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Selective removal of demineralized enamel using a CO₂ laser coupled with near-IR reflectance imaging

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Abstract

Detection and diagnosis of early dental caries lesions can be difficult due to variable tooth coloration, staining of the teeth and poor contrast between sound and demineralized enamel. These problems can be overcome by using near-infrared (NIR) imaging. Previous studies have demonstrated that lasers can be integrated with NIR imaging devices, allowing image-guided ablation. The aim of this study was to demonstrate that NIR light at 1500 – 1700 nm can be used to guide a 9.3- μ m CO₂ laser for the selective ablation of early demineralization on tooth occlusal surfaces. The occlusal surfaces of ten sound human molars were used in this *in-vitro* study. Shallow simulated caries lesions of varying depth and position were produced on tooth occlusal surfaces using a demineralization solution. Sequential NIR reflectance images at 1500 – 1700 nm were used to guide the laser for selective ablation of the lesion areas. Digital microscopy and polarization sensitive optical coherence tomography (PS-OCT) were used to assess the selectivity of removal. This study demonstrates that high contrast NIR reflectance images can be used for the image-guided laser ablation of early demineralization from tooth occlusal surfaces.

Keywords

caries removal; near-IR reflectance imaging; selective laser ablation

1. INTRODUCTION

Modern dentistry has evolved towards increasing preservation and prevention of sound tissue rather than on more invasive treatment options. New diagnostic tools have been developed to help identify caries lesions before they spread and aid in tooth preservation. Current methods of caries detection are confounded by factors such as tooth coloration and staining, leading to false diagnoses, and are incapable of monitoring lesion progression and assessing lesion severity. Previous studies have demonstrated that near-infrared (NIR) imaging at wavelengths from 1300-1700-nm is not confounded by tooth coloration and staining and reflectance measurements are able to achieve extremely high contrast between sound and demineralized tissue, particularly at wavelengths commensurate with higher water absorption, namely near 1450-nm and 1500-1700-nm [1-9]. The NIR from 1500 – 1700 nm achieves similar contrast to 1450-nm even though the mean water absorption

coefficient is lower and it is easier to achieve higher illumination intensities. It is likely that the lesion contrast is enhanced by higher water absorption because the deeply penetrating photons are absorbed by water in the underlying enamel and dentin lowering the reflectivity of sound areas. Dentin has a high water content and sound areas of the tooth with underlying dentin appear very dark at these near-IR wavelengths.

Specular reflection is a serious problem in imaging teeth due to the high refractive index of enamel, $n=1.63$. Therefore it is necessary to use polarization to reduce specular reflection and false positives. This study and previous studies have employed cross polarization to reduce specular reflection [6-8].

Previous approaches for guiding laser ablation have included fluorescence [10-12] and NIR transillumination [13]. NIR transillumination appears most promising for deep lesions (see manuscript Chung et al # 9306 -15 from this proceedings) but is insensitive to shallow demineralization. Fluorescence is limited by the interference of stains and the diffuse and delocalized nature of porphyrin fluorescence. In our previous study presented last year we showed that NIR reflectance in the wavelength regions 1450-nm and 1500-1700-nm were ideally suited for image-guided ablation using the CO₂ laser since the laser modified surfaces did not reduce the contrast to the degree that NIR reflectance was unsuitable for serial imaging during ablation [14]

Several studies have demonstrated that polarization sensitive optical coherence tomography can be used for the nondestructive assessment of lesion depth and severity. The integrated reflectivity (R) in the cross polarization OCT image (CP-OCT) over the lesion depth can be used as a measure of lesion severity, and it is analogous to the integrated mineral loss (Z) with depth, which is measured from microradiography, which is the gold standard for lesion severity. Previous studies have shown that the integrated reflectivity correlates well with integrated mineral loss, and thus can be used to monitor lesion severity. PS-OCT images also aid in the 3D reconstruction of the samples and the data can be used to estimate volumetric data. PS-OCT was used in this study to assess the lesion depth and severity before ablation, the depth of ablated enamel and the residual lesion depth and severity after removal.

In this study, a CO₂ laser operating at 9.3 μm coupled with near-IR reflectance imaging was used to selectively remove early demineralization. The CO₂ laser was guided by serial NIR reflectance images. NIR reflectance images were acquired using a polarized and filtered tungsten halogen source and an InGaAs area camera. Lookup tables (LUT) files were generated from NIR images based on the image contrast. Various image analysis methods including background thresholding, normalization and edge detection/enhancement were employed. The LUTs were subsequently used to control the laser scanning system.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Ten noncarious human molars were cleaned, sterilized with gamma radiation, and stored in a 0.1% thymol solution to prevent bacterial growth. Samples were selected based on visual

appearance and NIR images. The outlines of 4×4 mm windows approximately 50-μm deep were cut on the occlusal surface of each tooth using a CO₂ laser (Impact 2500, GSI Lumonics Rugby, UK) around the suspected lesion area. The channels cut by the laser serve as reference boxes for imaging and serial sectioning and are sufficiently narrow that they do not interfere with calculations of the image contrast. The reference boxes were cut into the samples using a laser spot size of 200-μm, a pulse separation of 50-μm and a pulse repetition rate of 50-Hz with 25-mJ per pulse with water spray (see section 2.2 below).

