Published in final edited form as:

J Biomed Mater Res B Appl Biomater. 2009 August ; 90(2): 802–812. doi:10.1002/jbm.b.31349.

Nondestructive assessment of dentin demineralization using polarization sensitive optical coherence tomography after exposure to fluoride and laser irradiation

Saman K. Manesh, Cynthia L. Darling, and Daniel Fried*

Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, CA 94143-0758

Abstract

Previous studies have demonstrated that polarization sensitive optical coherence tomography (PS-OCT) can be used to image natural and artificial caries in dentin. The purpose of this study was to measure non-destructively the severity of artificial caries lesions in dentin and determine the efficacy of intervention with anti-caries agents including fluoride and lasers. Although several studies have assessed the utility of PS-OCT to image caries lesions in enamel and to quantify the lesion severity, only a few studies have focused on lesions in dentin. In this study images of artificial dentin lesions on extracted human teeth were acquired with PS-OCT. Before exposure to an artificial demineralizing solution, three incisions were made on the sample surfaces using either Er:YAG, Nd:YAG ($\lambda=355\text{-nm}$), or TEA CO₂ lasers and selected areas were treated with topical fluoride to create six unique treatment areas for each of the three laser conditions investigated. The integrated reflectivity and depth of demineralization were calculated for each of the six areas on each sample using the PS-OCT images. Polarized light microscopy (PLM) and transverse microradiography (TMR) were used to measure lesion severity on histological thin sections for comparison. PS-OCT successfully measured the inhibition of demineralization by topical fluoride. Laser irradiation was not particularly effective in increasing or decreasing the rate of dentin demineralization. PLM and TMR corroborated those results. This study demonstrates that PS-OCT can be used to measure demineralization on dentin surfaces and determine the degree of inhibition of demineralization by anti-caries agents.

Keywords

optical coherence tomography; Er:YAG laser; CO₂ Laser; Nd:YAG laser dentin; laser ablation; artificial lesions; caries inhibition; thermal damage; topical fluoride

1. INTRODUCTION

Several studies over the past few years have demonstrated that optical coherence tomography (OCT) can be used for the assessment of the severity of early caries lesions. Optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structure 1–3. OCT has great potential for the non-destructive imaging of dental caries “*in vivo*” 4–6. Polarization-sensitive OCT (PS-OCT) is a form of OCT that is sensitive to changes in the polarization of the reflected light 7. Polarization sensitive optical coherence tomography (PS-OCT), has been successfully used to: acquire

Corresponding Author: Daniel Fried, PhD, Professor, Director, MS Program in Oral & Craniofacial Biology, Division Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, 707 Parnassus Ave., San Francisco, CA 94143-0758, Phone (415)502-6641, Fax (415) 476-0858, daniel.fried@ucsf.edu.

images of both artificial and natural caries lesions 8–10, assess their severity in depth, assess the remineralization of such lesions 3·4·5, and determine the efficacy of chemical agents in inhibiting demineralization 11.

Polarization-sensitive depth-resolved reflectivity measurements can provide a measure of the severity of natural and artificial caries lesions on smooth surfaces and in the occlusal pits and fissures 12–14. The high reflectivity of dentin and enamel surfaces produces a very strong reflection that can interfere with the measurement of early demineralization on the surface. This strong reflection can be reduced using polarized light. Moreover, demineralized areas on the tooth depolarize the incident linearly polarized light and the reflectivity in the orthogonal or perpendicular ($\perp$) polarization state to the incident polarization can be directly integrated to provide a measure of the lesion severity.

Although the penetration depth of near-IR light is considerably less in dentin than for enamel due to the markedly higher scattering coefficient of dentin, PS-OCT and OCT images of dentin with root caries show that penetration depths of ~ 1 mm can be achieved and demineralized dentin can be discriminated from sound dentin and cementum 10 and root canal walls and fractures can be imaged 15·16. In contrast to sound enamel that is highly transparent in the near-IR, sound dentin strongly scatters light in the near-IR and is highly birefringent 17·18. Therefore, it is not obvious that the same approach employed to assess the severity of demineralization on enamel surfaces can be successfully applied to the surface of dentin. Amaechi et al. 19 measured the % reflectivity loss due to demineralization on dentin surfaces and showed that this correlated well with mineral loss from microradiography. However, the validity of an approach that relies on the measurement of reflectivity loss is questionable since light scattering increases with demineralization for both enamel and dentin producing an overall increase in reflectivity from the area of the lesion, not a decrease in reflectivity. The approach that we have chosen is to measure the reflectivity from each layer of the lesion and subsequently integrate over a specific depth to yield the integrated reflectivity (ΔR) in units of $\text{dB} \times \mu\text{m}$ which is compared with the “gold standard”, the integrated mineral loss (ΔZ) in units of volume % mineral lost $\times \mu\text{m}$ that is measured using microradiography and is calculated in a similar fashion by integrating the volume % mineral loss over a specific depth 12–14·20·21.

