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Automated detection of remineralization in simulated enamel lesions with PS-OCT

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Abstract

Previous *in vitro* and *in vivo* studies have demonstrated that polarization-sensitive optical coherence tomography (PS-OCT) can be used to nondestructively image the subsurface structure and measure the thickness of the highly mineralized transparent surface zone of caries lesions. There are structural differences between active lesions and arrested lesions, and the surface layer thickness may correlate with activity of the lesion. The purpose of this study was to develop a method that can be used to automatically detect and measure the thickness of the transparent surface layer in PS-OCT images. Automated methods of analysis were used to measure the thickness of the transparent layer and the depth of the bovine enamel lesions produced using simulated caries models that emulate demineralization in the mouth. The transparent layer thickness measured with PS-OCT correlated well with polarization light microscopy (PLM) measurements of all regions ($r^2=0.9213$). This study demonstrates that PS-OCT can automatically detect and measure thickness of the transparent layer formed due to remineralization in simulated caries lesions.

Keywords

Enamel; caries; PS-OCT; image processing algorithms; edge-detection; remineralization

1. INTRODUCTION

New diagnostic tools are needed for the detection and characterization of caries lesions in the early stages of development [1]. 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. Therefore, one cannot overstate the importance of detecting the decay in the early stage of development at which point non-invasive preventive measures can be taken to halt further decay.

Accurate determination of the degree of lesion activity and severity is of paramount importance for the effective employment of new optical diagnostic technologies. Since optical diagnostic tools exploit changes in the light scattering of the lesion they have great

potential for the diagnosis of the current “state of the lesion”, i.e., whether or not the caries lesion is active and expanding or whether the lesion has been arrested and is undergoing remineralization. Therefore, new technologies should be able to determine whether caries lesions have been partially remineralized and have become arrested. Previous studies have demonstrated that polarization sensitive optical coherence tomography (PS-OCT) is uniquely capable of this task since it provides a measure of the reflectivity from each layer of the lesion and is able to show the formation of a zone of increased mineral density and reduced light scattering due to remineralization [2-4]. *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 [5, 6]. 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 [7, 8].

Polarization sensitivity is particularly valuable for imaging caries lesions due to the enhanced contrast of caries lesions caused by scattering of the incident light by the lesion and the confounding influence of the strong surface reflectance of the tooth surface is reduced in the cross-polarization image [9]. PS-OCT images are typically processed in the form of phase and intensity images, and such images best show variations in the birefringence of the tissues [10, 11]. Caries lesions rapidly scatter incident polarized light and the image orthogonal to that of the incident polarization can provide improved contrast of caries lesions. There are two mechanisms in which intensity can arise in the cross-polarization axis. The native birefringence of the tooth enamel can rotate the phase angle of the incident light beam between the two cross-polarization axes (similar to a wave-plate) as the light propagates through the enamel without changing the degree of polarization. The other mechanism is scattering of incident light in which the degree of polarization is reduced. It is this latter mechanism that is exploited to measure the severity of demineralization. Strong scattering of the incident linearly polarized light scrambles the polarization and leads to equal distribution of the intensity in both cross-polarization axes. Demineralization of the enamel due to dental decay causes an increase in the scattering coefficient by a 1-2 orders of magnitude, thus demineralized enamel induces a very large increase in the reflectivity along with scattering of the polarized light [12]. This in turn causes a large rise in reflectivity in the cross-polarization channel or axis.

This approach also has the added advantage of reducing the intensity of the strong reflection from the tooth surface for measurement of the lesion surface zone that can potentially provide information about the lesion activity and remineralization. A conventional OCT system cannot differentiate the strong reflectance from the tooth surface from increased reflectivity from the lesion itself. The reflectivity in the cross-polarization can be directly integrated to quantify the lesion severity, regardless of the tooth topography. By using this approach the difficult task of deconvolving the strong surface reflection from the lesion surface from reflectivity from within the lesion can be circumvented. By exploiting scattering of polarized light in the cross-polarization axis and strong surface reflection in the

co-polarization axis of the PS-OCT system we can quantify thickness of the transparent surface layer on highly convoluted surfaces.

We previously developed approaches to automatically quantify the severity of caries lesion and the depth of demineralized enamel lesions for rapid image processing of large 2D or 3D data sets [13]. This paper will present and evaluate an approach to automatically detect and measure the thickness of the transparent surface layer in simulated enamel lesions.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Simulated Lesion Models

