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Imaging natural and artificial demineralization on dentin surfaces with polarization sensitive optical coherence tomography

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Abstract

The purpose of this study was to assess the potential of polarization sensitive optical coherence tomography (PS-OCT) to measure non-destructively the severity of natural and artificial caries lesions in dentin and determine the efficacy of intervention with anti-caries agents. Although several studies have demonstrated the utility of PS-OCT to image caries lesions in enamel and to quantify the lesion severity, only a few studies have focused on lesions in dentin. In this study images of natural root caries lesions on extracted human teeth were acquired with PS-OCT. In addition artificial lesions were produced in dentin after 12 days of exposure to a demineralization solution at a pH of 5.0. Before exposure, three incisions were made on the sample surfaces using Er:YAG laser irradiation and selected areas were treated with topical fluoride. PS-OCT images were acquired using a high power (45-mw) 1310-nm superluminescent diode with a bandwidth of 35-nm. PS-OCT was able to measure demineralization in dentin to a depth of ~ 1 mm. Polarized light microscopy and microradiography were used to measure lesion severity on histological thin sections for comparison. PS-OCT successfully measured the inhibition of demineralization by topical fluoride. Er:YAG laser irradiation did not significantly increase or decrease the rate of dentin demineralization.

Keywords

polarization; optical coherence tomography; Er:YAG laser; dentin; laser ablation; caries inhibition; thermal damage; topical fluoride

2. INTRODUCTION

Several studies over the past few years have demonstrated that optical coherence tomography (OCT) can be used to measure natural and artificial caries lesions on enamel surfaces¹⁻¹³. Although the penetration depth of near-IR light is considerably less in dentin than for enamel due to the higher scattering coefficient of dentin, PS-OCT and OCT images of dentin with root caries show that penetration depths of ~ 1 mm can be achieved and demineralized dentin can be discriminated from sound dentin and cementum^{8,14}.

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Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structure^{15,16}. Depth-resolved measurements of caries lesions can be acquired on enamel surfaces^{17–21}. In contrast to sound enamel that is highly transparent in the near-IR, sound dentin strongly scatters light in the near-IR and is highly birefringent. Therefore, there is some question whether the same approach employed to assess the severity of demineralization on enamel surfaces can be applied to the surface of dentin. We have shown that for enamel surfaces demineralized areas on the tooth depolarize incident linearly polarized light and the reflectivity in the ($\perp$) polarization state can be directly integrated to provide a measure of the lesion severity.

PS-OCT is well-suited for imaging thermal changes in biological tissues such as dentin and another objective of this work was to use OCT to image ablation craters after treatment to show that there is minimal thermal modification of the dentin. Specific laser conditions were chosen for this study that have been found to produce minimal thermal modification of the dentin²². Laser irradiation can induce changes in the birefringence and roughness of the dentin surface that may interfere with the ability of PS-OCT to assess the lesion severity. In turn, such thermally induced changes can be measured using PS-OCT to assess the depth or thickness of the thermally modified dentin layer and any peripheral thermal damage or mechanical damages such as microcracks.

Current methods that are used to determine the efficacy of anti-caries agents require teeth scheduled for extraction. These methods include microradiography, microhardness or polarized light microscopy and all these methods require thin sectioning of the extracted tooth, hence the need to carry out studies on teeth scheduled for extraction.

It has been well established that topical fluoride agents can inhibit the demineralization of enamel, dentin and cementum²³. Several studies over the past thirty years have indicated that lasers can be used to thermally modify the chemical composition of dental enamel to render it more resistant to acid dissolution and potentially more resistant to dental caries^{24–30}. Moreover, other studies have indicated that topical fluoride treatments can work synergistically with the laser treatment to further enhance its inhibitory effect^{31–34}. However, it has yet to be demonstrated that lasers can be used to inhibit the demineralization of dentin and only a few papers have identified any beneficial effect of laser treatments on dentin^{35,36}. In this paper we used Er:YAG laser irradiation at high irradiation intensities to etch the dentin surface, remove the dentin smear layer, and increase the permeability of the dentin to fluoride.