Current methods that are used to determine the efficacy of anti-caries agents require teeth scheduled for extraction and partial destruction of the tooth through sectioning. These methods include microradiography, microhardness and polarized light microscopy. High resolution x-ray methods such as x-ray tomography (XTM) are capable of acquiring images of mineral density in caries lesions with resolutions on the order of a few microns 22·23. New high-resolution micro-CT systems are approaching the resolution needed to image early caries lesions. However, neither of these systems can be used in vivo. The most promising method that can be used in vivo for measurements of tooth demineralization is quantitative light fluorescence (QLF). Excellent correlation has been established between ΔF (fluorescence loss or radiance) and mineral loss (ΔZ) for shallow uniform artificial lesions on enamel surfaces 24–26. The QLF phenomenon, i.e., the loss of yellow/green fluorescence under illumination with blue light, can be solely explained by light scattering effects 27. A recent study published by Amaechi et al. 28 compared the loss of reflectivity with lesion depth as measured with optical coherence tomography (OCT) to the loss of fluorescence measured with QLF and achieved a very high correlation. Unfortunately, QLF has not been validated for use on dentinal lesions and is primarily used for incipient enamel lesions.

It has been well established that topical fluoride agents can inhibit the demineralization of enamel, dentin and cementum 29. 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 30–35. Other studies have indicated that topical fluoride treatments can work synergistically with the laser treatment to further enhance its inhibitory effect 36–37. However, it has yet to be demonstrated that lasers can be used to inhibit the demineralization of dentin and only a few papers have identified any beneficial effect of laser treatments on dentin 38–39. The three laser systems that were chosen for this study target different components of dentin and the mechanism of interaction varies with each system. The CO₂ laser is most strongly absorbed by the mineral phase of dentin and it heats the dentin surface to a high temperature during ablation leaving a melted and recrystallized thin layer of hydroxyapatite on the surface after laser irradiation. Er:YAG laser pulses are strongly absorbed by water and subsurface expansion of that water aids in the removal of the mineral and collagen without melting the mineral component. Nd:YAG ($\lambda=355\text{-nm}$) laser are absorbed by the collagen and preferentially etch the intertubular dentin. Only the Er:YAG and CO₂ lasers can efficiently remove sound dentin. The Nd:YAG ($\lambda=355\text{-nm}$) laser cannot and this is reflected in the depth of the incisions produced in this study. The incisions are deep for the erbium and carbon dioxide lasers and very shallow for the $\lambda=355\text{-nm}$ laser pulses. It was anticipated that each of these laser treated surfaces would remove the dentin smear layer and modify the permeability of the dentin to fluoride.

In summary, the principal objective of this study was to demonstrate that PS-OCT can be used as a nondestructive means for quantifying the severity of artificial demineralization and the inhibition potential of anti-caries agents such as fluoride and laser treatments on dentin surfaces. The integrated reflectivity in artificial lesion areas measured with PS-OCT was compared with the established methods of transverse microradiography and polarized light microscopy that require tooth extraction and thin sectioning.