Enamel blocks, approximately 8–12-mm in length with a width of 3-mm and a thickness of 2-mm were prepared from extracted bovine incisors acquired from a slaughterhouse. Each enamel and bovine sample was partitioned into six regions or windows (2 sound and 4 lesion regions) by etching small incisions 1.8-mm apart across each of the enamel blocks using a laser. Incisions were etched using a transverse excited atmospheric pressure (TEA) CO₂ laser, an Impact 2500, GSI Lumonics (Rugby, UK), operating at 9.3- μ m with a pulse duration of 15-s and a pulse repetition rate of 200-Hz. A thin layer of acid-resistant varnish in the form of red nail polish, Revlon (New York, NY) was applied to protect the sound control area on each end of the block before exposure to the demineralization solution. The enamel blocks were exposed to a demineralization solution at pH 4.9 composed of a 40-mL aliquot of 2.0-mmol/L calcium, 2.0-mmol/L phosphate, and 0.075-mol/L acetate for 24h. Acid-resistant varnish was applied to the windows for 0-day, 4-day, 8-day and 12-day periods of remineralization. The blocks were placed into the remineralization solution composed of 1.5-mmol/L calcium, 0.9-mmol/L phosphate, 150-mmol/L KCl, 2-ppm F⁻, and 20-mmol/L HEPES buffer maintained at pH 7.0 and 37° C. After the fourth period, the samples were removed from the remineralization solution, and the acid resistant varnish was removed using acetone in ultrasonic machine for 15 minutes. Each sample was then stored in 0.1% thymol solution to prevent fungal and bacterial growth. The groups overlapped each other by one period, so that they could be compared to ensure consistency.

2.2 PS-OCT System

An all fiber-based Optical Coherence Domain Reflectometry (OCDR) system with polarization maintaining (PM) optical fiber, high speed piezoelectric fiber-stretchers and two balanced InGaAs receivers that was designed and fabricated by Optiphase, Inc., Van Nuys, CA was used to acquire the images. This two-channel system was integrated with a broadband superluminescent diode (SLD) Denselight (Jessup, MD) and a high-speed XY-scanning system (ESP 300 controller & 850G-HS stages, National Instruments, Austin, TX) for *in vitro* optical tomography. This system is based on a polarization-sensitive Michelson white light interferometer. The high power (15-mW) polarized SLD source operated at a center wavelength of 1317 nm with a spectral bandwidth FWHM of 84 nm provided an axial resolution of 9- μ m in air and 6- μ m in enamel (refractive index = 1.6). This light was aligned with the slow axis of the PM fiber of the source arm of the interferometer. The sample arm was coupled to an AR coated fiber-collimator to produce a 6-mm in diameter, collimated beam. That beam was focused onto the sample surface using a 20-mm focal length AR

coated plano-convex lens. This configuration provided axial and lateral resolution of approximately 20 μm with a signal to noise ratio of greater than 40–50 dB. The all-fiber OCDR system is described in reference [14]. The PS-OCT system is completely controlled using Labview™ software (National Instruments, Austin, TX).

2.3 Polarized Light Microscopy

After sample imaging was completed, approximately 200 μm thick serial sections were cut using an Isomet 5000 saw (Buehler, IL), for polarized light microscopy (PLM). PLM was carried out using a Meiji Techno RZT microscope (Meiji Techno Co., LTD, Saitama, Japan) 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 crossed polarizers and a red I plate with 500-nm retardation.

2.4 Phase Adjustment

There is a difference in phase between the co- and cross-polarization. In order to account for this problem, a gold mirror was scanned at 100- μm intervals over the entire scan range to determine the required phase adjustments.

2.5 Image Processing

Images obtained from the PS-OCT scans were processed using a dedicated program constructed with Labview™ software (National Instruments, Austin, TX). Figure 1 illustrates diagram of steps involved in image processing. The improved automated image processing method is based on the previous study [13]. The cross-polarization scan images were adjusted to match the co-polarization scan images in z-axis position based on the phase adjustment Gaussian curve. The images were filtered with 5×5 median filter to reduce the speckle noise and convolved with 5×5 Gaussian matrix filter. Each scan had background removed by subtracting the mean reflectivity of the 200 data points measured in air from the unprocessed 50 a-scans. In addition, noise threshold was calculated and applied to images as 4-standard deviation of the same 200 data points acquired previously.

2.6 Edge Detection, Artifact Removal and Integrated Reflectivity

An edge locator was used to make two passes along each line. Each pass starts from the position of the maximum peak intensity and locates the first pixel, for which the intensity value is less than e^{-2} multiplied by the maximum peak intensity. All other pixels outside of the edges were set to zero. Lesion depth was estimated by measuring the distance between two edges. The distance between those positions was correlated and corrected with data previously analyzed [15]. The reflectivity was integrated over the lesion depth from the top edge to yield the integrated reflectivity (dB x microns), R . Previous studies have shown that R positively correlates with the integrated mineral loss (volume % mineral loss x microns), Z [16, 17].

2.7 Transparent Surface Layer Detection and Measurement

Our approach was based on the following suppositions;

1. The first peak at the $||$ axis represents the incident light surface reflection.

2. The second peak at the $\parallel$ axis represents the scattering at the edge of the lesion.
3. If there is only one strong peak at $\parallel$ axis, we assume that the two signals may be overlapping one another, or there is no transparent layer.
4. Strongest peak at the $\perp$ axis represents scattering at the lesion body.