In summary, the principal objectives of this project was to demonstrate that PS-OCT can be used as a nondestructive means for quantifying the severity of artificial and natural caries lesions and as a means of measuring the inhibition potential of anti-caries agents such as fluoride and laser treatments on dentinal surfaces. The integrated reflectivity in lesion areas on both natural and artificial caries lesion measured with PS-OCT was compared with the well established methods of transverse microradiography and polarized light microscopy that require tooth sectioning.

3. MATERIALS AND METHODS

3.1 Sample Preparation

Extracted teeth with natural root caries lesions were collected from patients in the San Francisco Bay Area. Teeth were sectioned in half and mounted on cylindrical delrin blocks with the exposed root caries lesion facing up to facilitate matching the position of the OCT scans and the sections to be cut for histology.

Dentin blocks, approximately $6 \times 3 \times 2 \text{ mm}^3$ were prepared from extracted human teeth for the artificial lesion studies. The dentin surfaces were serial polished to one micron using embedded diamond polishing discs. Each dentin sample was partitioned into three regions as shown in Fig. 1 with the center of each region being treated with the laser using the conditions specified in the next section. The middle partition of each sample was covered with a thin layer of acid resistant varnish in the form of red nail polish (Revlon, New York, New York) that served to protect the sound dentin as the control area before exposure to the demineralization solution. The middle partitions served as the negative control groups: protected (S) and protected + laser (S + L). A 1.23% acidulated phosphate fluoride foam, Oral B Minute Foam (Gillette, South Boston, MA), was applied only to the left side following treatment with the laser (F). After allowing exposure for one minute, the samples were rinsed with deionized water, taking care not to rinse fluoride onto the untreated side of the sample. The fluoride treated left partition served as the fluoride + lesion (F) and fluoride + laser + lesion (F + L) groups. The right side served as lesion (D) and lesion + laser (D + L) groups. The samples were then cemented to delrin blocks and the sample sides varnished to isolate the dentin surface. After treatment, samples were placed in a demineralization solution for twelve days consisting of a 40-mL aliquot of acetate buffer solution with 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate maintained at pH 5.0 and kept at a temperature of 37°C ³⁷. Having the six study groups within each sample, lesion (D), lesion plus laser (S + L), sound (S), sound plus laser (S + L), fluoride (F), and fluoride plus laser (F + L) allowed us to decrease inter-sample variability.

3.2 Laser Treatment

Samples were irradiated using an Er:YAG laser operating in free running mode originally manufactured by Schwartz Electro-optics (Orlando, FL). The “short” free-running laser pulse was generated using an Analog Modules (Longwood, FL) Model 8800 variable pulse power supply that generates a square shaped flash lamp drive pulse. The flash lamp drive pulse was set to 160- μs in order to generate the 35- μs pulse shown in Fig. 2. The laser pulse temporal profile was measured with a room temperature HgCdTe detector with a response time of a few ns Boston Electronics (Brookline, MA). The beam diameter at the position of irradiation was 400- μm , measured by scanning with a razor blade across the beam. The spot profile was single mode and fluences were defined using a Gaussian beam with a $1/e^2$ beam diameter. An irradiation intensity of 8 J/cm^2 was used to produce the three incisions on each sample. The sample was continuously scanned across the laser beam at a rate of 0.25 mm/sec and a pulse repetition rate of 5-Hz. A computer controlled motion control system ESP-300 (Newport, Irvine, CA) incorporating two 850G stages, was used to ensure uniform surface treatment. The samples were kept well-hydrated before irradiation and a computer

controlled pulsed air/water nozzle was used to apply a layer of water on the sample between laser pulses. A low volume / low pressure air-actuated fluid spray delivery system consisting of a EFD 780S-SS Spray valve, a Valvemate 7040 controller and a fluid reservoir from EFD Inc. (East Providence, RI) was used to provide a uniform continuous spray of fine water droplets on the tissue surface.