2. MATERIALS AND METHODS

2.1 Sample Preparation, topical fluoride treatment and artificial lesion creation

Forty-five dentin blocks, approximately $6 \times 3 \times 2 \text{ mm}^3$ were prepared from extracted human teeth for this study ($n=15$ per group). Teeth were collected with Committee on Research (CHR) approval under Exempt Certification Category 4. The dentin surfaces were serial polished to one micron using embedded diamond polishing discs. Each dentin sample was partitioned into three regions with the center of each region being treated with three types of laser using the conditions as specified in the next section. The middle partition of each sample was covered with a thin layer of acid resistant varnish (red nail polish (Revlon, New York, NY)) after laser irradiation that served to protect the sound dentin control area before exposure to the demineralization solution. The middle partition covered with varnish served as the two negative control groups: protected (S) and protected + laser (S + L). A 1.23% acidulated phosphate fluoride foam, Oral B Minute Foam (Gillette, South Boston, MA), was applied only to 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 onto the untreated side of the sample. The fluoride treated left partition served as the fluoride + lesion (F) and fluoride + laser + lesion (F + L) groups. The right unprotected 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 dentin surface. After treatment, samples were placed in a demineralization solution consisting of a 40-mL aliquot of 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate at pH 5.0, kept at a constant temperature of 37°C for twelve days to create artificial “surface softened” lesions. Having the six study groups within each sample, lesion (D), lesion plus laser (S + L), sound (S), sound plus laser (S + L), fluoride (F), and fluoride plus laser (F + L) allowed us to

decrease inter-sample variability. Groups were compared using repeated measures analysis of variance (ANOVA) with a Tukey multiple comparisons test.

2.2 Laser Irradiation

The 45 dentin samples were divided into three equal groups of fifteen for each set of laser parameters. 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 profiles were acquired using a Spirocon PyrocamTM I pyroelectric array (Logan, UT). The laser spatial beam profiles showed that the lasers were operating in a single spatial mode with a Gaussian shaped spatial beam profile. The laser pulse temporal profile was measured with a room temperature HgCdTe detector (Boston Electronics, Brookline, MA) with a response time of a few nanoseconds. Laser energy calibration and measurement was done using a laser energy/power meter, EPM 1000, Coherent-Moletron (Santa Clara, CA) with an ED-200 Joulemeter from Gentec (Quebec, Canada).

The laser irradiation parameters for the three laser systems are tabulated in Table I. The 1st group was irradiated with a transverse excited atmospheric pressure (TEA) CO₂ laser, Impact 2500 (GSI Lumonics, Rugby, UK) operating at $\lambda=9.3 \mu\text{m}$, with a fluence of 10 J/cm^2 , an energy of 7 mJ per pulse, and pulse duration of 15 μs . A ZnSe lens with a focal length of 100-mm was used to focus the laser to a beam diameter of approximately 300 μm on the sample surface. The laser was operated at a repetition rate of 100 Hz and the sample was scanned across the laser beam at a speed of 1.0 mm/sec across the surface of each of the fifteen dentin blocks three times to create the three irradiation zones. A water spray was used for all the samples in the CO₂ laser group.

The 2nd group was treated with a Tempest Q-switched Nd:YAG laser system (NewWave Research, Fremont, CA) with a pulse duration (FWHM) of 5 ns equipped with nonlinear optics for third-harmonic generation ($\lambda=355 \text{ nm}$). The Tempest laser was also equipped with a variable beam attenuator incorporating two linear polarizers with which to vary the incident intensity. Samples were irradiated at a fluence of 1.7 J/cm^2 , and a fixed repetition rate of 30 Hz. The samples were moved across the laser beam at a rate of 1.3 mm/sec during laser irradiation. Three incisions were produced on each sample using a laser beam that was focused to a spot size of 1000 μm with a $f=100\text{-mm}$ AR coated fused silica lens. Samples were irradiated without introducing water to the dentin surfaces since a previous study showed that the tamping effect of water enhances crack formation and minimum thermal damage is produced for these short laser pulses⁴⁰.

The 3rd group of samples was irradiated using an Er:YAG laser originally manufactured by Schwartz Electro-optics (Orlando, FL) operating in free running mode. The “short” free-running laser pulse was generated using an Analog Modules (Longwood, FL) Model 8800 variable pulse power supply that generates a square shaped flash lamp drive pulse. The flash lamp drive pulse was set to 160- μs in order to generate a laser pulse of 35- μs duration. The laser beam was focused to a spot size of 400- μm at the position of irradiation with an AR coated ZnSe lens with a focal length of 75-mm. An irradiation intensity of 8 J/cm^2 was used to produce the three incisions on each sample. Samples were scanned continuously across the laser beam at a rate of 0.25 mm/sec and a pulse repetition rate of 5-Hz.

Computer-controlled UTM150 and 850G stages and ESP300 controllers from Newport (Irvine, CA) were used to create controlled movement of the samples during laser irradiation. All samples were kept well-hydrated before laser irradiation and a computer controlled pulsed air/water nozzle was used to apply a layer of water at a flow rate of 2.4–2.8 mL/min to the samples of the TEA-CO₂ and Er:YAG laser groups. A low volume/low pressure air-actuated fluid spray delivery system consisting of a EFD 780S-SS Spray valve,

a Valvemate 7040 controller and a fluid reservoir from EFD Inc. (East Providence, RI) was used to provide a uniform continuous spray of fine water droplets on the dentin surface.