Three different cases are illustrated in Fig. 2; Case A where no transparent zone is present, Case B where two peaks are present in the $\parallel$ polarization scan clearly indicating the presence of a surface zone, and Case C where the two peaks overlap and the presence of a transparent surface zone is less obvious. In Fig. 2, α , the difference between the detected front edges of the scans for the two polarization axes is compared with β , the difference in position of the two peaks that represent the lesion body for both polarization axes. If α was greater than β , the transparent surface layer thickness was estimated to be α . Otherwise, there was no transparent surface layer detected.

2.8 Statistical Analysis

Sample groups were compared using repeated measures analysis of variance (ANOVA) with a Tukey–Kramer post hoc multiple comparison test. Pearson’s correlation was used to examine the relationship between data acquired from PS-OCT and PLM measurements, including sound, demineralization and remineralization areas. All statistical analyses were performed with 95% confidence with InStat™ (GraphPad software, San Diego, CA).

3. RESULTS AND DISCUSSION

The transparent surface layer detection algorithm was able to successfully distinguish lesions with a transparent surface layer. All windows that manifested the transparent surface layer with PLM were detected using the algorithm for PS-OCT ($n=13$). In addition the algorithm also correctly identified windows without the transparent layer with 3 false positives. However, detection of the transparent surface layer with PLM is limited for the shallow transparent surface layer less than $10\mu\text{m}$ due to “edge effect” and the non-uni form advancing front of the lesion on the $200\text{-}\mu\text{m}$ thick samples [18]. The algorithm detected the transparent surface layer with high sensitivity ($=1$) and high specificity ($=0.9286$). Figure 3 shows PS-OCT and PLM images from one of the samples. Linearly polarized light was incident on the sample and the reflected light with polarization ($\parallel$) and ($\perp$) to the incident light was measured, generating the $\parallel$ and $\perp$ images, respectively. There is minimal reflectivity in the sound regions outside the four lesion windows, while the lesions have much higher contrast in the cross-polarization images. It is obvious how the surface reflection interferes with resolution of the lesion in the images taken in the original polarization. The strong surface reflection of the co-polarization images can be used for resolving the outer surface of the transparent surface layer of the lesion. The transparent surface layer can be simply estimated by taking the differences in depth between of the top-edges of the two polarization images. However, this method overestimates the thickness of the transparent surface layer and it is unable to differentiate lesions with a transparent surface layer (arrested lesions) from demineralized and sound regions.

With our approach, the optical depth difference between the top-edges of $\parallel$ axis and $\perp$ axis as well as the difference between the lesion of the body and the edge of the lesion or the surface reflection can be calculated. These depth differences can be compared to differentiate the transparent surface layer from sound or demineralized areas. Figure 2 shows 3 different types of a-scan line profiles that can be observed after phase alignment and image processing. A plot of the estimated thickness of the transparent surface layer versus the thickness measured using PLM shown in Fig. 4 indicates strong correlation, Pearson $r^2=0.9213$. In addition, the depth of the lesion body under the transparent surface layer could also be estimated incorporating using the edge-detection algorithm. Although the algorithm was successful in differentiating the sound region from lesions, there was no significant difference in lesion depth measurements or lesion severity, R , among groups with different periods of remineralization due to the high variability in the degree of remineralization.

In this study, an algorithm was developed to detect and measure the thickness of the transparent surface zone formed due to remineralization using PS-OCT. It was successfully tested using an artificial remineralization model. PS-OCT can be used to non-destructively image the subsurface structure and measure the thickness of the highly mineralized transparent surface zone of caries lesions even with the presence of the very thin transparent surface layer. However, the method should be tested for a larger number of samples with varying thickness of the transparent surface layer in future studies. In addition, a cross-polarization (CP)-OCT system is being tested for clinical use and it will be crucial to develop an algorithm for detection of the transparent layer utilizing only the cross-polarization images.

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Fig 1.

Diagrammatical representation of the image processing steps used in this experiment.

Intensity scale is red-white-blue. (A) Original OCT b-scan; (B) Image adjusted with 5×5 median filter and 5×5 Gaussian convolution filter; (C) Image after background subtraction and thresholding; (D) Edges overlaid on C.

Fig 2.

If α was greater than β , the transparent surface layer thickness was detected. CASE A ($\alpha < \beta$, single peak): no transparent surface layer was detected. CASE B ($\alpha > \beta$, double peak): the transparent surface layer was detected. CASE C ($\alpha > \beta$, single peak): the transparent surface layer was detected.

Fig 3.

(A) ($\parallel$ -axis) PS-OCT b-scan of a sample before sectioning. Intensity scale is in red-white-blue scheme. (B) The corresponding ($\perp$ -axis) PS-OCT B-scan. (C) The corresponding PLM of 200- μ m-thick section in a similar orientation to the PS-OCT images.

Fig 4.

Plot of estimated transparent surface layer thickness from PS-OCT versus PLM images for the windows that manifested the transparent surface layer with PLM (n=13) with corresponding regression line ($p < 0.0001$, $r^2 = 0.9213$).