PS-OCT images are not only useful for providing improved contrast between sound and demineralized enamel. Polarization sensitivity is also useful for differentiating subsurface artifacts produced by the birefringence of sound enamel from caries lesions. It is not clear, however, that polarization sensitivity is necessary to solely detect the lesion for estimation of the depth of penetration. One objective of this study was to compare the lesion depth determined with and without polarization sensitivity with the lesion depth determined from

PLM and TMR to determine if polarization sensitivity is needed to make such a rough depth assessment.

In this study involving natural occlusal lesions, we focused on deeper occlusal lesions to determine whether we could determine if the lesions penetrate to the dentinal – enamel junction (DEJ). PS-OCT scans were compared with PLM and TMR images of histological thin sections to determine how well the OCT images reflect the actual lesion depth.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Teeth extracted from patients in the San Francisco Bay area were collected, cleaned and sterilized with Gamma radiation. Molars and premolars were visually inspected for caries lesions. On extracted molars these lesions are easily identified as white or brown/black (pigmented) spots on the tooth surface and specimens are readily available. Those samples with suspected lesions were further screened using a near-IR imaging system operating at 1310-nm. In the visible range it is difficult to differentiate between stains and actual decay and many of the teeth selected by visual inspection were only stained without decay. The organic molecules that cause pigmentation apparently do not strongly absorb near-IR light and staining does not interfere in the near-IR (28, 29). Twenty samples were selected with suspected deep natural existing occlusal decay using this screening technique.

The roots were cut off and the teeth were mounted on $1.2 \times 1.2 \times 3$ cm³ rectangular blocks of black orthodontic composite resin with the occlusal surface containing the lesion facing out from the square surface of the block. Each rectangular block fit precisely in an optomechanical assembly that could be positioned with micron accuracy. The rectangular symmetry of the mount facilitates matching the position of plano-parallel OCT *b-scans* to the serial thin sections produced for mineral density determination using a digital transverse microradiography system (TMR) and for histological examination using polarized light microscopy (PLM). Figure 1 shows one sample before and after sectioning. Additionally, the teeth were etched with a CO₂ laser to produce fiducial points for more precise matching of the PS-OCT scans with the sections. The CO₂ laser etched a 2×2 mm square with the incision approximately 200-μm across and 50–100-μm deep. Similar rectangular sample holders are mounted on both the PS-OCT scanning system and the microtome. Digital micrometers on both the microtome and the PS-OCT scanning stages were used to position the microtome saw blade and the PS-OCT scans.

2.2 PS-OCT System

A single-mode fiber, autocorrelator-based Optical Coherence Domain Reflectometry (OCDR) system with polarization switching probe, high efficiency piezoelectric fiber-stretchers and two balanced InGaAs receivers that was designed and fabricated by Optiphase, Inc. (Van Nuys, CA) was integrated with a broadband high power superluminescent diode (SLD), Denselight (Jessup, MD) with an output power of 45-mW and a bandwidth of 35 nm and a high-speed XY-scanning system, ESP 300 controller & 850-HS stages, Newport (Irvine, CA) and used for *in vitro* optical tomography. The system

was configured to provide an axial resolution at 22- μm in air and 14- μm in enamel and a lateral resolution of approximately 50- μm over the depth of focus of 10 mm. The all-fiber OCDR system has been previously described in greater detail (30, 31). The PS-OCT system was completely controlled using LabVIEW™ software, National Instruments (Austin TX). Figure 2 shows the scanning stage, sample holders and probe used for this study. Images were acquired in both orthogonal polarizations that were parallel ($\parallel$) and perpendicular ($\perp$) to the light incident on the tooth surface. The orthogonal polarization ($\perp$) is also called the cross polarization image and this image provides the best contrast between sound and demineralized enamel and dentin. We used the sum of both images to represent a “conventional” non-polarization sensitive OCT scan.

2.3 Polarized Light Microscopy (PLM) and Transverse Microradiography (TMR)

After the PS-OCT scanning, the teeth were serially cut into sections of ~200 μm thickness for polarized light examination and digital quantitative microradiography as shown in Fig. 1 using a linear precision saw, the Isomet 5000 (Buehler, Lake Buff, IL). The thickness of each section was measured with a digital micrometer with 1- μm resolution. Ideally, thin sections of 80-100- μm thickness should be used for both PLM and TMR, and thinner sections are more desirable for comparison with the 20- μm thick PS-OCT scans. However, enamel is very brittle and difficult to cut into thin sections without fracture, more so for carious sections, and attempts to produce such thin sections would result in an unacceptable high loss rate of samples/sections, therefore we choose a more reliable section thickness of 200- μm . Thin sections were imbibed in water and examined at up to 500 $\times$ with a polarizing microscope interfaced to a high-resolution digital camera. Demineralization due to strong scattering causes loss of birefringence in the lesion and it appears dark. Measurements of lesion depth were made with calibrated image analysis software that is capable of direct length and area measurements. Polarized light microscopy (PLM) was carried out using a Meiji Techno RZT microscope (Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the brightfield mode with cross polarizers and a red I plate with 500-nm retardation.

Thin sections used in PLM were also imaged using transverse microradiography (TMR). A custom-built digital TMR system was used to measure mineral loss in the different partitions of the sample (32). A high-speed motion control system with Newport (Irvine, CA) UTM150 and 850G stages and an ESP300 controller coupled to a video microscopy and laser targeting system was used for precise positioning of the tooth samples in the field of view of the imaging system. The volume percent mineral for each sample thin section was determined by comparison with a calibration curve of X-ray intensity vs. sample thickness created using sound enamel sections of 86.3 ± 1.9 vol.% mineral varying from 50 to 300 μm in thickness. The calibration curve was validated via comparison with cross-sectional microhardness measurements. The volume percent mineral determined using microradiography for section thickness ranging from 50 to 300- μm highly correlated with the volume percent mineral determined using microhardness $r^2 = 0.99$ (32).

