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Monitoring the inhibition of erosion by a CO_2 laser with OCT

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Abstract

Since optical coherence tomography (OCT) is well suited for measuring small dimensional changes on tooth surfaces, OCT has great potential for monitoring tooth erosion. Previous studies have shown that enamel areas ablated by a carbon dioxide laser manifested lower rates of erosion compared to the non-ablated areas. The purpose of this study was to develop a model to monitor erosion *in vitro* that could potentially be used *in vivo*. Teeth surfaces were irradiated with a carbon dioxide laser at low sub-ablative fluence to create an acid-resistant reference layer without damaging the enamel. The laser treated areas were compared with the unprotected areas using OCT during exposure to a pH cycling model for up to 6 days. The laser treated areas markedly reduced the rate of erosion.

Keywords

erosion; optical coherence tomography; caries prevention; carbon dioxide laser

1. INTRODUCTION

Optical coherence tomography is capable of measuring dimensional changes nondestructively on tooth surfaces. It appears to be an ideal tool for monitoring tooth erosion, which is the removal and softening of tooth surfaces due to a variety of factors including subsurface demineralization [1].

Previous studies have shown that polarization sensitive optical coherence tomography (PS-OCT) can nondestructively measure the severity of subsurface demineralization in enamel and dentin and is therefore well suited for this role [2–14]. Early *in vitro* studies of the use of OCT for monitoring tooth demineralization demonstrated that OCT can be used to measure the loss of enamel due to exposure to a demineralizing solution. In the study of Fried et al. [15], acid resistant varnish was added to a control surface and this surface served as a reference surface to quantify the loss of enamel exposed to erosion. Although this approach is simple to implement *in vitro*, more robust adhesives such as composites would be needed in order to employ it *in vivo* and they would have to be employed in areas that are not subject to wear.

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Studies have also employed OCT to monitor the remaining enamel thickness by using the dentinal enamel junction (DEJ) as a reference [16]. However, there are many challenges in accurately measuring the remaining enamel thickness. For example, the DEJ is scalloped and does not present a sharp boundary. If erosion is accompanied by subsurface demineralization or roughening of the surface, the strong increase in light scattering interferes with the ability to accurately resolve the DEJ [17]. Moreover, the tooth surface is often covered by a layer of saliva, which causes additional variation in the optical path length [17]. One can also achieve the inhibition of demineralization and erosion via laser irradiation [18–20]. In previous dissolution studies on human and bovine enamel blocks, we produced laser incisions to separate the areas of interest (Fig. 1). While investigating the use of OCT for quantifying the severity of tooth demineralization, we discovered that the area of enamel surrounding the laser irradiated reference incisions manifested increased resistance to erosion. In one such study, the demineralization model produced surface erosion instead of subsurface lesions, however the irradiated areas were preferentially protected and not eroded, and these areas protruded above the surrounding eroded untreated enamel erosion areas [21]. Last year, we carried out further studies exploiting the enhanced resistance to erosion of laser treated surfaces to assess the suitability of using it as a method to quantify the rate of erosion and found that the laser-irradiated areas appeared quite effective in inhibiting the surface loss in demineralization model [17]. The previous studies employed ablative laser irradiation intensities while an *in vivo* model requires a nondestructive approach with subablative laser irradiation intensities. The purpose of this study was to develop an *in vitro* model to monitor erosion with OCT that can potentially be translated to an *in vivo* setting, utilizing subablative laser irradiation intensities.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Erosion Model

Sixteen bovine enamel blocks, approximately 8–12 mm in length, 2-mm in width, and a thickness of ~1 mm of bovine enamel were prepared from extracted tooth incisors acquired from a slaughterhouse. Each enamel sample was partitioned into five regions or windows (two sound, two laser irradiated, and one unprotected) by etching 140 μm wide incisions 2-mm apart across each of the enamel blocks (see Fig. 1). Incisions were etched using a transverse excited atmospheric pressure (TEA) CO_2 laser operating at 9.3-μm with a fluence of 200 J/cm^2, Impact 2500, GSI Lumonics (Rugby, U.K.). In the windows adjacent to the center, a sub-ablative incident fluence of 2.4 J/cm^2 was used. The sub-ablative fluence was used to increase resistance to acid dissolution, protecting the region from further demineralization and erosion. In the outer most windows, a thin layer of acid resistant varnish, red nail polish, Revlon (New York, NY) was applied to protect the sound enamel control area. The center window was left unprotected.

All samples were exposed to a pH cycling model with a demineralization solution composed of a 40-ml aliquot of 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and a 0.075 mol/L acetate at pH 4.5 followed up with remineralization solution comprised of a 40-ml aliquot of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L potassium choloride, and 20 mmol/L HEPES at pH 7.0. Each of the cycles was repeated twice (2, 4, 6 cycles) for the central three

windows. Sixteen blocks were exposed to a daily pH cycling regimen consisting of immersion in a demineralization solution (pH 4.5) for 6 h, followed by a rinse with de-ionized water, and immersion in a remineralization solution (pH 7.0) for 17 h at 37°C. After every 2 cycles, PS-OCT scans were taken to assess the amount of erosion that had taken place by comparing the heights of the demineralized window and sub-ablative regions to that of the protected ends of the sample. After the last day of pH cycling, the acid resistant varnish was removed with acetone, Fisher Scientific (Hampton, NH) and then scanned.

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. 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 and 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 at half-maximum (FWHM) of 84 nm. 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 planoconvex lens. This configuration provided lateral resolution of approximately 20-μm and an axial resolution of 10-μm in air with a signal to noise ratio of greater than 40–50 dB. The PS-OCT system is completely controlled using Labview software (National Instruments, Austin, TX). The system is described in greater detail [22, 23]. Acquired scans are compiled into *b-scan* files. Image processing was carried out using Igor Pro, data analysis software (Wavemetrics Inc., Lake Oswego, OR).