2.3 PS-OCT System

A single-mode fiber autocorrelator-based Optical Coherence Domain Reflectometry (OCDR) system with polarization switching probe, high efficiency 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) (Denselight, Jessup, MD) with an output power of 45-mW and a bandwidth of 35-nm and a high-speed XY-scanning system, ESP 300 controller and 850-HS stages, Newport (Irvine, CA), and used for *in vitro* polarization sensitive optical coherence tomography (PS-OCT). This all-fiber OCDR system was configured to provide lateral resolution of approximately 50- μ m over the range of 10-mm, and an axial resolution of 22- μ m in air and 15- μ m in dentin 41. The PS-OCT system was completely controlled using LabVIEW™ software (National Instruments, Austin TX).

2.4 Polarized Light Microscopy (PLM) and Transverse Microradiography (TMR)

After the dentin samples were imaged with PS-OCT, they were cut into sections approximately 200–250- μ m thick using an Isomet 5000 saw (Buehler, Lake Bluff, IL) for polarized light microscopy (PLM) and digital transverse microradiography (TMR). 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 immersed in water and examined at 15–200 $\times$ magnification with crossed polarizers and a red I plate (500-nm retardation). 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. The volume percent mineral of the three partition areas on each sample was determined by comparison with a calibration curve of x-ray intensity versus sample thickness created using sound enamel sections of $86.3 \pm 1.9\%$ volume percent mineral varying from 50 to 300 μ m in thickness. The mineral content of the enamel samples used in the calibration curve was confirmed using transverse microhardness measurements employing a x-ray digital imager. The volume percent mineral determined using microradiography for section thickness ranging from 50 to 300 μ m highly correlated with the volume percent mineral determined using microhardness, $r^2 = 0.99$ which validates this approach 13,18.

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

The integrated reflectivity and integrated mineral loss were calculated in each of the six areas on the sample. Line profiles were taken from the orthogonal polarization ($\perp$ -axis) PS-OCT images in each of the six regions and the reflectivity was integrated from the dentin surface to a real depth of 500- μ m, yielding the integrated reflectivity, ΔR , of the regions in units of (dB $\cdot\mu$ m). The real depth was calculated by dividing the optical depth by the refractive index of dentin, 1.542. Previous studies of demineralization on enamel surfaces have shown that ΔR correlates directly with the integrated mineral loss (vol. % mineral $\cdot\mu$ m) called ΔZ 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 sound enamel calibration curve. The line-profiles in the four treatment (lesion) areas were subtracted from the sound mineral profiles to yield the integrated mineral loss. Demineralized lesions will shrink in dry conditions and

this can be seen in the PS-OCT and TMR scans; hence, the line profiles were integrated from an extrapolated edge of the sample to a depth of 500- μm to give units of ΔZ (vol.% $\bullet \mu\text{m}$). The integrations were performed using Igor Pro software (Wavemetrics, Lake Oswego, OR). Prior to taking the line profiles for the OCT images, a 10×10 convolution filter was applied to smooth out speckle in the images. Sample groups were compared using repeated measures analysis of variance (ANOVA) with a Tukey-Kramer post hoc multiple comparisons test. A paired t-test was used when only two groups were under consideration. InstatTM from GraphPad software (San Diego, CA) was used for the statistical calculations.

3. RESULTS

Reflected light images of dentin samples irradiated by the three laser systems are shown in Fig. 1. PS-OCT scans were acquired along the long axis of each sample near the center and sectioned for PLM and TMR in the same orientation. The areas treated by the laser do not exhibit any discoloration upon visual examination which suggests minimal thermal damage (charring). The cross sections do not show peripheral areas of birefringence loss (appear black) around the laser ablation craters which is characteristic of thermal damage to the collagen matrix under PLM examination. Areas of birefringence loss greater than 10- μm thick are typically seen for longer laser pulses that produce more extensive peripheral thermal damage 40.