The lesion depths measured using PLM and TMR were compared with the depths indicated with OCT via linear correlation plots (Fig. 4).

3. RESULTS

PS-OCT scans show deep lesion zones penetrating to the dentin-enamel junction for many of the samples. PS-OCT images along with the corresponding PLM and TMR images of the matching thin section are shown in Fig. 3. Each figure represents a PS-OCT b-scan, and PLM and TMR images from one matching section from one tooth sample. The black and gray boxes demarcates the areas of the PS-OCT b-scan and the PLM image represented in the corresponding TMR image. In the PS-OCT images demineralized zones or areas of high reflectance appear as white-red in this color scheme. In the PLM images demineralized zones appear as dark black areas. In the TMR images mineral content is represented by a 16 color rainbow false color table.

One section from each tooth that was cut through the 2×2 mm window matching the PS-OCT scan was chosen for PLM and TMR analysis. The position of measurement was chosen as the most severe lesion area of the OCT scan. It is important to point out that in many areas of each section the OCT scans did not show the full depth of penetration of the lesion. For most of the samples the corresponding PS-OCT and TMR images correspond well showing that the increase in reflectivity follows the increase in mineral loss. The penetration depth reaches beyond the DEJ in many cases. Fig. 4 shows linear correlation plots comparing the lesion depth measured using OCT with the lesion depth measured using PLM and TMR. The correlation coefficient (Pearson) was $r = 0.63$ ($P < 0.01$) for OCT and PLM, and $r = 0.75$ ($P < 0.001$) between OCT & TMR.

4. DISCUSSION

This study indicates that optical coherence tomography has great clinical potential for the nondestructive assessment of the depth of occlusal lesions and for the detection of hidden lesions under the enamel.

The requisite optical penetration/imaging depth for the detection and diagnosis of occlusal lesions is to the DEJ. If the lesion is present in the underlying dentin and the enamel above is sound, OCT works quite well in resolving that lesion and the images confirm penetration to the DEJ. If extensive demineralization is present from the enamel surface all the way down to the DEJ the results are quite mixed, i.e., sometimes the entire lesion is visible from the enamel surface to the DEJ, while more typically only the outer surface of the lesion is visible or the area where the lesion has reached the DEJ (lower part) can be seen. Additional studies are needed to determine why the optical penetration depth through the lesions is so variable. Most lesions extend outward upon reaching the underlying dentin, therefore we anticipate that OCT should be able to determine whether most lesions have reached the DEJ. However, because of the complex nature of the OCT images it will probably be necessary to acquire 3D images of each lesion or several b-scans across different regions of the lesion in order to make a reliable assessment of the lesion. Our time-domain OCT system utilized a fairly high power optical source (45 mW) with a fairly narrow bandwidth with lower depth

resolution. We have found that the best optical penetration is achieved with higher power sources. Similar improvements in performance should be attainable with higher sensitivity systems along with better signal to noise. It is anticipated that future improvements in OCT technology in such areas should provide more improved optical penetration for even better performance.

One limitation of radiography is that ionizing radiation is required, however it is even more important to emphasize that radiography is insensitive for the detection of early occlusal lesions and that by the time they show up on a radiograph they have typically spread extensively throughout the dentin and are far too late for surgical intervention. Therefore, the performance of OCT in this study is a marked improvement over existing technology.

The utility of PS-OCT for the measurement of early demineralization on tooth surfaces has been clearly established from previous studies (16#x2013;20). However, in this study with the aim of assessing the depth of deeply penetrating caries lesions the advantage of having polarization sensitivity was less evident. Although the polarization sensitivity did provide an apparent improvement in contrast, most of the benefit is near the tooth surface. Polarization sensitivity also helps differentiate subsurface artifacts from lesions. However, PS-OCT systems are more expensive and difficult to use and this study suggests that it is not absolutely necessary to have polarization sensitivity to detect deep occlusal lesions. The correlation of OCT with PLM and TMR was encouraging. But it is important to stress the point that the comparison is based on the deepest lesion penetration in the OCT image and does not reflect the overall correlation of the OCT images with PLM and TMR. If the comparison was based on a random position of each lesion initially chosen from the PLM images the correlation coefficients are likely to be lower.

In summary, these measurements show that PS-OCT images reflect the structure, depth and severity of deep natural carious lesions in occlusal surfaces and have considerable potential as a non-destructive tool to assess the severity of caries lesions in the important occlusal surfaces where most decay is found.

5. ACKNOWLEDGMENTS

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Fig. 1.

A photograph series of a prepared sample mounted on the acrylic base with a laser etched 2×2 mm square and serial sections prepared for histological examination with PLM and TMR.

Fig. 2.

A photograph of the PS-OCT systems showing the relative positions of the sample holder with tooth, scanning stages and probe. The incident beam is aligned with one corner of the brass holder so that the scan position can be matched to the position of serial sectioning for histology.

Fig. 3.

Image series of PS-OCT ($\perp$ -axis) (left), PLM (center), and TMR (right) for four different teeth, A–D. The lesion is demarcated by the black and gray boxes in the OCT and PLM images. OCT images are shown in a red-white-blue false color scale in dB with high reflectivity in red. The TMR false color scale is 16 color Rainbow representing mineral density with purple (high) to orange (low).

Fig. 4.

Lesion depths measured using OCT, PLM and TMR for 20 teeth. Correlation plots are also shown for (OCT & PLM, solid line) $r=0.63$ (Pearson), $P < 0.01$ and (OCT & TMR – dotted line) $r = 0.75$, $P < 0.001$.