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Assessment of Dentin Remineralization with PS-OCT

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Abstract

Previous studies have demonstrated that polarization sensitive optical coherence tomography (PS-OCT) can be used to image natural root caries lesions, measure non-destructively the severity of dentin demineralization and determine the efficacy of intervention with anti-caries agents including fluoride and lasers. The objective of this study was to determine if PS-OCT could be used to nondestructively measure the formation of a layer of remineralized dentin on the surface of dentin lesions after exposure to a remineralization solution. In this study images of artificial dentin lesions on extracted human teeth were acquired using PS-OCT after exposure to an artificial demineralizing solution at pH 4.9 for six days and after subsequent exposure to a remineralizing solution at pH 7.0 for 20 days. Polarized light microscopy and microradiography were used to examine histological thin sections from the samples for comparison. PS-OCT successfully measured the formation of a layer of increased mineral content near the lesion surface. PLM and TMR corroborated those results. This study demonstrates the potential use of PS-OCT for the nondestructive measurement of the remineralization of dentin surfaces.

Keywords

polarization sensitive optical coherence tomography; dentin; early demineralization; remineralization

1. INTRODUCTION

Several studies over the past few years have demonstrated that optical coherence tomography (OCT) can be used for the assessment of the severity of early caries lesions. Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structure¹⁻³. It is nondestructive and has great potential for imaging “*in vivo*”. Polarization-sensitive OCT (PS-OCT) is a form of OCT that is sensitive to changes in the polarization of the reflected light⁴. Polarization sensitive optical coherence tomography (PS-OCT) has been successfully used to: acquire images of both artificial and natural caries lesions, assess their severity in depth, assess the remineralization of such lesions on enamel^{3,4,5}, and determine the efficacy of chemical agents in inhibiting demineralization⁵. Recent studies in our laboratory on dentin and cementum surfaces

demonstrate that PS-OCT is also well suited to assess the severity of demineralization on those surfaces as well and that it can be used to determine the efficacy of anti-caries agents such as fluoride and lasers ^{6,7}.

One of the most promising applications of PS-OCT is the nondestructive measurement of the remineralization of existing caries lesions. “In vitro” measurements of the remineralization of artificial lesions produced on enamel surfaces demonstrated that the thickness of the thin layer of higher mineral content that is typically formed near the lesion surface during the remineralization process can be measured with PS-OCT^{8,9}. This layer is of considerable importance since the formation of this layer of fluoroapatite limits diffusion into the lesion leading to the arrest of lesion progression and the lesion becomes inactive and there is no further need for intervention.

Polarization-sensitive depth-resolved reflectivity measurements can provide a measure of the severity of natural and artificial caries lesions on smooth surfaces and in the occlusal pits and fissures ^{10–14}. The high reflectivity of dentin and enamel surfaces produces a very strong reflection that can interfere with the measurement of early demineralization on the surface. This strong reflection can be reduced using polarized light. Moreover, demineralized areas on the tooth depolarize the incident linearly polarized light and the reflectivity in the orthogonal or perpendicular polarization state ($\perp$ -axis) to the incident polarization can be directly integrated to provide a measure of the lesion severity.

Although the penetration depth of near-IR light is considerably less in dentin than for enamel due to the markedly higher scattering coefficient of dentin, PS-OCT and OCT images of dentin with root caries show that an effective imaging depth of ~ 1 mm can be achieved and demineralized dentin can be discriminated from sound dentin and cementum ¹⁵ and root canal walls and fractures can be imaged ^{16,17}. Amaechi et al.¹⁸ measured the % reflectivity loss beneath the lesion due to demineralization on dentin surfaces and showed that this correlated well with mineral loss from microradiography. However, the validity of an approach that relies on the measurement of reflectivity loss is questionable since light scattering increases with demineralization for both enamel and dentin producing an overall increase in reflectivity from the area of the lesion, not a decrease in reflectivity. The approach that we have chosen is to measure the reflectivity from each layer of the lesion and subsequently integrate over a specific depth to yield the integrated reflectivity (R) in units of $\text{dB} \times \mu\text{m}$ which is compared with the “gold standard”, the integrated mineral loss (Z) that is calculated in a similar fashion by integrating the mineral loss over a specific depth ^{10–14}. 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 ^{19,20}. Even with this limitation, we have successfully demonstrated that a similar approach to that used to assess the severity of demineralization on enamel surfaces can be applied to the dentin surfaces as well ^{6,7}.

