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Imaging laser irradiated enamel surfaces with polarization sensitive optical coherence tomography

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Abstract

Several studies have shown that lasers can be used to modify the surface morphology and chemical composition of tooth enamel to render it less soluble. Other studies have shown that Polarization Sensitive Optical Coherence Tomography (PS-OCT) can be used to non-destructively measure the efficacy of fluoride in inhibiting the development of artificial caries lesions. The purpose of this study was to determine if PS-OCT can be used to measure inhibition of enamel demineralization after CO₂ laser irradiation. Polarized light microscopy and microradiography were used to measure lesion severity on histological thin sections for comparison. PS-OCT was able to measure a significant reduction in the integrated reflectivity due to inhibition by the laser even though the laser modification of the enamel surface caused a slight increase in reflectivity. This study shows that the PS-OCT is well-suited for *in vivo* measurements of caries inhibition after laser treatments.

2. INTRODUCTION

Several studies over the past thirty years have indicated that lasers can be used to thermally modify the chemical composition of dental enamel to render it more resistant to acid dissolution and potentially more resistant to dental caries.^{1–7} Carbon dioxide laser wavelengths of 9.3 and 9.6- μ m have the strongest absorption in dental hard tissues and a pulse duration in the microsecond range is well-matched to the thermal relaxation time of the absorbed laser energy near the tooth surface for those wavelengths. Moreover, other studies have indicated that topical fluoride treatments can work synergistically with the laser treatment to further enhance its inhibitory effect^{8–11}. Current methods of evaluating the efficacy of chemical and thermal methods of anti-caries agents requires teeth scheduled for extraction followed by assessment with either microradiography, microhardness or polarized light microscopy. Such methods require thin sectioning of the extracted tooth, hence the need to carry out studies on teeth scheduled for extraction. One major limitation of this approach is that these studies must be limited in duration to a maximum of a few months and that natural caries lesions, particularly in occlusal surfaces, require a more extended time for development. One promising new method for the assessment of early caries lesions “*in vivo*” is polarization sensitive optical coherence tomography (PS-OCT), which has been

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successfully used to acquire images of both artificial and early natural caries lesions, assess their severity, as well as measure the remineralization of such lesions^{12,13}.

Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structure^{14,15}. We have demonstrated that polarization-sensitive depth-resolved reflectivity measurements can be used to measure the severity of natural and artificial lesions on extracted teeth in the laboratory^{16–20}. The reflectance at the air-enamel interface of the tooth surface is very strong due to the high refractive index of enamel, $n=1.63$. It poses a particular challenge for OCT because the surface reflectivity can be much more intense than the sub-surface scattering, and it can interfere and overlap the reflectivity from the lesion area and prevent accurate assessment of the lesion depth and severity. If the incident light is linearly polarized, surface reflections do not depolarize light so the surface reflection does not interfere as strongly in the orthogonal ($\perp$) polarization state that can be measured using a polarization sensitive OCT system. Demineralized areas on the tooth depolarize incident linearly polarized light and the reflectivity in the ($\perp$) polarization state can be directly integrated to provide a measure of the lesion severity. By exploiting polarization sensitivity, the reflectivity from all lesion areas can be directly integrated — including the very important surface zones—thus overcoming the interference of strong surface reflections at tooth surfaces, a serious limitation of conventional OCT systems. Another concern is that thermal changes in the enamel can induce changes in the birefringence and light scattering properties of the enamel that may interfere with the ability of PS-OCT to assess the lesion severity. In turn, such thermally induced changes can be measured using PS-OCT to assess the depth or thickness of the thermally modified enamel layer and any peripheral thermal damage.

Quantitative light fluorescence (QLF) has been used to quantify the severity of incipient caries lesions on smooth surfaces *in vivo*^{21–28}. The method measures the loss of the enamel native fluorescence as the lesion increases in severity²⁹. The pores in the lesion formed due to demineralization, scatter and attenuate the fluorescence that originates from the underlying sound enamel and dentin. The fluorescence loss (%) has been compared with the mineral loss integrated with depth, Z (Vol.% mineral $\times\mu\text{m}$) to give a measure of the lesion severity. However, this method has only been validated for use on smooth surfaces and it has not been determined that it will work on laser treated surfaces.

In a recent study, we demonstrated that PS-OCT can be used to assess the inhibitory effect of fluoride agents and sealants peripheral to orthodontic brackets³⁰. The purpose this paper is to demonstrate that PS-OCT can be used as a nondestructive means of measuring the efficacy of fluoride and CO₂ laser treatments and for measuring the inhibition of lesion formation.

