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Nondestructive monitoring of the repair of enamel artificial lesions by an acidic remineralization model using polarization – sensitive optical coherence tomography

Hobin Kang, Cynthia L. Darling, and Daniel Fried*

University of California, San Francisco, San Francisco, CA 94143-0758

Abstract

Objectives—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.

Methods—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 using PS-OCT.

Results—Automated integration routines worked well for assessing the integrated reflectivity for the lesion areas after remineralization. Although there was a high degree of remineralization, there was still incomplete remineralization of the body of the lesion.

Conclusion—This study demonstrated that PS-OCT can be used to non-destructively measure changes in lesion structure and severity upon exposure to an acidic remineralization model. This study also demonstrated that automated algorithms can be used to assess the lesion severity even with the presence of a weakly reflective surface zone.

Keywords

polarization; optical coherence tomography; tooth demineralization; dental caries; remineralization; enamel

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 trials [1]. Several studies have demonstrated that polarization-sensitive optical coherence tomography (PS-OCT) can be used to nondestructively measure

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Corresponding author: Dr. Daniel Fried, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, San Francisco, CA 94143-0758, daniel.fried@ucsf.edu, Phone: 415-502-6641, Fax: 415-476-0858.

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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] in order to highlight variations in the birefringence of the tissues. However this approach is not particularly advantageous for monitoring dental caries. 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 quantify the severity of caries lesion by integrating the reflectivity of the orthogonal axis ($\perp$) or cross polarization OCT image (CP-OCT) [3]. 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 1–2 orders of magnitude [15], thus mineral loss 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 interfere with measurement of the area of the lesion 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 remineralization[16]. Arrested lesions and developmental defects have a zone of higher mineral content on the outside of the lesion which can readily be imaged using PS-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- μ m), the intensity decreases only by 3dB at 10- μ m below the surface, however if the surface reflection is very strong (~20–30 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 20–30 dB through use of cross polarization, 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 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 PS-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 enamel[6] 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 growth [21]. 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 diffusion [21]. 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 reflectivity [7]. 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, $\Delta R(\text{dB} \times \mu\text{m})$, 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 Margolis [22] yielded more complete remineralization of the lesion body. The lower pH of the remineralization solution inhibited the formation of the impermeable outer layer of high mineral content allowing greater mineral deposition in 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 PS-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 make it challenging to define the lesion depth. Once the lesion depth is 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 and integration over greater depths than necessary can lead to a significant overestimate of the lesion severity. 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.

MATERIALS AND METHODS

Sample Preparation

Enamel blocks, approximately 8 to 12-mm in length with a width of ~ 3-mm and a thickness of 2-mm 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, an Impact 2500, GSI Lumonics (Rugby, UK), operating at 9.3- μm with a pulse duration of 15- μs and a pulse repetition rate of 200 Hz. Incisions were produced by delivering a series of overlapping laser pulses at 100- μm intervals across each sample. The laser energy was 12 mJ per pulse, with a spot diameter of 325- μm and a fluence of 14 J/cm². 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- μ m deep.

The blocks were placed into acidic remineralization solution [22], and acid-resistant varnish was applied to the 0-day window for three 4-day periods of remineralization and the appropriate windows were covered with acid-resistant varnish after each subsequent 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 50 mmol/L lactic acid [22]. 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.

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 full-width-half – maximum (FWHM) of 84 nm to provide 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 anti-reflectance (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 μ m 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.

Calculation of Integrated Reflectivity and Lesion Depth

The integrated reflectivity, ΔR in units of ($\text{dB} \times \mu\text{m}$) was calculated for each of the four lesion areas on the samples from the cross-polarization OCT images. Previous studies have shown that ΔR can be correlated with the integrated mineral loss (volume % mineral $\times$ microns) called ΔZ [4,5].

