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Repair of Artificial Lesions using an Acidic Remineralization Model Monitored with Cross – Polarization Optical Coherence Tomography

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Abstract

It is difficult to completely remineralize carious lesions because diffusion into the interior of the lesion is inhibited as new mineral is deposited in the outermost layers. In previous remineralization studies employing polarization sensitive optical coherence tomography (PS-OCT), two models of remineralization were employed and in both models there was preferential deposition of mineral in the outer most layer. In this study we attempted to remineralize the entire lesion using an acidic remineralization model and demonstrate that this remineralization can be monitored using PS-OCT. Artificial lesions approximately 100–150 μm in-depth were exposed to an acidic remineralization regimen and the integrated reflectivity from the lesions was measured before and after remineralization. Automated integration routines worked well for assessing the integrated reflectivity for the lesion areas after remineralization. Although there was a higher degree of remineralization, there was still incomplete remineralization of the body of the lesion.

Keywords

polarization; optical coherence tomography; tooth demineralization; dental caries

1. INTRODUCTION

New tools are needed to non-destructively assess carious lesion depth and severity, efficacy of chemical intervention, and testing of anti-caries agents to serve as a likely surrogate end point in dental clinical trials¹. Several studies have demonstrated that polarization sensitive optical coherence tomography (PS-OCT) can be used to nondestructively measure the severity of subsurface demineralization in enamel and dentin and is therefore well suited for this role^{2–9}.

Baumgartner et al.^{10–12} presented the first polarization resolved images of dental caries. PS-OCT images are typically processed in the form of phase and intensity images^{13, 14}, such images best show variations in the birefringence of the tissues. Caries lesions rapidly depolarize or scramble the polarization of incident polarized light and the image of the orthogonal polarization to that of the incident polarization can provide improved contrast of

caries lesions. We developed an approach to quantifying the severity of caries lesion by integrating the reflectivity of the orthogonal axis ($\perp$) or cross polarization (CP) image³. There are two mechanisms in which intensity can arise in the cross polarization image. The native birefringence of the tooth enamel can rotate the phase angle of the incident light beam between the two orthogonal axes (similar to a waveplate) as the light propagates through the enamel without changing the degree of polarization. The other mechanism is depolarization or polarization scrambling from scattering in which the degree of polarization is reduced. It is this later mechanism that is exploited to measure the severity of demineralization. Demineralization of the enamel due to dental decay causes an increase in the scattering coefficient by a 1–2 orders of magnitude¹⁵, thus demineralized enamel induces a very large increase in the reflectivity along with depolarization. This in turn causes a large rise in intensity in the cross polarization image. This approach also has the added advantage of reducing the intensity of the strong reflection from the tooth surface that can prevent resolution of the lesion area near the tooth surface. This surface zone is of particular importance for differentiating active lesions from developmental defects (hypomineralization typically caused by fluorosis) and those lesions that have become arrested due to remineralization¹⁶. Arrested lesions and developmental defects have a zone of higher mineral content on the outside of the lesion which can readily be imaged using OCT. It is difficult for conventional OCT systems to differentiate the strong reflectance from the tooth surface from increased reflectivity from the lesion itself^{17, 18}. This problem is further compounded by the specular reflection from the tooth surface which can vary by several orders of magnitude > 30 dB depending on the angle of incidence. Even if a high resolution OCT system is used (axial resolution $< 10\text{-}\mu\text{m}$), the intensity decreases only by 3dB at $10\text{-}\mu\text{m}$ below the surface, however if the surface reflection is very strong ($\sim 20\text{--}30$ dB) the intensity “bleeds” into several layers below the surface and this prevents reliable quantitative measurements of the reflectivity. By reducing the surface reflection by $20\text{--}30$ dB through use of cross polarization (CP) OCT the difficult task of having to deconvolve the strong surface reflection from the lesion surface can be circumvented and direct integration of the lesion reflectivity is feasible to quantify the lesion severity, regardless of the tooth topography. Longitudinal studies have demonstrated that PS-OCT or CP-OCT can be used for monitoring erosion, demineralization and remineralization^{2–9}. The progression of artificially produced caries lesions in the pit and fissure systems of extracted molars can also be monitored non-destructively and the integrated reflectivity in the cross polarization image correlates well with the growth of the lesion^{4, 19}. Since the most important information about the lesion is near the surface, a polarization sensitive OCT system is invaluable for imaging dental caries particularly early lesions.

