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Investigation of Acid-Etched CO₂ Laser Ablated Enamel Surfaces Using Polarization Sensitive Optical Coherence Tomography

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Abstract

A carbon dioxide laser operating at the highly absorbed wavelength of 9.3 μ m with a pulse duration of 10–15 μ s is ideally suited for caries removal and caries prevention. The enamel thermally modified by the laser has enhanced resistance to acid dissolution. This is an obvious advantage for caries prevention; however, it is often necessary to etch the enamel surface to increase adhesion to composite restorative materials and such surfaces may be more resistant to etching. The purpose of the study was to non-destructively measure the susceptibility of laser-ablated enamel surfaces to acid dissolution before and after acid-etching using Polarization Sensitive Optical Coherence Tomography (PS-OCT). PS-OCT was used to acquire images of bovine enamel surfaces after exposure to laser irradiation at ablative fluence, acid-etching, and a surface softened dissolution model. The integrated reflectivity from lesion and the lesion depth were measured using PS-OCT. Samples were also sectioned for examination by Polarized Light Microscopy (PLM). PS-OCT images showed that acid-etching greatly accelerated the formation of subsurface lesions on both laser-irradiated and non-irradiated surfaces ($P < 0.05$). A 37.5% phosphoric acid etch removed the laser modified enamel layer after 5–10 seconds.

Keywords

laser ablation; optical coherence tomography; tooth demineralization; tooth erosion; acid-etching

1. INTRODUCTION

Several studies have demonstrated that carbon dioxide lasers operating at wavelengths of $\lambda = 9.3$ and $9.6\text{-}\mu\text{m}$ are ideally suited for the efficient ablation of dental caries and for surface treatments to increase the resistance to acid dissolution.^{1–5} If pulse durations in the range of 5–20- μ s are used, efficient ablation can be achieved with minimal peripheral thermal damage^{3, 5, 6}. Another advantage of CO₂ laser systems is that they are capable of operating efficiently at high pulse repetition rates and laser systems can be combined with laser beam scanning systems for high-speed precision removal of dental caries. One concern at higher repetition rates is the increased potential for peripheral thermal damage due to heat accumulation from multiple laser pulses delivered in rapid succession. Much of the heat accumulation is offset by rapidly scanning the laser beam. Some residual heat deposition

after laser ablation is advantageous for caries inhibition around cavity preparations but the thermally modified layer may reduce the bond strength to composite⁷⁻¹⁰. In a previous study, we demonstrated that this laser could be used to rapidly remove enamel while maintaining interpulpal temperatures at safe levels¹¹. Studies employing this laser also indicated that high shear bond strengths can be achieved on both enamel and dentin surfaces irradiated by this laser if those surfaces were subsequently acid-etched^{7, 8, 12-14}. The shear bond strength of the laser treated surfaces that were not acid etched indicated low bond strength, ~ 6 MPa. This contradicted early studies in which we achieved markedly higher bond strengths for the laser irradiated surface even without water-cooling. These lasers produced a multimode spatial profile and the ablated enamel surfaces were rough and not very uniform^{9,13}. This led to a subsequent study that was presented here last year in which we investigated the influence of surface roughness¹⁵. The surface roughness did not increase the adhesive strength, however deliberate patterning of the surface with retention holes did significantly increase the bond strength without having to acid etch the surface.

The purpose of this study was to evaluate the effect of acid etching on the acid resistance of the layer of enamel modified by the CO₂ laser. Our objectives are to establish that acid etching rapidly removes the modified enamel layer thus removing the acid protection and determine if the acid resistant layer interferes with the ability to acid etch the enamel surface.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Thirty-four bovine enamel blocks(~2 × 10 mm) were prepared and mounted on delrin blocks. For the first set of fifteen samples (Set I), each sample was partitioned into five windows as shown in the diagram below. Two of the five windows were treated by the laser. One of those two windows was etched with phosphoric acid (37.5%). One of the remaining windows was covered with acid resistance varnish, another window was acid-etched and the last window was untreated. The samples were subsequently exposed to a surface-softened dissolution model to generate subsurface lesions. Another set of 17 blocks (Set II) were divided into eight windows that were laser-irradiated and acid-etched for various time periods before exposure to the surface softened dissolution model^{16, 17}. The remaining 2 blocks (Set III) were divided and etched in the same way as Set II but only one of them underwent ablation with the laser before applying the etchant. These two blocks were not put into surface-softened dissolution model. Automated methods of analysis were employed to calculate the depth and severity of the subsurface lesions after scanning by PS-OCT. Blocks were placed into surface softened dissolution model for 8 days. The solution was composed of a 40-mL aliquot of 20 mmol/L calcium, 10 mmol/L phosphate, and 0.1 mol/L lactic acid set at pH 4.6 and the samples were incubated at 37°C.