3. MATERIALS AND METHODS

3.1 Sample Preparation

Twelve enamel blocks, approximately $5 \times 3 \times 2 \text{ mm}^3$, of bovine enamel were prepared from extracted bovine tooth incisors acquired from a slaughterhouse. The enamel surfaces were serial polished to a one micron finish using embedded diamond polishing discs. Each

enamel sample was partitioned into three regions with the center of each region being treated with the laser using the conditions specified in the next section. The middle partition of each sample was covered with a thin layer of acid resistant varnish in the form of red nail polish (Revlon, New York, New York) that served to protect the sound enamel control area before exposure to the demineralization solution. The middle partitions served as the negative control groups: protected (P) and protected + laser (P + L). A 1.23% acidulated phosphate fluoride foam, Oral B Minute Foam (Gillette, South Boston, MA) was applied to only one side, the left side, following treatment with the laser. After allowing exposure for one minute, the samples were rinsed with deionized water, taking care not to rinse fluoride on to the untreated side of the sample. The fluoride treated partition served as the fluoride + lesion (F) and fluoride + laser + lesion (F + L) groups. The final side served as lesion (D) and lesion + laser (D + L) groups. The samples were then cemented to delrin blocks and the sample sides varnished to isolate the enamel surface. After treatment, samples were placed in a demineralization solution for seven days to approximately 40-mL aliquot of acetate buffer solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate maintained at pH 4.9 and kept at a temperature of 37°C designed to generate lesions approximately 100- μ m deep¹³. Having the six study groups within each sample, lesion (D), lesion plus laser (D + L), sound (P), sound plus laser (P + L), fluoride (F), and fluoride plus laser (F + L) allowed us to decrease intersample variability.

3.2 Laser Treatment

The enamel samples were irradiated with a transverse excited atmospheric pressure (TEA) CO₂ laser, Impact 2500 (GSI Lumonics, Rugby, United Kingdom) operating at 9.3 μ m, a fluence of 1 J/cm², an energy of 8 mJ per pulse, and pulse duration of 15 μ s. Laser energy calibration and measurement was done using a laser energy/power meter, EPM 1000, Coherent-Molelectron (Santa Clara, CA) with an ED-200 Joulemeter from Gentec (Quebec, Canada). A planoconvex ZnSe lens with focal length 100-mm was used to focus the laser to a beam diameter of approximately 1 mm. The laser beam diameter ($1/e^2$) at the sample position was determined by scanning with a razor blade across the beam. Two and three dimensional images of the laser spatial profile was acquired using a Spirocon PyrocamTM I pyroelectric array (Logan, UT). Both laser beam profile and spatial profile showed that the laser was operating in a single spatial mode, Gaussian spatial beam. Irradiation was performed by scanning the laser operating at a repetition rate of 200-Hz continuously at a scanning rate of 3.0 mm/sec across the surface of the bovine blocks to create the three irradiation zones (Fig. 1). A computer-controlled stage high-speed motion control system with Newport (Irvine, CA) UTM150 and 850G stages and an ESP300 controller was used to create controlled movement of the samples for the laser irradiations. For this study, the samples were irradiated without introducing water to the enamel surfaces.

3.3 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 integrated with a broadband high power superluminescent diode (SLD) DL-CS31159A, (Denselight, Singapore) and a high-speed XY-scanning system (ESO 300

controller & 850-HS stages, National Instruments, Austin, TX) and used for *in vitro* optical tomography. The system was configured to provide axial and lateral resolution of approximately 20–25- μm with a signal to noise ratio of greater than 40–50 dB. The all-fiber OCDR system is described in more detail in reference ³¹. The PS-OCT system was completely controlled using LabVIEWTM software (National Instruments, Austin TX).

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

After the samples were imaged with PS-OCT, the bovine samples were cut into sections approximately 200- μm thick using an Isomet-5000 saw (Buehler, Lake Bluff, IL) for polarized light microscopy (PLM) and digital transverse microradiography (TMR). During this step, samples were lost due to the inherent brittleness of enamel coupled with the existence of the induced artificial lesions which made the samples even more susceptible to fracturing. Polarized light microscopy was carried out using a Scientific Series 7 optical microscope (Westover Scientific, Mill Creek, WA) 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 cross polarizers and a red I plate with 500-nm retardation. The lesions appear dark due to the demineralization causing strong scattering which results in a loss of birefringence. A custom-built digital transverse microradiography (TMR) system was used to measure mineral loss in the different partitions of the sample. 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.