In previous studies we investigated the remineralization of smooth enamel surfaces employing two caries models. The first model involved pH cycling to produce lesions with a well-defined surface zone of intact enamel⁶ while the 2nd model used a different demineralization model to produce a *surface softened lesion*^{7, 20}. Both models showed markedly different outcomes after exposure to the remineralization solution. Studies have shown that remineralization requires the presence of residual partially dissolved crystals to serve as a template for growth²¹. Furthermore, remineralization has been observed to proceed from the outside of the lesion towards the lesion body, therefore as the

remineralization takes place in the surface zone of the lesion the diffusion pathways to the lesion body are blocked thus preventing further remineralization of the lesion body. However, the lesion does become arrested since further dissolution in the lesion body is also blocked. This is typically how lesions are arrested naturally. We observed that the *surface softened lesion* model yields the greatest change in mineral content upon remineralization since it does not contain a well-defined surface layer that inhibits diffusion²¹. PS-OCT images of a *surface softened lesion* before and after remineralization have shown that there was significant growth in the thickness of a layer of remineralized enamel along with a concomitant decrease in the integrated reflectivity⁷. The surface layer thickness increased significantly from $10 \pm 4 \mu\text{m}$ for the lesion before remineralization to $33 \pm 5 \mu\text{m}$ after remineralization, $p < 0.05$, $n=10$. The mean integrated reflectivity of the lesion, $R(\text{dB} \times \mu\text{m})$, also decreased significantly after 20 days of immersion in a remineralization solution at neutral pH by 31 %.

The acidic pH remineralization model of Yamazaki and Margolis²² yielded more complete remineralization of the lesion body. One objective of this paper is to utilize this model to investigate remineralization of the body of the lesion in addition to the surface zone and demonstrate that CP-OCT can monitor that enhanced remineralization.

Last year we demonstrated that automated algorithms can be applied successfully to calculate the depth of demineralization and the overall or integrated reflectivity from the zone of demineralization at the earliest stages of demineralization^{23–25}. This approach has significant advantages because PS-OCT can be used to rapidly acquire 2D and 3D tomographic images of areas of early demineralization on tooth surfaces. In order to rapidly process the images and effectively quantify the lesion severity, algorithms are needed to automatically extract lesion depth and severity information. Moreover, the high dynamic range of the reflectivity and the lack of a sharp demarcation between the sound and demineralized enamel at the lesion margins makes it challenging to define the lesion depth and we have found that edge finding algorithms are suitable for determining the lesion depth. Once the lesion depth is accurately calculated the lesion severity is computed by integrating the reflectivity over that depth. In previous studies we integrated over a fixed lesion depth that was chosen to be greater than any of the simulated lesions in the study. This latter approach is more accurate since the reflectivity of sound enamel is not zero. A second goal of this paper is to demonstrate that the same automated methods used to assess artificial demineralization in CP-OCT images can be applied to lesions that have undergone remineralization.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Enamel blocks, approximately 8 to 12-mm in length with a width of ~ 3-mm and a thickness of 2-mm of bovine enamel were prepared from extracted bovine tooth incisors acquired from a slaughterhouse. Each enamel sample was partitioned into six regions or windows (two sound and 4 lesion areas) by etching small incisions 1.4-mm apart across each of the enamel blocks using a laser (see Fig. 1). Incisions were etched using a transverse excited atmospheric pressure (TEA) CO₂ laser operating at 9.3- μm , Impact 2500, GSI Lumonics

