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Nondestructive Monitoring of the Repair of Natural Occlusal Lesions using Cross – Polarization Optical Coherence Tomography

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Abstract

Previous remineralization studies employing cross polarization sensitive optical coherence tomography (CP-OCT), have been limited to the repair of artificial enamel-like lesions. In this study we attempted to remineralize existing occlusal lesions on extracted teeth. Lesions were imaged before and after exposure to an acidic remineralization regimen and the integrated reflectivity and lesion depth was calculated. Automated integration routines worked well for assessing the integrated reflectivity for the lesion areas after remineralization. Polarized light microscopy was also used to examine the lesions areas after sectioning the teeth. An acidic remineralization solution was used to remineralize the lesions. The integrated reflectivity significantly increased after exposure to the remineralization solution which suggests that the acidic solution caused additional demineralization as opposed to the desired remineralization.

Keywords

polarization; optical coherence tomography; demineralization; remineralization; 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-carries 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 role²⁻⁹.

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}, where 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 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- 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 (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 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 acidic pH remineralization model of Yamazaki and Margolis ²² has yielded more complete remineralization of the lesion body in in vitro studies. We employed this model last year to remineralize artificial lesions produced using a surface softened lesion model. Overall the integrated reflectivity decreased by 35 % for the ten samples after a period of only 12 days²³. This exceeds the degree of remineralization that we had observed in prior studies.

The purpose of this study was to monitor the remineralization of natural lesions using an acidic remineralization model.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Fifteen 3rd molars with suspected occlusal lesions were selected from extracted teeth collected in the San Francisco bay area. The teeth were mounted in rectangular blocks of orthodontic composite to facilitate matching of OCT scans and histological sectioning. A region of interest or a 3 by 3 mm square window was cut around an area of the occlusal surface containing a suspected lesion as shown in Fig. 1. Incisions were etched using a transverse excited atmospheric pressure (TEA) CO₂ laser operating at 9.3- μ m, 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 ²⁵. A thin layer of acid resistant varnish in the form of red nail polish, Revlon (New York, NY) was applied to tooth areas outside the window to protect those areas from the remineralization solution. The samples were immersed in an acidic remineralization solution ²² for 14-days at pH 4.8 composed of a 40-mL aliquot of 0.1 M lactic acid calcium, 0.018 M CaCl₂, and 0.0078 M K₂PO₄. The pH was adjusted to 4.8 using NaOH. 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 exposure to the remineralization solution 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 OCT Systems

Two OCT systems were used to scan the samples. A TD-OCT system with PM-fiber and a cross polarization SS-OCT system.

The 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- 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 .

The cross-polarization system is Model IVS-3000-CP from Santec (Komaki, Aichi, Japan) and utilizes a swept laser source, Santec Model HSL-200-30 operating with a 30 kHz sweep rate. The Mac-Zehnder interferometer is integrated into the handpiece which also contains the microelectromechanical (MEMS) scanning mirror and the imaging optics. The handpiece body is 7 $\times$ 18 cm with an imaging tip that is 4 cm long and 1.5 cm across. This system operates at a wavelength of 1321-nm with a bandwidth of 111-nm with a measured resolution in air of 11.4 μm (3 dB). The lateral resolution is 80- μm ($1/e^2$) with a transverse imaging window of 6 mm $\times$ 6 mm and a measured imaging depth of 7-mm in air. The extinction ratio was measured to be 32 dB.

2.3 Calculation of Integrated Reflectivity and Lesion Depth

The integrated reflectivity, R in units of (dB $\times \mu\text{m}$) was calculated for the 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 $\times$ 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 enamel²⁶. 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. The 3-mm box area demarcated by the laser was integrated for each sample (see Fig. 1). Therefore, 5625 a-scans (SS-OCT) and 22,500 a-scans (TD-OCT) were analyzed for each group. InStatTM from GraphPad software (San Diego, CA) was used for statistical calculations.

2.4 Polarized light microscopy

Teeth were serial sectioned (200- μm thickness) using a linear precision saw, the IsoMet 5000 (Buehler, Lake Bluff, IL). Polarized light microscopy (PLM) was carried out using a Meiji Techno RZT microscope (Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT from Canon Inc. (Tokyo, Japan). The sample sections were imbibed in water and examined in the brightfield mode with cross polarizers and a red I plate with 500-nm retardation.