After completion of the specified exposure periods to demineralization solution and etchant, 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 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 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 ¹⁸. 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).

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 ^{19, 20}.

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 microscopy with measurements using OCT in order to avoid overestimation of

lesion depth due to weak signals caused by birefringence in sound enamel²¹. 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. InStatTM from GraphPad software (San Diego, CA) was used for statistical calculations.

2.4 Polarized light microscopy

The blocks were serial sectioned to sections 200- μ m thick 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

The first set of bovine enamel blocks, set #1, compared the acid resistance of surfaces with and without laser ablation after the standard 37.5% phosphoric etch for 30 seconds. Figure 2 shows an image of a thin section viewed under polarized light along with a cross polarization b-scan image of one of the samples from set #1. The lesions in the groups that were acid etched with and without laser irradiation show more severe demineralization for both imaging modalities. The mean $\pm$ s.d of the integrated reflectivity from the CP-OCT scans are shown in Fig. 3.

The second set of bovine blocks, set #2, was used to compare the resistance of the laser ablated surfaces after different time periods of phosphoric acid etching. Figure 4 shows PLM and-OCT b-scan images of a sample from set #2. The lesions appear to be of similar severity for all the acid etched groups. The mean $\pm$ s.d of the integrated reflectivity from the CP-OCT scans are presented in Fig. 5.

The co-polarization and cross-polarization OCT scans from the two bovine blocks of Set #3 representing different etching times with and without laser ablation are shown in Fig. 6. Both orthogonal PS-OCT images are shown because it is easier to identify the degree of erosion in the co-polarization image. In the co-polarization images the dashed line demarcates the original position of the enamel surface so the degree of erosion can be estimated.

4. DISCUSSION

The formation of subsurface lesions was greatly accelerated after acid etching for both laser-irradiated and non-irradiated surfaces. Non-destructive and non-invasive OCT measurements of erosion and demineralization matched polarized light measurements and OCT was able to show non-destructively erosion and formation of subsurface lesions. These results were confirmed using PLM. These results suggest that acid etching should be avoided unless necessary and the area that is acid etched should be minimized. Enamel surfaces are etched prior to the placement of Orthodontic brackets and care should be taken to minimize the area exposed to the etchant. This is particularly important since areas peripheral to the bracket manifest increased plaque retention and increased susceptibility to demineralization and white spot lesions.

The laser-modified layer was rapidly etched away after only 5–10 seconds of phosphoric acid etching. This result suggests that any acid-etching with 37.5% phosphoric acid will remove the acid resistant modified enamel layer. Acid etching may be unavoidable for the restoration of many surfaces where high bond strength is required. Since 80% of decay begins in the pits and fissures, a fissurotomy or enameloplasty is often recommended; namely the cleaning of such surfaces with a small burr, if the fissures are deep or are stained or demineralized prior to sealant placement. Such surfaces are difficult to etch due to prism orientation and high fluoride content. The CO₂ laser is ideally suited to perform this task since it leaves the irradiated area with increased resistance to acid dissolution. Studies are needed comparing the efficacy and longevity of conventional sealants vs. CO₂ laser treated surfaces with and without subsequent placement of sealants. This study suggests that acid etching should be avoided after CO₂ laser irradiation if preservation of the modified enamel layer with increased acid resistance is desired.

The thermally modified enamel layer manifests increased reflectivity which does interfere with OCT measurements. Moreover, the roughened surface from ablation also interferes. The interference is substantial and prevented the measurement of a significant difference in the integrated reflectivity for the laser ablated surfaces vs. the non-irradiated surfaces. In previous studies we were able to measure a significant difference between laser treated surfaces and non-treated surfaces but the lesions produced in those studies were more severe and they were easier to differentiate^{22, 23}.

Acknowledgments

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Fig. 1. Diagram showing the layout of the different treatment groups on the bovine enamel blocks. The inset in lower left shows an image of the sample and sample holder.

Fig. 2. PLM image of a thin section along with a cross polarization OCT scan of the groups from Set #1.

Fig. 3.

The mean $\pm$ s.d (n=15) integrated reflectivity (dBx μ m) of each group calculated from the cross polarization OCT images for Set #1. Bars of the same color are statistically similar, $P>0.05$.

Fig. 4. PLM image of a thin section along with a cross polarization OCT scan of the groups from Set #2. Bars of the same color are statistically similar, $P > 0.05$.

Fig. 5. The mean±s.d (n=15) integrated reflectivity (dBxμm) of each group calculated from the cross polarization OCT images for set #2.

Fig. 6.
 (//) co-polarization and (⊥) cross polarization OCT scan of the groups from Set #3 which represent enamel surfaces with and without laser ablation for different periods of acid-etching with H_3PO_4 .