3.3 PS-OCT System

A single-mode fiber autocorrelator-based Optical Coherence Domain Reflectometry (OCDR) system with a polarization switching probe, high efficiency piezoelectric fiber-stretchers and two balanced InGaAs receivers that was designed and fabricated by Optiphas, Inc., Van Nuys, CA was used for these measurements. A description of the scanning autocorrelator is described in reference³⁸. The OCDR 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 & 850G-HS stages, National Instruments, Austin, TX) for *in vitro* optical tomography. The probe was designed to provide a spot diameter of 50- μ m over a range of 10-mm.

3.4 Polarized Light Microscopy (PLM) and Digital Transverse Microradiography (TMR)

After the samples were imaged with PS-OCT, they were cut into sections approximately 200–250- μ m thick using an Isomet 5000 saw (Buehler, IL), for polarized light microscopy (PLM) and digital transverse microradiography (TMR). Polarized light microscopy was carried out using a Scientific Series 7 optical microscope (Westover Scientific, Mill Creek, WA) with an integrated digital camera, Canon EOS Digital Rebel XT (Canon Inc., Tokyo, Japan). The sample sections were imbedded in water and examined in the brightfield mode with cross polarizers and a red I plate with 500-nm retardation. A custom-built digital transverse microradiography (TMR) system was used to measure mineral loss in the different partitions of the sample. A high-speed motion control system with Newport 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.

3.5 Integrated Reflectivity, Quantitative Mineral Loss Profiles and Lesion Depth Measurements

The integrated reflectivity and integrated mineral loss were calculated in each area of interest on the samples. Line profiles were taken from the orthogonal polarization ($\perp$ -axis) PS-OCT images to a real depth of 500- μ m, yielding the integrated reflectivity, R , of the regions in units of (dB $\cdot\mu$ m). Previous studies have shown that R correlates directly with the integrated mineral loss called Z for enamel⁶. Similarly, line profiles were taken from the digital transverse microradiographs (TMR) of the thin sections cut along the same position scanned by OCT. X-ray attenuation was converted to volume percent mineral using a calibration curve. The line profiles were integrated from the edge of the sample to a depth of 500- μ m to give units of Z (vol.% $\cdot\mu$ m). The integrations were performed using Igor Pro software (Wavemetrics, Lake Oswego, OR). Prior to taking the line profiles for the OCT

images, a 10×10 convolution filter was applied to smooth out the images using the Convolution Filtering function from the Image Processing package of Igor Pro.

4. RESULTS AND DISCUSSION

4.1 Natural Root Caries Lesions

A PS-OCT b-scan of a natural carious lesion is shown in Fig. 3 along with the corresponding digital transverse microradiograph. The integrated reflectivity, R , representing the lesion severity was calculated to a depth of 500 μm for 11 teeth with root caries lesions. Five line profiles were extracted from areas of each lesion in regions of varying severity including one line profile from a sound region. After imaging the teeth, they were sectioned across the lesion areas to generate thin slices of ~200–250- μm thickness for examination by polarized light microscopy (PLM) and microradiography (TMR). The integrated mineral loss, Z , was determined using TMR for the matching line profiles from the lesion areas. A plot of the integrated reflectivity vs. the integrated mineral loss for the 55 line profiles is shown in Fig. 4. The multi-colored points correspond to the five profiles taken from the sample shown in Fig. 3 while the other points are taken from the other 10 samples. Although the integrated reflectivity of the natural lesions increased with increasing mineral loss the correlation was fairly low ($P_r = 0.419$). This low correlation is mainly due to fracture of the fragile carious lesions during the histological sectioning, since it is extremely difficult to acquire intact thin sections of root caries lesions without damaging the fragile lesions. The ability of PS-OCT to measure the depth and severity of fragile root caries lesions further underscores the utility of OCT for caries imaging lesions since it may be next to impossible to acquire intact thin sections of severe lesions in dentin without embedding the samples before sectioning. Moreover, embedding the highly porous lesions may interfere with assessment by TMR and PLM.