2.3 Analysis of Erosion rates from OCT Scans

Co-polarization OCT scans were used to determine the depths of both the sub-ablative regions and the central unprotected region. Depths were compared with the central unprotected window and the two outer most protected windows for each sample. These depths were calculated by measuring slopes determined from two points arbitrarily chosen within each window. The four points from the two sub-ablative regions determined a best-fit line, which was used to determine the depth of the unprotected central region. Points chosen in the unprotected region were mapped accordingly to a point directly above on the best-fit line, resulting in a depth difference measured using Igor Pro. The depths of erosion measured in microns were averaged across all samples on the last day of pH cycling and compared to the protected sound regions to determine the amount inhibited.

3. RESULTS AND DISCUSSION

One sample that was imaged with digital microscopy after the completion of the study is shown in Fig. 2. The three center windows are exposed to demineralization without an acid protective varnish. One cannot resolve differences between the laser treated and unprotected windows by visual inspection alone. Sequential parallel-axis OCT *b-scans* were taken at

different pH cycling time periods (0, 2, 4, 6 cycles) and examples for one sample are shown in Fig. 3. The goal of our study was to investigate the use of laser-modified enamel surfaces or marks to serve as a reference for the nondestructive assessment of erosion rates *in vivo*. The laser irradiated enamel surfaces manifested little erosion after 6 cycles of exposure; while the unprotected region of the samples exhibited markedly higher erosion, ~ 100-μm. These results suggest that it may be feasible to utilize these laser-irradiated (protected) areas generated with sub-ablative fluence to monitor erosion. Our studies indicated that after 6 days of pH cycling, laser irradiated enamel inhibited erosion by 74%. It is important to note that these samples were done using bovine enamel blocks, which are more susceptible to demineralization and erosion than human enamel.

Previous studies have found that the layer of modified enamel after 9.3-μm CO_2 laser pulses of 10–15-μs duration is only 10–20-μm thick [20]. However our study indicates that the laser irradiated areas underwent an average loss of 30-μm of enamel, suggesting complete loss of the protected layer. It is interesting that the laser modified zone provided such a high degree of protection even after the depth of erosion exceeded the depth of the laser modified enamel.

As mentioned in previous studies, the efficacy of protection against erosion from the laser modified area depends on the severity of demineralization. The pH cycling model proved to be quite severe in our study, since it eroded roughly 100-μm of enamel. Clinical studies will be needed to establish whether this method will work *in vivo* or *in situ* over a wide range of acid or erosive challenges.

Acknowledgments

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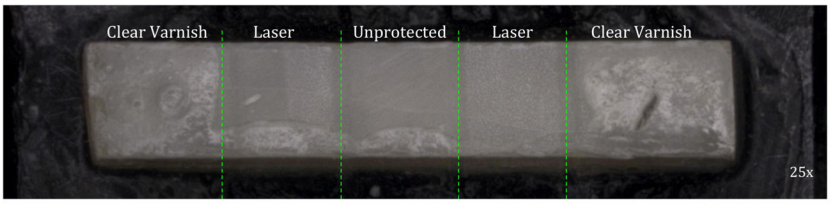

Fig. 1.
Bovine enamel block with the 5 treatment windows (25x).

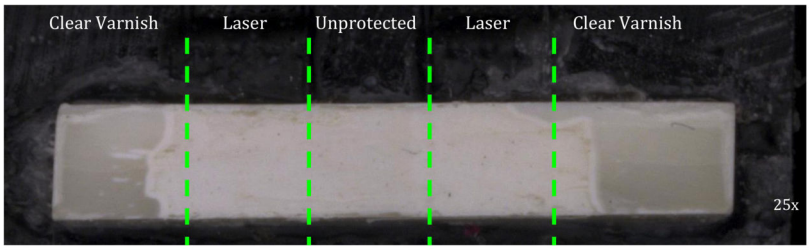

Fig. 2.
Bovine enamel block with the 5 treatment windows (25x) after 6 pH cycles.

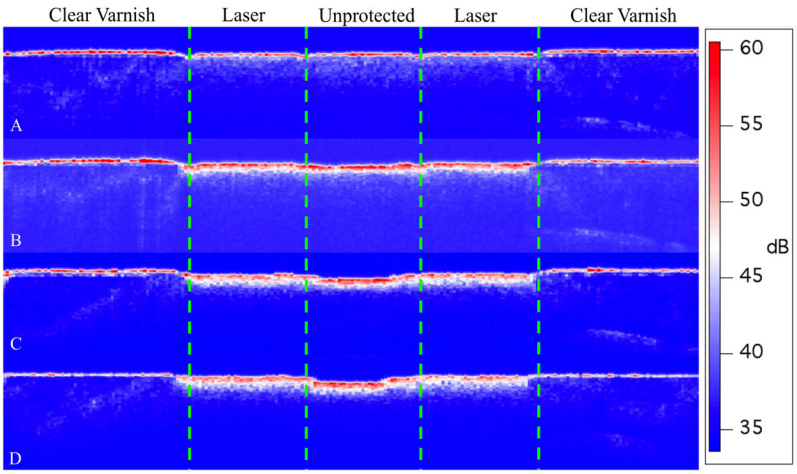

Fig. 3.

Co-polarization OCT *b-scans* during consecutive pH cycling time periods. (A) initial scan, (B) 2 pH cycles, (C) 4 pH cycles, (D) 6 pH cycles. The laser treated regions markedly inhibits erosion compared to unprotected regions.