It has been well established that topical fluoride agents can inhibit the demineralization of enamel, dentin and cementum²¹. Current methods that are used to determine the efficacy of anti-caries agents require teeth scheduled for extraction and partial destruction of the tooth through sectioning. These methods include microradiography, microhardness and polarized

light microscopy. PS-OCT is ideally suited to assess the efficacy of anti-caries agents non-destructively both *in vitro* and *in vivo*.

In summary, the principal objective of this study was to demonstrate that a significant reduction in the reflectivity from the area of dentin lesions can be measured using PS-OCT after exposure of those lesions to a remineralization solution. PS-OCT measurements were also compared with the well-established methods of transverse microradiography and polarized light microscopy that require tooth extraction and thin sectioning.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Fifteen dentin blocks, approximately $6 \times 3 \times 2 \text{ mm}^3$ were prepared from extracted human teeth for this study. The dentin surfaces were serial polished to one micron using embedded diamond polishing discs. Two windows on each sample were exposed to the demineralization solution and one window was exposed to the remineralization solution (Fig. 1) The middle partition of each sample and both ends was covered with a thin layer of acid resistant varnish, red nail polish from Revlon, (New York, NY)) to protect the sound dentin control area before exposure to the demineralization solution. Samples were placed in a demineralization solution for six days with both windows exposed consisting of a 40-mL aliquot of 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate at pH 4.9, kept at a constant temperature of 37°C. One window was covered with acid resistant varnish leaving the other window exposed and the samples were placed in a solution for remineralization consisting of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl, 20 mmol/L cacodylate buffer maintained at pH 7.0 and 37°C for 20 days. Fluoride, as NaF, was added at the level of 2 ppm F to enhance remineralization ²².

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) from 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 and 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 16- μm in dentin and a lateral resolution of approximately 50- μm over the depth of focus of 10-mm. The all-fiber OCDR system is described in more detail in reference ²³. The PS-OCT system was completely controlled using LabVIEW™ software from National Instruments (Austin TX).

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

After the dentin samples were imaged with PS-OCT, they were cut into sections approximately 200–250- μm thick using an Isomet 5000 saw from Buehler (Lake Bluff, IL) for polarized light microscopy (PLM) and digital transverse microradiography (TMR). PLM was carried out using an RZT microscope from Meiji Techno Co., LTD (Saitama, Japan)

with an integrated digital camera, Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the brightfield mode with crossed 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 (see manuscript #7162-30 in this proceeding volume). 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. The volume percent mineral of the three partition areas on each sample was determined by comparison with a calibration curve of x-ray intensity versus sample thickness created using sound enamel sections of $86.3 \pm 1.9\%$ volume percent 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 $r^2 = 0.99$.

3. RESULTS AND DISCUSSION

Reflected light images of one of the dentin samples after exposure to the demineralization and remineralization solutions is shown in Fig. 1. The demineralization window (D) appears whiter or more opaque than the remineralization window (R) as would be expected if additional mineral has been deposited in the area exposed to remineralization. PS-OCT scans were acquired along the long axis of each sample near the center and sectioned for PLM and TMR in the same orientation.

Figures 2 & 3 each contain a (A) ($\perp$ -axis) PS-OCT b-scan image and the corresponding (B) transverse microradiography (TMR) and (C) polarized light microscopy (PLM) images for a $\sim 200\text{-}\mu\text{m}$ thick section cut from the same sample with a similar orientation. A red-white-blue false color scheme is used for the PS-OCT images with the intensity scale in decibel units (dB), where red and white areas have the highest reflectivity. A similar red-white-blue false color scheme was also used for the volume % mineral content in the TMR images. The reflectivity of sound dentin is relatively high due to its high scattering coefficient. The lower range of the intensity scale for the PS-OCT images was intentionally set to maximize the contrast of the demineralized dentin in Figs. 2 & 3. Therefore, the sound areas appear blue. The demineralization was most severe in the region marked (D) as anticipated while the areas exposed to the remineralization solution (R) typically had a layer of intact mineral of enhanced mineral content.

