Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2017 January 28; 10044: . doi:10.1117/12.2256739.

Synergistic effect of fluoride and laser irradiation for the inhibition of the demineralization of dental enamel

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Abstract

Both laser irradiation and fluoride treatment alone are known to provide increased resistance to acid dissolution. CO₂ lasers tuned to a wavelength of 9.3 μm can be used to efficiently convert the carbonated hydroxyapatite of enamel to a much more acid resistant purer phase hydroxyapatite (HAP). Further studies have shown that fluoride application to HAP yields fluoroapatite (FAP) which is even more resistant against acid dissolution. Previous studies show that CO₂ lasers and fluoride treatments interact synergistically to provide significantly higher protection than either method alone, but the mechanism of interaction has not been elucidated. We recently observed the formation of microcracks or a “crazed” zone in the irradiated region that is resistant to demineralization using high-resolution microscopy. The microcracks are formed due to the slight contraction of enamel due to transformation of carbonated hydroxyapatite to the more acid resistant pure phase hydroxyapatite (HAP) that has a smaller lattice. In this study, we test the hypothesis that these small cracks will provide greater adhesion for topical fluoride for greater protection against acid demineralization.

Keywords

optical coherence tomography; caries prevention; carbon dioxide laser

1. INTRODUCTION

Previous studies indicated that CO₂ laser irradiation renders enamel more resistant to acid dissolution. At wavelengths between 9–11 μm where CO₂ lasers operate, enamel strongly absorbs laser energy resulting in the conversion of carbonated hydroxyapatite to a purer phase hydroxyapatite [1–5]. In this study, we test the hypothesis that caries inhibition can be imparted without changing the appearance of the enamel surface. 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 softening and loss of tooth surfaces due to a variety of factors including subsurface demineralization [6].

Previous studies have also 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 [7–9]. Since OCT measures changes in the reflectivity of the enamel it is capable of measuring both changes in the

enamel due to thermal modification by laser irradiation and changes in the reflectivity due to acid demineralization.

In a study several years ago, we demonstrated that PS-OCT could be used to nondestructively assess caries inhibition after carbon dioxide laser irradiation and the application of topical fluoride [10]. PS-OCT was used to measure the integrated reflectivity of the lesion area and it was significantly lower in the laser and fluoride treated areas. We also found that the laser irradiation alone caused changes in the reflectivity of the enamel and caused an increase in the integrated reflectivity from those areas. This interfered with assessment of the enamel lesions but was not large enough to prevent resolution of differences between the treated and untreated groups. It appeared that at incident fluence below the melting threshold for enamel at the “wings” of the laser treated zones where there were minimal changes in enamel reflectance, the laser was still effective in inhibiting demineralization. Last year we carried out a follow up study using a new RF-excited laser operating at 9.4- μm that is capable of generating short 26- μs laser pulses with high single pulse energies ($>100\text{-mJ}$), delivered at high pulse repetition rates. With the Gaussian spatial profile and the high single pulse energies, a large beam diameter ($\sim 1.4\text{ mm}$) was used, and we were able to observe the influence of the continuous varying laser intensity profile on the surface morphology and the resistance to acid dissolution. The resistance to acid dissolution was assessed with varying fluence using PS-OCT [11]. There was significant protection from the laser in areas that were not visually altered. By utilizing the laser beam intensity profile, we were able to identify the incident fluence necessary for protection while not markedly altering the surface reflectivity. In the areas irradiated at lower irradiation intensities where protection occurred without melting there were small microcracks. We hypothesized that these areas would enhance the retention of topical fluoride for increased protection in these areas. Topical fluoride has been found to interact synergistically with laser irradiation to inhibit acid dissolution but the mechanism of synergistic interaction is not known [10, 12–15]. In this study, we carried out similar laser treatments and added topical fluoride after laser irradiation to test that hypothesis.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Bovine enamel blocks, approximately 10 mm in length, 2-mm in width, and a thickness of $\sim 1\text{ mm}$ of enamel were prepared from extracted tooth incisors acquired from a slaughterhouse. Each enamel sample was partitioned into three regions or windows (two laser irradiated, and one protected) by etching 140 μm wide incisions 2-mm apart across each of the enamel blocks. Fluence values of 0.9 J/cm^2 were used. In the center 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.

Two sets of bovine blocks (20 per set) were irradiated and exposed to an acid challenge that produces subsurface demineralization while retaining an intact surface. In addition, topical fluoride was also added to the left side of the block and the same laser irradiation parameters were applied to both the left and right windows. Samples were exposed to a surface softened 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 5.0 at 37 °C for 48 hours. After exposure the acid resistant varnish was removed with acetone, Fisher Scientific (Hampton, NH) and then scanned with PS-OCT.

For the later two sets of blocks, the protected windows were located on the extreme left and right sides of the blocks and there was no protected window in the center. In the first set of 20 bovine blocks, a fluence of 0.9 J/cm² was used with a laser scanning rate of 1 mm/sec, pulse repetition rate of 100 Hz and a spot diameter of 3.1 mm so that a narrow melt zone was created at the center of the laser profile similar as shown in Fig. 1A. For the second set, the same parameters were used, however a higher laser-scanning rate of 2 mm/sec was used which did not produce a continuous melt zone at the center of the laser spot. The area was irradiated by two laser scans separated by 250-μm to create a larger area with crazing as shown in Fig. 1B which is below the melting threshold, however microcracks were clearly visible in the surface. Acidulated Phosphate Flouride (APF) gel 1.23% from Keystone (Gibbstown, NJ) was added to the left side of each of the samples for one minute and it was rinsed off with water. OCT scans were taken after every 24 hours so that images could be acquired before erosion if erosion did occur. After 72 hours, subsurface lesions approximately 100-μm deep were produced with an intact surface and without erosion.