Demineralized areas of enamel of varying severity were produced in the 4×4 windows on the samples. First the areas were cleaned with pumice Whip Mix (Louisville, KY). After cleaning the sample occlusal surfaces, clear acid-resistant varnish (Revlon, New York) was randomly applied within the 4×4 mm window to expose certain areas for lesion generation. This was done to produce non-uniform patterns of demineralization. The enamel surrounding the 4×4 windows created by the laser was covered with a red acid-resistant varnish (Revlon, New York).

Artificial lesions were created within the 4×4 windows by immersing each tooth into a 50 ml aliquot of a Ca/PO₄/acetate solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate maintained at pH 4.5 and a temperature of 37 °C for 3-days. After the last day of demineralization the acid resistant varnish was removed with acetone.

2.2 Laser Irradiation

Samples were irradiated using an industrial marking laser, Impact 2500 from GSI Lumonics (Rugby, United Kingdom) operating at a wavelength of 9.3 μm. The laser was custom modified to produce a Gaussian output beam (single spatial mode) and a pulse duration between 10-15-μs. The laser energy output was monitored using a power meter EPM 1000, Coherent-Molelectron (Santa Clara, CA), and the Joulemeter ED-200 from Gentec (Quebec, Canada). Computer-controlled XY galvanometers 6200HM series with MicroMax Series 671 from Cambridge Technology, Inc. (Cambridge) were used to scan the laser beam over the sample surfaces. A repetition rate of 100-Hz was used, and the laser beam was scanned point to point over a 100-μm grid using a ZnSe scanning lens of 90-mm focal length. The laser spot size was ~350-μm (note 100-μm spacing between pulses for high overlap) and the incident fluence was 20 J/cm² (19 mJ per pulse). An air-actuated fluid spray delivery system consisting of a 780S spray valve, a Valvemate 7040 controller, and a fluid reservoir from EFD, Inc. (East Providence, RI) was used to provide a uniform spray of fine water mist onto the tooth surfaces at 2 mL/min.

2.3 Near-IR Cross Polarization Reflectance Images (NIR)

In order to acquire reflected light images, linearly polarized and collimated light from a 150-W fiber-optic illuminator, Model FOI-150 from the E Licht Company (Denver, Colorado) was used to illuminate the samples at an incident angle of 30°. Polarizers were placed after the light source and before the detector to remove specular reflection (glare) that interferes with measurements of the lesion contrast. The NIR reflectance images were captured using an InGaAs area camera, Model SU320-KTSX (320 × 240 - 25-μm pixel pitch) from Sensors Unlimited (Princeton, NJ) sensitive from 900-1700-nm, equipped with a Model SWIR-35

lens from Navitar, Inc. (Rochester, NY). Reflectance measurements were taken using a 1500-nm long-pass filter, the FEL 1500 from Thorlabs (Newton, NJ).

2.4 Digital Microscopy (DCDM)

Tooth surfaces were examined after laser irradiation using an optical microscopy/3D surface profilometry system, the VHX-1000 from Keyence (Elmwood, NJ). Two lenses were used, the VH-Z25 with a magnification from 25 to 175 $\times$ and the VH-Z100R with a magnification of 100-1000 $\times$. Depth composition digital microscopy images (DCDM) were acquired by scanning the image plane of the microscope and reconstructing a depth composition image with all points at optimum focus displayed in a 2D image. Images of the samples were acquired before and after ablation at 25 $\times$ and 50 $\times$ magnification. The Keyence 3-D shape measurement software, VHX-H3M, was used to correct the tilt of the sample and measure the variation in depth over the enamel in the ablated areas.

2.5 NIR Guidance (Description of Procedures)

The CO₂ laser was guided by NIR reflectance images of the samples (see flowchart in the manuscript Chan et al. # 9306-15 in this proceedings). An initial NIR background image was acquired that was used for illumination nonuniformity correction. Each sample was then placed on a magnetic sample holder that could be switched back and forth between magnetic mounts on the laser and imaging setups. The initial background image was subtracted from each subsequent image acquired. Maximum pixel intensity values in the 4 $\times$ 4 mm ROI (83 $\times$ 83 pixels) and average sound values from the outside the ROI were calculated from the first corrected image and used for subsequent assessments of the post ablation images. The image/lesion contrast was calculated for each pixel in the ROI using $(I_{ROI} - I_{MS})/I_{ROI}$ where I_{MS} is the mean intensity of the sound pixels outside the ROI and I_{ROI} is the value of the individual pixel in the ROI to yield values between 0 and 1. A threshold demarcating the lesion area in the ROI was manually selected after comparing the NIR images acquired before and after generation of the artificial lesions. Once the threshold was selected, the ROI image was then converted to binary and stored as a look-up table (LUT) file. The look-up table (LUT) was used to scan the laser beam to ablate matched areas on the sample.