3. RESULTS AND DISCUSSION

Figure 2 shows PS-OCT images from one of the fifteen samples taken before and after exposure to the remineralization solution. The lesions actually appear slightly more extensive after exposure to the remineralization solution which was contrary to what was anticipated. Volumetric images are also shown for the sample in Fig. 2 and it is difficult to resolve any changes in the lesion. Polarized light microscopy was used to examine some of the samples after remineralization. Figure 3 shows a PLM image of a thin section cut from one of the samples. There is no evidence of new mineral deposition on the surface of the lesion. OCT images are also shown which were taken at a matching position before sectioning the tooth. The PLM and OCT images match extremely well. The two fiducial marks are visible in the images and it is important to point out that the left cut shows a ring of slight demineralization visible in both the PLM and OCT images. This demineralization occurred after the incision was cut which suggests that the low pH remineralization solution did produce some demineralization. We have previously observed accelerated demineralization around laser incisions. This likely occurs because the highly acid resistant outer layer of fluorapatite rich mineral is removed. The enamel around the incision is a purer phase more acid resistant layer but it may be less resistant than the fluorapatite rich layer at the tooth surface.

Figures 4 and 5 show the mean depth and integrated reflectivity of the lesion in the ROI before and after exposure to the remineralization solution for both systems. The lesion depth did not change significantly and the mean reflectivity increased after exposure to the remineralizing solution but the change was not significant. Although it appears that the lesion depth was slightly higher for the SS-OCT system, comparison of all four groups (both OCT systems) indicates no significant difference in the depth.

The two OCT systems produced similar results in depth and integrated reflectivity. However, it appears that the TD-OCT system provided better contrast than the SS-OCT between the before and after exposure to the remineralizing solution. This is most likely due to the higher lateral resolution of the TD-OCT vs. the SS-OCT system (20- μm vs. 80 μm). If this hypothesis is correct then it may be advantageous to modify the imaging optics of the SS-OCT/MEMS system for a higher lateral resolution if the intending imaging target is restricted only to shallow demineralization/remineralization. It will be necessary to sacrifice imaging range if higher lateral resolution is required.

PLM and OCT images suggest that the acidic remineralization did not appear to be effective on existing enamel lesions. This model was used last year on artificial lesions produced

using the surface softened dissolution model and it did result in a significant decrease in the reflectivity (38% reduction)²³. Those lesions are highly porous and represent fairly active lesions. In a previous study we were unable to measure a significant reduction in the reflectivity of artificial enamel lesions produced using the pH cycling model. That model results in a more definitive surface zone that inhibits diffusion into the lesion. In a recent study by Churchley et al. employing terahertz imaging they were able to almost completely remineralize shallow (<50- μ m) artificial lesions using a pH cycling model involving dentifrices, natural and simulated saliva and an acid challenge.²⁴ This latter model may be more successful on natural lesions.

Reflectivity appeared to increase (not significant $P > 0.05$) which suggests either an increase of demineralization or non-epitaxial precipitation of mineral. Neither the PLM and OCT images show a highly scattering surface layer indicative of non-epitaxial precipitation of mineral on tooth surfaces. The PLM image (Fig. 3) shows subtle signs of increased demineralization, which suggests that the lesions demineralized as opposed to the desired remineralization.

The negative results of this study underscore the problems associated with both the nondestructive assessment of remineralization, the development of artificial caries models and the difficulty in completely remineralizing existing lesions. These studies are also important because new tools are needed to assess the status of existing lesions to determine whether such lesions are already arrested and cannot be remineralized, and also differentiate active caries lesions from developmental defects. Methods such as PLM and TMR are reliable but they are destructive and cannot be used to measure mineral changes. X-ray tomography can be used but it is difficult to quantify small changes in mineral content with μ CT and it cannot be used for in vivo imaging.

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Fig. 1.
Molar tooth showing 3×3 mm box demarcating the region of interest for remineralization.

Fig. 2. CP-OCT b- scans using the TD-OCT system (A) and SS-OCT system (B) for one of the samples. Volumetric scans for the samples are shown (SS-OCT system) in (C) before and after exposure to the remineralization solution.

Fig. 3. Cross sectional images taken after exposure to the remineralization solution using polarized light microscopy after sectioning (A) and with TD-OCT (B) and SS-OCT (C) before sectioning.

Fig. 4. The mean lesion depths ($\pm$ standard deviation) for the samples before and after exposure to the remineralization solutions for both OCT systems, $n=15$.

Fig. 5.

The mean integrated reflectivity ($\pm$ standard deviation) for the samples before and after exposure to the remineralization solutions for both OCT systems, $n=15$.