Figures 2–4 show a composite of the (A) PS-OCT image, orthogonal polarization ($\perp$), and the matching (B) polarized light microscopy (PLM) and (C) transverse microradiography (TMR) images for a $\sim 200\text{-}\mu\text{m}$ thick section cut from the same sample. A red-white-blue false color scheme is used for the PS-OCT images with the intensity scale in decibel units (dB), where red and white areas have the highest reflectivity. The same red-white-blue false color scheme was used for volume % mineral in the TMR images. The reflectivity of sound dentin is relatively high due to its high scattering coefficient, thus the lower range of the intensity scale for the PS-OCT images was intentionally set to maximize the contrast of the demineralized dentin in Figs. 2–4. Therefore, the sound areas as well as the background appear blue. In most of the samples, the unprotected area on the right side that was exposed to demineralization manifested a higher reflectivity in the orthogonal ($\perp$) polarization than the protected area (center) and fluoride treated region (left). The demineralization was most severe in the region marked (D) where topical fluoride was not applied and the lesion depth typically exceeded 200 μm . Demineralization was less severe in the area treated with fluoride (F), left side. The reflectivity is lower and the depth of the lesion appears to be approximately half the depth of the untreated side. Laser treated areas are indicated by the shallow depressions where ablation occurred and indicated by white arrows. These are best seen in the PLM image. The original incision depth before exposure is shown in the sound area. The areas of the laser incisions exposed to the demineralization solution do not show a dramatic reduction in the lesion severity as has been observed for enamel³⁸. This suggests that the laser is not as effective at inhibiting decay as it is on enamel. On the other hand it also shows that the laser does not make things worse by increasing the solubility of dentin that could occur with thermal damage to the collagen matrix³⁹ and the laser modified surfaces do not inhibit imaging of the sound and demineralized dentin beneath the incision areas.

Dentin contains a high percentage of collagen and demineralized dentin, therefore it tends to shrink considerably when exposed to air, i.e., if it is not immersed in water. This can be seen in Figs. 2–4. PS-OCT and TMR were carried out with wet samples in air while for PLM the samples were immersed in water. There is considerable shrinkage in the (F) and (D) regions for the PS-OCT and TMR images while the PLM images show minimal shrinkage in those regions.

Individual line-profile were chosen from the center of each area on the sample and the mean integrated reflectivity in units of $\text{dB} \cdot \mu\text{m}$ was calculated by integrating the reflectivity in the orthogonal polarization ($\perp$) image in units of decibels (dB) to a depth of 500- μm . The volume % mineral loss was measured in the same location for TMR and integrated over the same depth, 500- μm , to yield the integrated mineral loss in $\text{Vol.}\% \cdot \mu\text{m}$. The lesion depth was also measured with PLM and PS-OCT and these measurements are tabulated in Table II for the TEA-CO₂, Nd:YAG and Er:YAG laser groups respectively. Table III shows the mean values for the combined three groups comparing lesions with no laser treatment (F, S and D). The mean integrated reflectivity measured with PS-OCT is plotted in Figs. 5–7. There was a significant reduction ($p < 0.05$) in the integrated reflectivity between regions treated with fluoride (F) versus regions that were completely untreated (D) as well as between the protected region (S) and the demineralized regions (F and D). In the fluoride treated areas, PS-OCT was unable to detect a significant effect of the laser irradiation with the exception of the Nd:YAG irradiated samples where (F+L) lesion was considerably higher in R than (F). The differences in integrated reflectivity of the protected laser (S + L) and sound dentin (S) regions were also not statistically significant ($p > 0.05$) for any of the laser conditions.

The integrated mineral loss or ΔZ values are also plotted in Figs. 5–7 for TEA-CO₂, Nd:YAG ($\lambda=355\text{-nm}$) and Er:YAG laser groups respectively. These measurements were taken from the 200- μm thick transverse sections cut from the samples at similar positions to the OCT b-scans. The integrated mineral loss or ΔZ manifests similar behavior between groups to the integrated reflectivity ΔR , demonstrating that ΔR reflects the severity of demineralization. One source of concern is the effect of shrinkage on the ΔZ calculations. A calibration curve was used for determination of the mineral content and that curve is only accurate for volume percent mineral content above 10%. The dentin shrinkage introduces an error since void areas due to shrinkage are counted as 10% mineral as opposed to 0% mineral content in the calculation of the integrated mineral loss, therefore shrinkage of the lesions (see Fig. 2–4) leads to an overestimation of the mineral loss by TMR. Therefore, the ΔZ values in lesion areas such as groups (D) and (F) are most likely inflated due to the effect of shrinkage. TMR analysis does clearly show the effect of topical fluoride in enhancement of inhibiting demineralization regardless of laser treatments.