(Rugby, UK). The incision area also has an increased resistance to acid dissolution that serves to more effectively isolate each group ²⁶. A thin layer of acid resistant varnish in the form of red nail polish, Revlon (New York, NY) was applied to protect the sound enamel control area on each end of the block before exposure to the demineralization solution. The samples were immersed in a demineralization solution maintained at 37 °C for 8–days at pH 4.6 composed of a 40-mL aliquot of 18 mmol/L calcium, 8 mmol/L phosphate, and 0.1 mol/L lactic acid with 3 mmol/L sodium azide added to inhibit bacteria growth. This surface softened lesion model, produces subsurface demineralization without erosion of the surface ²⁷. The mineral loss profiles are fairly uniform in these lesions and they emulate an active lesion. Surface softened lesions were produced on ten bovine enamel blocks. The lesions produced in the four windows were approximately 140-μm deep.

The blocks were placed into acidic remineralization solution ²² with acid resistant varnish covering the 0-day window for three 4-day periods, covering the appropriate windows with acid resistant varnish after each 4-day period. The acidic remineralization solution was at pH 4.8 and it was composed of a 40-mL aliquot of 4.1 mmol/L calcium, 15 mmol/L phosphate, and 0.05 mol/L lactic acid. Fluoride, 20 ppm, was added to enhance remineralization and 3 mmol/L sodium azide was also added to inhibit bacteria growth and the samples were incubated at 37°C.

After the fourth period, the samples were removed from the remineralization solution and the acid resistant varnish was removed using acetone. Each sample was then stored in a 0.1% thymol solution to prevent fungal and bacterial growth.

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 to provide an axial resolution of 9-μm in air and 6-μm 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. Both orthogonal polarization states of the light scattered from the tissue are coupled into the slow and fast axes of the pm- fiber of the sample arm. A quarter wave plate set at 22.5° to horizontal in the reference arm rotated the polarization of the light by 45° upon reflection. After being reflected from the reference mirror and the sample, the reference beams were recombined by the pm fiber-coupler. A polarizing cube splits the recombined beam into its

horizontal and vertical polarization components or “slow” and “fast” axis components, which were then coupled by single mode fiber optics into two detectors. The light from the reference arm was polarized at 45° and therefore split evenly between the two detectors. Readings of the electronically demodulated signal from each receiver channel represent the intensity for each orthogonal polarization of the backscattered light. Neutral density filters are added to the reference arm to reduce the intensity noise for shot limited detection. The all-fiber OCDR system is described in reference ²⁸. The PS-OCT system is completely controlled using Labview™ software (National Instruments, Austin, TX). Acquired scans are compiled into *b-scan* files. Image processing was carried out using Igor Pro™, data analysis software (Wavemetrics Inc, Lake Oswego, Oregon).

PS-OCT scans acquired from PM fiber based PS-OCT systems typically contain artifacts (additional peaks) due to cross-talk and the limited extinction ratio of the fiber that may confound analysis. Automated removal of such artifacts can be carried out successfully with a few extra data alteration steps after data collection. A reference a-scan was acquired from a mirror prior to scanning the samples. The reference a-scan contains several weak artifact signals along with the primary reflection. A smaller 400-point a-scan array was extracted from the 2000-pt reference a-scan containing the principal artifacts. The reference array was normalized to the intensity of the point of interest and subtracted to selectively remove the artifacts.

2.3 Calculation of Integrated Reflectivity and Lesion Depth

The integrated reflectivity, R in units of (dB $\times \mu\text{m}$) was calculated for each of the four lesion areas on the samples. Previous studies have shown that R can be correlated with the integrated mineral loss (volume % mineral $\times$ microns) called Z ^{4,5}.