Dentin contains a high percentage of collagen and demineralized dentin tends to shrink considerably when exposed to air, i.e., if it is not immersed in water. This can be seen in Figs. 2 & 3. PS-OCT and TMR were carried out with wet samples in air while for PLM the samples were immersed in water. There is considerable shrinkage in the demineralized regions for the PS-OCT and TMR images while the PLM images show minimal shrinkage in those regions. It is interesting to note that shrinkage typically did not occur in the remineralized window (R) since the highly mineralized intact surface zone prevented loss of water from the lesion area.

The reflectivity from the artificial lesion areas exposed only to the demineralization solution were higher than the lesion areas exposed to the same demineralization solution followed by subsequent exposure to a remineralization solution for 20-days, thus demonstrating that PS-OCT can successfully be used to measure the deposition of new mineral in lesion areas particularly near the surface. In many of the lesion areas exposed to remineralization there was a highly transparent zone (PLM) of high mineral content that was deposited on the surface of the lesion. TMR indicated that the mineral content of that outer zone was higher than 60% mineral by volume, which is higher than sound dentin. The high transparency and mineral density of this zone suggests the growth of a highly orientated crystalline phase of apatite. A simple precipitation of calcium phosphate or hydroxyapatite on the surface would produce a rough and opaque, white polycrystalline deposit. We have observed both transparent and opaque surface layers formed on the surface of enamel after exposure to a similar regimen of remineralization ^{8,9,24}. PLM images show that the layer of higher mineral extends above the original dentin surface. This might suggest that this layer may only be a simple, non-integrated precipitate on the lesion surface. However, the surface layer had sufficient mechanical integrity to remain intact during the sectioning process, which indicates that the newly deposited mineral layer is well integrated into the underlying dentin. It is interesting to note that the zone of remineralization is thicker and more pronounced on dentin than we have observed previously on enamel surfaces. Moreover, a definitive zone of remineralization was present on virtually all the dentin samples while the formation of the outer remineralization layer was less consistent on enamel ^{8,9}.

One can postulate that the presence of the outer zone of high mineral content indicates that the active lesion has become arrested and that further chemical intervention is not necessary. If this is validated clinically then PS-OCT imaging would present a valuable nondestructive method for assessing non-surgical intervention.

The excellent agreement of PLM, TMR and PS-OCT further demonstrates the diagnostic potential of PS-OCT for root caries lesions. Statistical comparisons of the PS-OCT scans, PLM images and Microradiographic scans for all the samples will be submitted for publication in a future article to be submitted for publication in the future.

In conclusion, this study demonstrates that PS-OCT can be used to measure remineralization on dentin surfaces and detect the formation of a zone of highly mineralized dentin on the lesion surface after exposure to a remineralization solution. Further work is needed to demonstrate that PS-OCT can be used to measure the remineralization of natural caries lesions both *in vitro* and *in vivo*.

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Fig. 1.

Reflected light image of a representative tooth sample after demineralization and remineralization studies introducing 3 study groups of D, S, and R. The remineralized layer is clearly visible on the (R) group as compared to its (D) group counterpart.

Fig. 2.

(A) ($\perp$ -axis) PS-OCT scan taken along long axis of a sample before sectioning. Intensity scale is shown in red-white-blue in units of decibels (dB). Remineralized layer is visible as a blue gap above the remineralized lesion. (B) The corresponding transverse microradiograph (TMR) of thin section cut in a similar orientation to the PS-OCT image. The intensity scale is also shown in red-white-blue with units in volume % mineral. (C) The PLM image of the same section is shown at 15 $\times$ magnification.

Fig. 3.
 (A) ($\perp$ -axis) PS-OCT scan taken along long axis of another sample before sectioning.
 Remineralized lesion has a significant lower intensity compared to the demineralization
 lesion. TMR (B) and PLM (C) images of the corresponding thin section.