A combined plot comparing the integrated reflectivity and the integrated mineral loss ($n=108$) shows that ΔR manifests a positive correlation with ΔZ (Pearson correlation coefficient of 0.65, $p < 0.05$) for dentin.

The lesion depth was also calculated from the PS-OCT and PLM images and those values are tabulated in Table II. Both PS-OCT and PLM indicate that there was a significant reduction in lesion depth in the groups with topical fluoride application. A plot of the lesion depth measured using both methods for all of the samples ($n = 72$) indicates that there is a positive correlation between the two methods ($r=0.57$, $p < 0.05$).

4. DISCUSSION

The integrated reflectivity from the artificial lesion areas measured with PS-OCT was significantly higher than the sound dentin. Moreover, PS-OCT was able to measure significant differences in the integrated reflectivity between unprotected dentin and dentin treated with topical fluoride which reduced the lesion severity (ΔZ) by $\sim 35.5\%$. It was not obvious from prior PS-OCT imaging studies that this approach would be successful due to the high reflectivity of sound dentin. Table II shows that the mean integrated reflectivity of sound dentin exceeds a 1000 ($\text{dB} \cdot \mu\text{m}$) for a depth of 500- μm . This is more than twice the integrated reflectivity from an equivalent thickness of sound enamel. However, it is apparent

from these measurements that the reflectivity of dentin increases sufficiently enough with demineralization to enable differentiation. Further refinements in our approach, such as only integrating over the PS-OCT determined lesion depth—instead of a fixed value of 500- μm —should significantly improve sensitivity by reducing the magnitude of the contribution from sound dentin. A significant positive correlation between ΔZ and ΔR suggests that PS-OCT can be used for the nondestructive assessment of dentin demineralization, however the correlation is not high enough to claim that PS-OCT can be used to accurately measure the mineral content of dentin.

There was also a significant positive correlation between the lesion depth measured with PLM and PS-OCT, however the lesion depths estimated using PS-OCT were $\sim 35\%$ lower than for PLM. This is not surprising since PLM is extremely sensitive and optical changes appear in PLM well before there are sufficiently large changes in mineral to be visible with TMR. For this reason lesion depths calculated using PLM and TMR seldom agree. The imaging geometry also influences the sensitivity to optical changes, in PLM a thin section of the tooth is being imaged while in PS-OCT images are acquired from an intact sample normal to the surface.

Laser irradiation was not particularly effective in increasing or decreasing the rate of dentin demineralization. PLM and TMR corroborated those results. This finding is consistent with similar studies that were conducted using the same TEA CO_2 laser and another cw- CO_2 laser that failed to show inhibition on dentin surfaces 43·44. Studies have shown using the same TEA CO_2 laser that CO_2 laser irradiation is highly effective for inhibiting demineralization on enamel surfaces at both ablative and sub-ablative fluence 35·45·46. Erbium lasers are effective on inhibiting decay on enamel surfaces if water cooling is not used 47 but this approach typically results in inefficient ablation and excessive peripheral thermal damage. Even though CO_2 laser irradiation converts the surface of dentin to a highly mineralized enamel-like composition the contraction due to the removal of the collagen leaves many small cracks that probably increase the permeability, thus negating the influence of the thin veneer of converted enamel-like mineral 48. TMR and PS-OCT showed that there was a statistically significant increase in inhibition by the Er:YAG laser in combination with the fluoride group. It is possible that removal of the smear layer enhanced the retention of the topical fluoride. However the % inhibition is not much larger than $\sim 25\%$ compared to fluoride alone and that decrease in mineral loss still falls within one standard deviation of the mean and should be interpreted with caution since the lesions in the fluoride group for the Er:YAG laser were more severe than for the corresponding fluoride only groups for the other two laser groups.

PS-OCT images did not show significant differences in the integrated reflectivity between the sound dentin (S) and the laser treated sound areas (S+L) and it was successful in assessing the severity of demineralization beneath areas treated by all three of the laser conditions employed. PS-OCT is also useful for measuring thermal changes in dentin that increase the dentin reflectivity, however there was no evidence of thermal damage in the PS-OCT images of the laser treated areas nor is there any indication that stress cracks were created by the laser irradiation. This was not unexpected since laser conditions were chosen that minimized thermal changes to the dentin. It would be interesting to employ OCT in future studies in which laser conditions are chosen to deliberately cause thermal and mechanical damage.