An initial background subtraction was carried out for each OCT scan and a 2×2 convolution filter was applied to remove speckle noise. In the edge-detection approach, the enamel edge and the lower lesion boundary were determined by applying an edge locator. Two passes were required for each a-scan to locate each respective boundary with each pass starting from opposite ends of the a-scan and identifying the first pixel that exceeds the threshold of e^{-2} of the maximum value. The minimum threshold values for edge detection were previously experimentally determined by comparison of lesion depths measured using polarized light microcopy with measurements using OCT in order to avoid overestimation of lesion depth due to weak signals caused by birefringence in sound enamel ²³. Distance (micron) per pixel conversion factor was obtained experimentally by system calibration. The two cutoff points for the lesion surface and endpoint represent the calculated lesion depth and the integration between these two positions represents the integrated reflectivity. A 1-mm square area was chosen for analysis in the center of each of the 1.4-mm by 3-mm areas demarcating each group on each sample. Therefore, 400 a-scans were analyzed for each group.

Typically there are large variation in the depth and integrated mineral loss from sample to sample for these types of demineralization experiments resulting in large standard deviations for each group. Sample groups were compared using Repeated Measures Analysis of Variance (ANOVA) with a Tukey–Kramer post hoc multiple comparison test. Having all the

study groups (5) for each series on each sample allowed us to decrease intersample variability. InStat™ from GraphPad software (San Diego, CA) was used for statistical calculations.

3. RESULTS AND DISCUSSION

Figure 2 shows PS-OCT images from two of the ten samples. Linearly polarized light was incident on the sample and the reflected light was measured in the same polarization ($\parallel$) to the incident light and also in the orthogonal polarization ($\perp$) to the incident light. There is minimal reflectivity in the sound regions demarcated by the S region, while the lesions have much higher contrast in the ($\perp$) or CP-OCT image. It is obvious how the surface reflection interferes with resolution of the lesion in the images taken in the original polarization, ($\parallel$) or co-polarization image. However, the ($\parallel$) images are useful for increased resolution of the tooth surface and one can make out the thickness of the transparent surface zone. It is much harder to see differences in the lesion severity in the body of the lesion over time in the ($\parallel$) images versus the ($\perp$) images. In both of the samples the zone of remineralization is very thick, approximately 30- μm . The overall lesion depth was 150- μm for the first sample and 130- μm for the second sample. The integrated reflectivity decreased by $\sim 50\%$ in the first sample which shows less reflectivity from the body of the lesion while the reflectivity decreased by $\sim 25\%$ in the second sample that shows a smaller change in reflectivity in the body of the lesion. Overall the integrated reflectivity decreased by 35% for the ten samples after a period of only 12 days. This exceeds the degree of remineralization that we have observed in prior studies. We have yet to analyze the change in mineral content after remineralization using transverse microradiography. The TMR results may show a much higher degree of remineralization. A relatively small loss of mineral or increase in porosity causes large changes in light scattering¹⁵, therefore it is possible that a 35% decrease in reflectivity may indicate a high degree of remineralization. It appears the acidic remineralization model resulted in increased remineralization of the lesions over previous studies, however it appears that the majority of the lesion body has not been remineralized.

There was a high degree of variability among the samples and four out of the ten samples exhibited little change in reflectivity after 12 days of exposure to the remineralization solution. One explanation may lie with the nature of the samples employed in this study, Yamasaki and Margolis employed tooth thin sections ($\sim 100\text{-}\mu\text{m}$ thick) covered with acid resistant varnish, and apparently achieved more complete remineralization²².

One very encouraging result of this study was that the automated integration routines worked well for assessing the integrated reflectivity for the lesion areas after remineralization. The transparent surface zones did not inhibit analysis which means that those routines show work for a wide range of different lesion types.

Acknowledgments

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Fig. 1.
Sample block connected to a jig for scanning by the PS-OCT system. Inset: Sample with two exposed windows surrounded by acid resistant varnish.

Fig. 2.

PS-OCT b-scan images of for two of the samples after exposure to the solution. Areas labeled (S) are sound, demineralized areas were exposed to increasing periods of time to the remineralization solution, 0, 4, 8, and 12-days. The period label 0 corresponds to the demineralized area prior to remineralization. The (||) image represents the light reflected in the original polarization while the ($\perp$) image is the orthogonal polarization or cross polarization image which was used for analysis in these studies. The incisions are 50- μ m deep and separated by 1.4-mm.