3.5 Integrated Reflectivity, Quantitative Mineral Loss Profiles and Lesion Depth Measurements

The integrated reflectivity and integrated mineral loss were calculated for each of the six areas on all the samples. Line profiles were taken from the orthogonal polarization ($\perp$ -axis) PS-OCT images or b-scans in each of the six regions and the reflectivity was integrated from the enamel surface to a real depth of 200- μm , yielding the integrated reflectivity of the regions in units of (dB $\cdot\mu\text{m}$). Previous studies have shown that R correlates directly with the integrated mineral loss called Z ⁶. Similarly, line profiles were taken from the digital transverse microradiographs (TMR) of the thin sections cut along the same position scanned by OCT. X-ray attenuation was converted to volume percent mineral using a calibration curve. The line-profiles in the four treatment (lesion) areas were subtracted from the sound mineral profiles to yield the integrated mineral loss. The line profiles were integrated from the edge of the sample to a depth of 200- μm to give units of Z (vol.% $\cdot\mu\text{m}$). In some cases where samples had cavitated, the edge of the sample was linearly extrapolated by calculating the slope along the sample surface and the integration started from the calculated point. This yields a mineral value of ~20–25% in areas where mineral was completely lost and consistent with the mineral volume at which the enamel collapses. The integrations were performed using Igor Pro software (Wavemetrics, Lake Oswego, OR). Prior to taking the line profiles for the OCT images, a 10 $\times$ 10 convolution filter was applied to smooth out the images using the Igor Pro image processing package.

4. RESULTS AND DISCUSSION

Sample PS-OCT images of two of the enamel specimens are shown in Figs. 2 and 3. Optical changes caused by melting and recrystallization due to the laser beam are visible to the naked eye and are visible in the PS-OCT images before exposure to the demineralization solution. The lesions were typically less severe in the laser treated areas and on the side exposed to the fluoride.

The inhibition by the laser is clearly evident in the PS-OCT scan of Fig. 2, which shows that both the lesion depth and severity are less at the position of the laser treatments, the left and right black arrows. On some samples the lesions were quite severe and there was cavitation due to excessive mineral loss. Other lesions disintegrated due to the thin sectioning process. One example of such a sample is shown in Figs. 3&4. The right side of the sample in Fig. 3 has a severe lesion that has cavitated in the area not treated by fluoride outside the laser treated area due to excessive demineralization while the laser treated zone in between the cavitated area has remained intact. We know this area didn't fall apart during sectioning because the area of cavitation is clearly visible on the OCT image. On the left side or fluoride treated side the OCT scan shows that the lesion is quite severe just to the right side of the laser treated area in the circled area. That zone is intact in the OCT scan but has fractured off during sectioning. The lesion in the fluoride + laser treated area is less severe than the surrounding area. A composite of digital microradiographs taken across another sample with cavitation in the unprotected lesion area is shown in Fig. 4. The laser irradiation zones were not visible in the TMR images (Fig. 4) since they do not produce a reduction in the mineral density.

The laser greatly inhibited lesion progression on the fluoride side of this sample as well. Similar results were acquired for the other samples and we plan on performing a thorough statistical analysis of the results in the future.

The laser treated enamel zone was readily visible in many of the OCT images and this laser irradiated zone did reduce the intensity of the signal from the underlying sound enamel and the dentinal-enamel junction (DEJ) as can be seen in the center of the PS-OCT scans of Figs. 2 & 3. However the depth of the laser-modified zone is relatively small compared to the typical lesion depth, and the reflectivity from the thermally modified enamel is less than that from the demineralized enamel. Therefore, although the thermally modified enamel does influence the OCT signal one is still able to differentiate the underlying demineralized enamel in the laser-treated lesion area because the reflectivity is much higher.

These results suggest that PS-OCT can be used to assess the severity of early demineralization and measure the inhibition of that demineralization by fluoride and CO₂ laser treatments. Since PS-OCT is not destructive, it does not require teeth scheduled for extraction for clinical studies that are required for more conventional techniques such as TMR and PLM.

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Fig. 1. Bovine enamel sample after irradiation showing the position of the three laser treated areas that were subsequently treated with topical fluoride (F), protected with varnish (S) and unprotected (D).

Fig 2.

Enamel block after exposure to the demineralization solution. The red line on the left shows the position of the ($\perp$ -axis) PS-OCT scan that is shown on the right. Areas of high reflectivity such as lesion areas and the dentinal enamel junction (DEJ) appear in red according to the false color scheme as shown on the right. The black arrows show the positions of the laser treated areas. Fluoride (F) was applied to the left side of the sample and the lesion is more severe on the right (D).

Fig 3.

An enamel block after exposure to the demineralization solution that exhibited cavitation in the unprotected lesion area (right-side). The PLM (A) shows the cavitation on both sides however the OCT image (B) shows cavitation on only the right side indicating that the cavitation on the left side (black circle) occurred during sectioning. The black arrow shows where the laser treatment prevented

Fig 4.

A composite of transverse microradiographs taken of a sample with cavitation and severe demineralization in the unprotected area. The left and right black arrows mark the position of the laser-treated areas. Note the right unprotected side has cavitation in the area not protected by the laser.