The laser was operated at a pulse repetition rate of 100 Hz and the laser beam focused to a spot size of 350- μ m. The beam was scanned from point to point in 100- μ m increments with $\sim$ 4 laser pulses per position. All designated areas of demineralization were irradiated during each iteration (scan) and new NIR images were acquired after every two iterations. This process was repeated until a final NIR image indicated that all demineralized areas were removed.

2.6 PS-OCT System (OCT)

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. 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

coherence tomography. The spectral output of the 15-mW SLD was centered at 1317 nm with a spectral bandwidth full-width at half-maximum (FWHM) of 84 nm. This configuration provided a lateral resolution of approximately 20 μm and an axial resolution of 10 μm in air. The system is described in greater detail in reference [15]. The PS-OCT scans were used to confirm the presence of the occlusal lesions, assess the depth and severity of the artificial lesions and assess the depth ablated.

2.7 Polarized Light Microscopy (PLM)

After sample imaging was completed, approximately 200 μm thick serial sections were cut using an Isomet 5000 saw (Buehler, IL), for polarized light microscopy (PLM). PLM was carried out using a Meiji Techno RZT microscope (Meiji Techno Co., LTD, Saitama, Japan) 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 crossed polarizers and a red I plate with 500-nm retardation.

2.8 Image Analysis

Images were acquired before and after artificial lesion removal using three different modalities: Digital microscopy (DCDM), PS-OCT, and PLM. The volume of the lesions was measured using CP-OCT before and after removal by the laser. Statistical calculations were carried out using Prism (Graphpad Software, La Jolla, CA). Teeth were sectioned after lesion removal and the 200- μm thick sections were examined using polarized light microscopy (PLM). Images were then compared to their respective CP-OCT images to confirm existence of artificial lesions. All image analysis was carried out using Igor pro software (Wavemetrics, Lake Oswego, OR). A repeated measures one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc multiple comparison test was used to compare groups employing Prism software (GraphPad, San Diego, CA).

3. RESULTS AND DISCUSSION

PS-OCT images were compared before and after ablation to assess the selectivity of the artificial removal process. These images represent cross sections of the tooth samples and indicate the initial lesion depth and the depth of ablation. CP-OCT images clearly show the removal of artificial lesions; however, there are still areas with higher “residual” reflectivity caused by the increased surface roughness produced by laser irradiation. Images from two of the tooth samples are shown in Figs. 1 & 2. DCDM images before and after the laser scans clearly show that the majority of artificial lesions were removed. As can be seen in A & B most of the whiter areas (higher reflectivity) indicative of demineralization have been removed after scanning. Image C & D are the NIR reflectance images before and after removal. In C, the artificial lesions have high contrast as indicated by the white patches, while D shows that the artificial lesions were removed within the area of the box. There is still some residual contrast even though the lesion was removed. The higher reflectivity in irradiated areas is due to increased surface roughness due to the laser and is not residual demineralization. In our study presented last year, we evaluated the effects of the CO₂ laser modified surfaces on the contrast in NIR reflectance. There were changes but they did not

reduce the contrast to the degree that NIR reflectance was unsuitable for serial imaging during ablation [14].

Cross sectional CP-OCT scans across the sample at the positions indicated by the yellow dotted lines in A - D are shown in E & F. The yellow arrows in all images indicate the beginning and end of the ROI window corresponding to the CP-OCT images. From the left yellow arrow to the right arrow, artificial lesions are present along the surface of the scan as indicated by the higher reflectivity in E. After ablation, the artificial lesions have disappeared as indicated by the rough surface in F.

The increase in reflectivity caused by surface modification manifests itself in all of the image modalities, NIR reflectance, visible reflectance and OCT and presents a challenge in automating the caries removal process.

Another challenge was maintaining alignment of the samples while switching back and forth between the laser-ablation and NIR imaging setups. The magnetic holder worked well for the samples however there were some slight displacements. A total of 10 samples were used in this study and a complete analysis of the results for all the samples will be submitted for future publication in a peer reviewed manuscript.

One goal of this work is to fully automate the caries removal process by developing methods for image analysis that are capable of accurately identifying the appropriate contrast thresholds in the NIR images as opposed to having the operator manually set thresholds as was done in this study. For a practical clinical image-guided ablation system, the clinician would likely desire to manually select or approve the computer selected area to be ablated initially. An automated approach is more important for determination of the end point of removal, i.e., accurately identifying when caries removal is complete.

In conclusion, this study demonstrates that high contrast NIR reflectance images can be used for the image-guided laser ablation of early demineralization from tooth occlusal surfaces.

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Figs. 1 & 2.

DCDM images before (A) and after (B) removal, NIR reflectance images before (C) and after (D) removal, and corresponding CP-OCT cross sectional images (B-scans) before (E) and after (F) removal. The yellow dotted lines indicate the position of the CP-OCT B-scans on the occlusal tooth surface, while the yellow arrows indicate the beginning and end of the scan from left to right.