PS-OCT is ideally suited as an *in situ* diagnostic of laser ablation and to specifically guide the laser beam for selective ablation of dental caries. It will be necessary to acquire images of the dentin surfaces before and after laser irradiation in order to employ PS-OCT effectively in this manner. Therefore it is encouraging that changes in the optical properties

of the laser irradiated dentin under the laser irradiation conditions employed in this study did not inhibit imaging the dentin beneath the laser treated area. In conclusion, PS-OCT was successfully used to measure demineralization on dentin surfaces and determine the inhibition of demineralization by anti-caries agents. This is a significant step forward since these measurements provide depth resolved information about the lesion and are non-destructive in nature and can therefore be applied *in vivo*. Other PS-OCT studies are underway in our laboratory involving other dentin demineralization models such as pH cycling, the demineralization of root surfaces with a layer of cementum, and the measurement of remineralization of dentin.

Acknowledgments

The authors acknowledge the support of NIH R01 grants R01-DE017869 and R01-DE14698 and the help of Dennis Hsu, Chulsung Lee, Michal Staninec and John Featherstone.

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Figure 1.

Reflected light images of representative tooth samples after irradiation with (A) TEA-CO₂, (B) Nd:YAG, ($\lambda=355\text{-nm}$) and (C) Er:YAG lasers introducing 6 study groups of fluoride only F, F+L, S, S+L, D, and D+L where F is the fluoride treated area exposed to the demineralization, S is the sound area protected with varnish, D is the unprotected area exposed to demineralization, and L indicates the position of each of the three “tracks” due to the laser irradiation (L).

Figure 2.

A sample irradiated with the TEA-CO₂ laser ($\lambda=9.3\text{-}\mu\text{m}$). (A) Polarization sensitive optical coherence tomography (PS-OCT) scan in the orthogonal polarization ($\perp$) taken along long axis of the sample before sectioning the tooth. Intensity scale is shown in red-white-blue in units of decibels (dB). (B) The corresponding transverse microradiograph (TMR) of a 200- μm thick section in a similar orientation to the PS-OCT image. The intensity scale is also shown in red-white-blue with units in volume % mineral. (C) The polarized light microscopy (PLM) image of the same section is shown at 15 $\times$ magnification. Note the vertical shrinkage in the fluoride treated area (F) and unprotected lesion area (D) compared to the sound (S) area.

Figure 3. Sample irradiated with the Nd:YAG laser ($\lambda=355\text{-nm}$). (A) Orthogonal polarization ($\perp$) PS-OCT scan taken along long axis of the sample before sectioning the tooth. (B) The corresponding TMR image of a 200- μm thick section. (C) The PLM image of the same section is shown at 15 $\times$ magnification.

Figure 4. Sample irradiated with the Er:YAG laser ($\lambda=2.94\text{-}\mu\text{m}$). (A) Orthogonal polarization ($\perp$) PS-OCT scan taken along long axis of the sample before sectioning the tooth. (B) The corresponding TMR image of a 200- μm thick section. (C) The PLM image of the same section is shown at 15 $\times$ magnification.

Figure 5.

(top graph) Mean $\pm$ s.d. values of the integrated reflectivity, ΔR , measured with PS-OCT for the six treatment groups of the TEA-CO₂ laser ($\lambda=9.3\text{-}\mu\text{m}$) treated samples ($n = 12$). (bottom graph) Mean $\pm$ s.d. values of the integrated mineral loss, ΔZ , for the same samples measured with TMR. The integrated reflectivity and mineral loss values were calculated to a depth of 500 μm . Bars sharing the same color or pattern are statistically similar ($p > 0.05$).

Figure 6. (top graph) Mean±s.d. values of the integrated reflectivity, ΔR , measured with PS-OCT for the six treatment groups of the Nd:YAG ($\lambda=355$ -nm) laser treated samples ($n = 12$). (bottom graph) Mean±s.d. values of the integrated mineral loss, ΔZ , for the same samples measured with TMR. The integrated reflectivity and mineral loss values were calculated to a depth of 500 μm . Bars sharing the same color or pattern are statistically similar ($p > 0.05$).

Figure 7.