4.2 Artificial Lesions of Dentin

Artificial lesions were produced by exposing the dentin samples shown in Fig. 1 to a demineralization solution at pH 5.0 for 12-days. Fig. 5 shows the corresponding PS-OCT b-scan ($\perp$ -axis), PLM image and digital microradiograph of one sample. The demineralization was most severe in the region marked (D) where topical fluoride was not applied and the lesion exceeds a depth of 200 μm . Demineralization is less severe in the area treated with fluoride (F). The reflectivity is lower and the depth of the lesion appears to be approximately half the depth. Laser treated areas are indicated by the shallow depressions where ablation occurred. These are best seen in the PLM image. The original incision depth before exposure is shown in the sound area. The areas of the laser incisions exposed to the demineralization solution do not show a dramatic reduction in the lesion severity as has been observed for enamel (see the other manuscript in this proceedings by Hsu et al.) that suggests that the laser is not as effective at inhibiting decay as it is on enamel. It also shows that the laser does not make things worse by increasing the solubility of dentin which could occur with thermal damage to the collagen matrix (see the other paper in this proceeding by Featherstone et al. that clearly shows that thermal damage to collagen accelerates the demineralization of dentin). Since dentin contains a high percentage of collagen it shrinks considerably with loss of mineral in air. This can be seen in Fig. 5. PS-OCT and TMR are

carried out with the dentin samples in air while for PLM the samples were immersed in water. There is considerable shrinkage in the F and D regions for the PS-OCT and TMR images while the PLM images show minimal shrinkage in those regions. The integrated reflectivity and the integrated mineral loss are significantly lower ($p < 0.05$) for the fluoride treated areas (F) vs. the untreated areas (D). The laser treated areas had slightly lower reflectivity compared to the rest of the lesion zones, however, those areas were not significantly lower. The integrated reflectivity correlated well with integrated mineral loss of all six regions on the 12 samples with a Pearson correlation coefficient of 0.776. With subtraction of the baseline reflectivity of sound dentin, this coefficient was a bit higher ($P_r = 0.854$).

In summary, PS-OCT was capable of measuring differences in reflectivity between sound and carious/demineralized dentin. We attempted to correlate the integrated reflectivity of different lesion areas as measured with PS-OCT with the integrated mineral loss measured with TMR for several root caries lesions, however, most of the extremely fragile root caries lesions were damaged during the sectioning process. This underscores the value of PS-OCT as a non-destructive imaging tool since it is extremely challenging to acquire intact thin sections of caries lesions. PS-OCT performed well in its ability to measure the severity of artificially demineralized dentin and the PS-OCT, polarized light, and microradiography images matched consistently. We will submit a future more extensive manuscript for peer review summarizing the results employing PS-OCT to measure the effects of fluoride in conjunction with Er:YAG, CO₂ and frequency-tripled Nd:YAG ($\lambda = 355\text{-nm}$) laser irradiation on inhibiting artificial lesion development in dentin.

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Fig. 1. Human dentin sample after irradiation showing the position of the three laser treated areas that were subsequently treated with topical fluoride (F), protected with varnish (S) and left unprotected (D).

Fig. 2.
Temporal profile of the Er:YAG laser used in this study with the 35- μs laser pulse.

Fig. 3. PS-OCT ($\perp$ -axis) scan and microradiograph of a root caries lesion. Integrated reflectivity and integrated mineral loss are calculated at indicated line profiles. The red-white-blue false color is used to show the reflectivity in (dB) and volume % mineral content for PS-OCT and TMR, respectively

Fig. 4.

Integrated reflectivity vs. integrated mineral loss values plotted from line profiles of 11 root caries samples. The corresponding Pearson correlation coefficient was 0.419. The colored points were taken from the lesion of figure 3.

Fig. 5. PS-OCT, PLM, and TMR images of the same dentin sample with artificial lesions. Er:YAG laser irradiated areas are indicated by the shallow incisions with the fluoride treated region (F) is on the left, the protected region (S) is in the middle and the untreated region (D) is on the right.