An initial background subtraction was carried out for each CP-OCT image 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 microscopy 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 windows 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. Each of the five study groups were represented on each sample which reduced the inter-sample variability and allowed comparison using Repeated Measures Analysis of Variance (ANOVA) with a Tukey–Kramer post hoc multiple comparison test. InStat™ from GraphPad software (San Diego, CA) was used for statistical calculations.

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

The samples were serial sectioned to a thickness of 200- μm along the long axis using an Isomet 5000 saw from Buehler (Lake Bluff, IL) for polarized light microscopy (PLM) and transverse microradiography (TMR). PLM was carried out using a Meiji Techno RZT microscope from Meiji Techno Co., Ltd. (Saitama, Japan) with an integrated digital camera, EOS Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the bright field mode with crossed polarizers and a red I plate with 500-nm retardation.

A custom built digital microradiography (TMR) system was used to measure the volume percent mineral content in the areas of demineralization on the tooth sections [29]. High-

resolution microradiographs were taken using Cu K α radiation from a Philips 3100 x-ray generator and a Photonics Science FDI x-ray digital imager, Microphotonics, (Allentown, PA). The x-ray digital imager consists of a 1392 $\times$ 1040 pixel interline CCD directly bonded to a coherent micro fiber-optic coupler that transfers the light from an optimized gadolinium oxysulphide scintillator to the CCD sensor. The pixel resolution is 2.1 μ m, and the images can be acquired in real time at a frame rate of 10 fps. A high-speed motion control system with Newport (Irvine, CA) UTM150 and 850G stages and an ESP 300 controller coupled to a video microscopy and laser targeting system was used for precise positioning of the tooth sample in the field of view of the imaging system.

RESULTS

Figure 2 shows PS-OCT images from one of the ten 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 co-polarization and cross polarization images, respectively. There is minimal reflectivity in the sound regions outside the four windows, 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 are particularly valuable for resolving the outer surface of the transparent outer layer of the lesion for easier measurement of 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. The transparent outer layer formed after exposure to the remineralization solution was very thick and exceeded 50- μ m. The depth of the lesion shown in Fig. 2 was $\sim$ 140- μ m and the integrated reflectivity for this sample decreased by $\sim$ 50% after 12-days and showed less reflectivity from the body of the lesion. The degree of remineralization was highly variable from sample to sample and this sample provided one of the best examples of a distinct surface layer formed due to remineralization.

The mean ($\pm$ s.d) values determined from PS-OCT, PLM, TMR measurements for the integrated reflectivity, lesion depth, integrated mineral loss (ΔZ) and the thickness of the transparent layer formed after remineralization for each of the time periods of exposure to the remineralization solution are listed in Table I. PS-OCT showed that there were significant changes in the lesion depth, overall reflectivity from the lesion area and thickness of the transparent outer layer after exposure to the remineralization solution. However, the lesions did not completely remineralize and the integrated (overall) reflectivity decreased by only 35% for the ten samples after a period of 12 days.

PLM showed that the mean lesion depth was 117- μ m after exposure to demineralization and that the lesion depth decreased to 100- μ m after exposure to the remineralization solution, however the difference was not significant. Figure 3 shows PLM images of one of the samples. TMR indicated that the lesions were very shallow and demineralization was not severe. The mean ΔZ was only 594- μ m and the contrast of the lesions was very low in the TMR images. The mineral loss in the lesion area was $\sim$ 5% which is very difficult to resolve with TMR. There was a 46% decrease in the mean ΔZ value after 12 days exposure to the remineralization solution, however none of the groups were statistically significantly different. Only in a few of the samples were we able to resolve the formation of a distinct surface zone.