(top graph) Mean±s.d. values of the integrated reflectivity, ΔR , measured with PS-OCT for the six treatment groups of the Er:YAG laser ($\lambda=2.94\text{-}\mu\text{m}$) treated samples ($n = 12$). (bottom graph) Mean±s.d. values of the integrated mineral loss, ΔZ , for the same samples measured with TMR. The integrated reflectivity and mineral loss values were calculated to a depth of 500 μm . Bars sharing the same color or pattern are statistically similar ($p > 0.05$).

Table I

Laser irradiation parameters for the three lasers used in this study.

Laser (Wavelength)	Fluence (J/cm ²)	Pulse Duration	(1/e ²) Beam Diameter (μm)	Energy pulse (mJ)	Repetition Rate (Hz)	Scan Rate (mm/sec)	Water Spray
Er:YAG(2.94-μm)	8	35-μs	400	10	5-Hz	0.25	yes
Nd:YAG(355-nm)	1.7	5-ns	1000	13	30-Hz	1.3	no
CO ₂ (9.3-μm)	10	15-μs	300	7	100-Hz	1.0	yes

Table II

Polarization sensitive optical coherence tomography (PS-OCT), polarized light microscopy (PLM) and transverse microradiography (TMR) measurements for all six groups mean $\pm$ s.d. Groups in the same row with the same letter are statistically similar ($p>0.05$) $n=15$.

	F	F+L	S	S+L	D	D+L
TEA-CO₂ Laser						
PS-OCT (dB• μ m)	1678 $\pm$ 721 a	1609 $\pm$ 793 a	1046 $\pm$ 425 c	975 $\pm$ 478 c	2221 $\pm$ 774 b	2007 $\pm$ 695 a,b
TMR (Vol.%• μ m)	3399 $\pm$ 1068 a,c	2708 $\pm$ 967 a	-	-	5596 $\pm$ 1273 b	4347 $\pm$ 1155 c
PS-OCT (μ m)	113 $\pm$ 20 a	118 $\pm$ 23 a	-	-	166 $\pm$ 18 b	153 $\pm$ 24 b
PLM (μ m)	148 $\pm$ 14 a	132 $\pm$ 25 a	-	-	208 $\pm$ 35 b	187 $\pm$ 24 b
Nd:YAG Laser						
PS-OCT (dB• μ m)	1553 $\pm$ 610 a	2069 $\pm$ 664 b	947 $\pm$ 308 c	1068 $\pm$ 333 c	2157 $\pm$ 584 b	1973 $\pm$ 696 a,b
TMR (Vol.%• μ m)	3826 $\pm$ 1512 a	4686 $\pm$ 1891 a	-	-	6540 $\pm$ 1113 b	7021 $\pm$ 1236 b
PS-OCT (μ m)	97 $\pm$ 38 a	125 $\pm$ 30 b	-	-	145 $\pm$ 38 b	146 $\pm$ 31 b
PLM (μ m)	179 $\pm$ 39 a	205 $\pm$ 42 c	-	-	240 $\pm$ 24 b	252 $\pm$ 24 b
Er:YAG Laser						
PS-OCT (dB• μ m)	2097 $\pm$ 712 a	1964 $\pm$ 760 a	1136 $\pm$ 494 c	1187 $\pm$ 550 c	2693 $\pm$ 696 b	2494 $\pm$ 968 a,b
TMR (Vol.%• μ m)	4858 $\pm$ 1006 a	3650 $\pm$ 142 c	-	-	6626 $\pm$ 1339 b	5646 $\pm$ 899 a,b
PS-OCT (μ m)	109 $\pm$ 13 a	66 $\pm$ 14 c	-	-	147 $\pm$ 25 b	130 $\pm$ 27 b
PLM (μ m)	176 $\pm$ 13 a	121 $\pm$ 36 a	-	-	242 $\pm$ 25 b	201 $\pm$ 25 c

Table III

PS-OCT, PLM and TMR measurements of for the three groups without laser treatment, mean $\pm$ s.d. Groups in the same row that have the same letter are statistically similar, ($p>0.05$) $n=15$.

	F	S	D
PS-OCT (db•μm)	1776 $\pm$ 704 a	1043 $\pm$ 412 c	2357 $\pm$ 711 b
TMR (Vol.% •μm)	4028 $\pm$ 1332 a	-	6254 $\pm$ 1299 b
PS-OCT (μm)	106 $\pm$ 26 a	-	153 $\pm$ 29 b
PLM (μm)	167 $\pm$ 28 a	-	230 $\pm$ 32 b