2.2 Laser Irradiation Parameters

An RF-excited laser prototype, Diamond J5-V from Coherent (Santa Clara, CA) operating at a wavelength of 9.4 μm, was used with a pulse duration of 26-μs and pulse repetition rates of up to 3 kHz. The laser had a Gaussian shaped spatial profile. The laser energy output was monitored using a power/energy meter, ED-200 from Gentec (Quebec, Canada). The laser beam was focused to a beam diameter of 3.1 mm using a ZnSe scanning lens of f=125-mm. A razor blade was scanned across the beam to determine the diameter (1/e²) of the laser beam. A computer-controlled high-speed motion control system with Newport (Irvine, California) UTM150 and 850G stages and an ESP300 controller was used to create controlled movement of the samples during laser irradiation. Samples were scanned across the laser beam at the rate of 2 mm/sec and the pulse repetition rate was varied. Incisions separating the windows were created using the same laser system but a f=25-mm aspheric ZnSe lens was used to create incision ~ 150-μm in diameter. A low volume/low pressure 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 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[12] 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- μ m and an axial resolution of 10- μ m in air with a signal to noise ratio of greater than 40–50 dB. The PS-OCT system is completely controlled using Labview software from National Instruments (Austin, TX). The system is described in greater detail [16, 17]. Acquired scans are compiled into *b-scan* files. Image processing was carried out using Igor Pro, data analysis software from Wavemetrics Inc. (Lake Oswego, OR).

2.4 Analysis of PS-OCT Scans

In previous studies [18–20], we established automated methods for determining the depth of demineralization (LD) and integrating over that depth to calculate the integrated reflectivity with depth which we call (R) which is analogous to the integrated mineral loss called Z which is measured with microradiography and is the gold standard for quantification of the severity of demineralization. The demineralization can be represented by 2D projection maps of R . In this study, R maps were created for all the windows so that changes with laser intensity along the laser profile.

3. RESULTS AND DISCUSSION

A 2D surface projection image of R , the integrated reflectivity in the cross polarization OCT image over the lesion depth is shown in Fig. 2 for one of the samples from the first group of bovine blocks after exposure to the dissolution solution. Higher values for R represent more severe demineralization and are shown in red while the areas protected by laser irradiation are shown in blue. Mean R values for the area between the lines are plotted below the 2D projection image. The reduction in the lesion severity follows the Gaussian profile of the laser intensity profile. There does not appear to be any effect of the topical fluoride on inhibiting acid dissolution and the lesion severity appears similar on both sides of the sample. The small central peak is due to protection peripheral to the laser incision separating the two windows.

The mean values for R at the center of each window are plotted at the bottom of the image. The sample groups were compared using RM-ANOVA and the means for the control & fluoride groups were significantly higher ($P < 0.05$) than for the two laser groups but neither the laser groups (L, L+F) or non-laser groups (C, F) were significantly different than each other.

Similar data is shown in Fig. 3 for one of the samples from the second group of bovine blocks after exposure to the dissolution solution. That set was irradiated at a fluence of 0.9 J/cm² with a laser scanning rate of 2 mm/sec and a spot diameter of 3.1 mm and there were two parallel scans offset by 250- μ m to create a flatter intensity profile without a definitive melt zone. For this sample the profile of the protected area is larger and not as sharply peaked as in Fig. 2 reflecting the flatter intensity profile. The mean values for R at the center of each window are plotted at the bottom of the Fig. 3. The sample groups were compared using RM-ANOVA and the means for the control & fluoride groups were

significantly higher ($P < 0.05$) than for the two laser groups but neither the laser groups or non-laser groups were significantly different from each other.

The lack of efficacy of topical fluoride in this study was unanticipated and we have no explanation for the lack of effect. We had anticipated that the fluoride would stick to the prism boundaries and microcracks formed in the laser affected zones and that a positive result would provide an explanation for the synergistic effect that has previously been observed for laser irradiation and fluoride. The effect of topical fluoride was observed in our previous enamel dissolution studies and we were previously able to differentiate the effect using PLM. This was the first time we employed a topical fluoride gel as opposed to a topical fluoride foam but both systems should behave similarly [10].

Acknowledgments

We would like to thank Robert Lee for his contributions and acknowledge the support of NIH/NIDCR grants R01-DE017869 and R01-DE019631.

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Fig. 1.
Bovine enamel block with the 2 treatment windows with a superimposed diagram of the incident fluence distribution for different irradiation parameters.

Fig. 2.

(top) 2D projection image of the integrated reflectivity with lesion depth (ΔR) from a bovine block after exposure to the demineralization solution for an incident fluence of 0.9 J/cm², a spot diameter of 3.1 mm, a 100 Hz repetition rate, and a scanning speed of 1 mm/sec. A red-white-blue intensity scale is used where red indicates higher reflectivity. The mean ΔR values in the window between the two lines is plotted below. (bottom) Measured mean integrated reflectivity $\pm$ S.D. after laser irradiation and exposure to the demineralization solution (Bars of the same color are statistically similar ($P > 0.05$)).

Fig. 3.

(top) 2D projection image of ΔR from a bovine block after exposure to the demineralization solution for an incident fluence of 0.9 J/cm^2 , a spot diameter of 3.1 mm, a 200 Hz repetition rate, and a scanning speed of 2 mm/sec. Two overlapping spots were used separated by 250- μm . A red-white-blue intensity scale is used where red indicates higher reflectivity. The mean ΔR values in the window between the two lines is plotted below. (bottom) Measured mean integrated reflectivity $\pm$ S.D. after laser irradiation and exposure to the demineralization solution (Bars of the same color are statistically similar ($P>0.05$)).