DISCUSSION

One aim of this study was to determine if PS-OCT could be used to monitor more extensive remineralization in the lesion surface zone and in the lesion body. One advantage of using light-scattering (reflectance) changes to monitor early demineralization over TMR—in addition to the capability of use *in vivo*—is that it is very sensitive. Small changes in volume % mineral cause large changes in light scattering/reflectivity. This can also be easily seen by comparing PLM and TMR. However, a major disadvantage of using light scattering changes/reflectivity as a monitor of mineral loss is that there is minimal change in intensity beyond a 10–20% loss of the mineral volume[15]. Therefore it is difficult to resolve changes in the lesion in which the mineral content varies in the range beyond 10 or 20% mineral content using optical methods that rely on light scattering changes, such as OCT or quantitative light fluorescence (QLF). In a prior study of remineralization employing a pH cycling regimen to produce lesions, TMR showed remineralization in the lesion body while there was no decrease in the reflectivity measured using PS-OCT[6]. pH cycling produces lesions with a very distinct surface zone of higher mineral content. Mineral deposition takes place to a large degree in the lesion body and there is little change to the surface zone. Both TMR and OCT indicated only partial remineralization of the lesions, 54% and 35%, respectively. However, it did appear that the acidic remineralization model resulted in increased remineralization of the lesions over previous studies. Unfortunately, it appears that the majority of the lesion body has not been remineralized. We were surprised that the initial lesions were not as severe as anticipated. The same demineralization solution resulted in more severe lesions in prior studies [25]. 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 (~100-µm thick) covered with acid-resistant varnish in their study, and apparently achieved more complete remineralization [22].

Another aim was to determine if the automated integration routines could successfully assess the integrated reflectivity for the lesion areas after remineralization. OCT is rapidly capable of collecting entire 3D images of samples, resulting in large data files. In order to effectively implement OCT to monitor the severity of demineralization, algorithms are needed to calculate the reflectivity and depth of the lesions. In this study, the transparent surface zones did not inhibit analysis which suggest that those routines will work effectively for a wide range of different lesion types.

CONCLUSION

This study demonstrated that PS-OCT can be used to non-destructively measure changes in lesion structure and severity upon exposure to an acidic remineralization model. This study also demonstrated that automated algorithms can be used to assess the lesion severity even with the presence of a weakly reflective surface zone.

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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 one of the samples showing the sound (protected) regions located on the extreme left and right side of the sample, the lesion area (0-days exposed to remin. soln) and the areas exposed for increasing periods of time to the remineralization solution, 4, 8, and 12-days. The (II) image represents the light reflected in the original polarization while the (⊥) image is the orthogonal polarization or cross polarization image which was used for analysis in these studies. The incisions are ~ 100- μ m deep and separated by 1.4-mm.

Fig. 3. Polarized light micrographs (PLM) of a cross section cut from the sample imaged in Fig. 2. The top image shows the entire sample and the lower four images show each window at a higher magnification. The lesion window represents the original area of demineralization, 0-days exposure to the remineralization solution.

Table I

The PS-OCT, PLM, TMR measurements, mean ($\pm$ s.d.) n=10 for the four periods of exposure to the remineralizing solution. Groups with the same letter are statistically similar $P > 0.05$.

Measurement	0 - days	4 - days	8 - days	12 - days
Lesion Depth (PS-OCT)	138 (18.4) ^a	124 (14.4) ^{a,b}	118 (22.5) ^{a,b}	113 (24.9) ^b
Reflectivity (PS-OCT)	257 (60.2) ^{a,b}	236 (73.8) ^{a,b,c}	206 (96.0) ^{b,c,d}	168 (58.5) ^{c,d}
Transparent Layer Thickness (PS-OCT)		41.6 (9.7) ^{a,b}	46.1 (9.5) ^{a,b,c}	54.3 (10.8) ^{b,c}
Lesion Depth (PLM) [*]	117 (27.3) ^a	113 (15.0) ^a	101 (9.6) ^a	100 (12.9) ^a
Transparent Layer Thickness (PLM)		22.5 (8.9) ^a	30.4 (8.6) ^b	39.5 (10.1) ^c
Microradiography (ΔZ) [*]	594 (296) ^a	469 (387) ^a	307 (463) ^a	274 (168) ^a

^{*} n=9, demin area of lesion lost in sectioning for 1 sample.