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Assessment of remineralization in simulated enamel lesions via dehydration with near-IR reflectance imaging

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Abstract

Previous studies have demonstrated that near-IR imaging can be used to nondestructively monitor the severity of enamel lesions. Arrested lesions typically have a highly mineralized surface layer that reduces permeability and limits diffusion into the lesion. The purpose of this study was to investigate whether the rate of water loss correlates with the degree of remineralization using near-IR reflectance imaging. Artificial bovine (n=15) enamel lesions were prepared by immersion in a demineralization solution for 24 hours and they were subsequently placed in an acidic remineralization solution for different periods. The samples were dehydrated using an air spray for 30 seconds and surfaces were imaged using an InGaAs camera at 1300-1700 nm wavelengths. Near-IR reflectance intensity differences before and after dehydration decreased with longer periods of remineralization. This study demonstrated that near-IR reflectance imaging was suitable for the detection of remineralization in simulated caries lesions and near-IR wavelengths longer than 1400 nm are well suited for the assessment of remineralization.

Keywords

Dental caries; lesion activity; remineralization; near-IR imaging

1. Introduction

Caries lesions can be arrested by the preferential deposition of mineral at the lesion surface that inhibits diffusion into the lesion and reduces the rate of water loss [1-3]. Since arrested lesions do not need further intervention, an accurate assessment of lesion activity is paramount for clinical diagnosis. Gold standards for lesion assessment such as transverse microradiography (TMR) and polarized light microscopy (PLM) require destruction of the tooth, and are unsuitable for use *in-vivo*. New non-destructive diagnostic tools are needed that can assess lesion activity with a single measurement (time-point).

The optical changes associated with water loss have been exploited via quantitative light-induced fluorescence (QLF), thermal imaging and near-IR imaging [4-6]. Near-IR imaging is advantageous for caries detection because sound enamel is transparent in the near-IR and

the scattering coefficient increases exponentially with increasing mineral loss [7]. Wu et al. and Lee et al. reported that reflectance and transillumination imaging were able to show demineralization on buccal and occlusal surfaces with high contrast at near-IR wavelengths [8, 9]. Zakian et al. carried out near-IR reflectance measurements from 1000–2500 nm using a hyperspectral imaging system and showed that the reflectance from sound tooth areas decreases at longer wavelengths where water absorption is higher [10]. Recent studies at near-IR wavelengths coincident with high water absorption, i.e. 1460 nm and > 1500-nm for artificial and natural lesions demonstrated high lesion contrast [11–13].

The magnitude of water absorption increases between 1400 and 1700 nm and we hypothesize that these near-IR wavelengths are more sensitive to changes in lesion hydration [11]. The objective of this work is to demonstrate that near-IR reflectance imaging can be used to monitor the remineralization of simulated enamel lesions.

2. Materials and Methods

2.1. Sample preparation

Enamel blocks ($n = 15$), approximately 8–12 mm in length with a width of 2 mm and a thickness of 3 mm were prepared from extracted bovine incisors acquired from a slaughterhouse. The surfaces of the bovine enamel blocks were ground to a 9 μm finish. Each sample was partitioned into six regions or windows (1 sound control, 4 lesion and 1 remineralization control) by etching small incisions 1.8 mm apart across each of the enamel blocks using a laser. Incisions were etched using a transverse excited atmospheric pressure (TEA) CO_2 laser, an Impact 2500, GSI Lumonics (Rugby, UK), operating at 9.3 μm with a pulse duration of 15 μs , a pulse repetition rate of 200 Hz. A thin layer of acid-resistant varnish in the form of nail polish, Revlon (New York, NY), was applied to protect the sound and remineralization control windows before exposure to the demineralization solution. Samples with four exposed lesion windows were immersed in 45 mL aliquots of the demineralization solution for 24 hours. The demineralization solution, which was maintained at 37°C and pH 4.8, was composed of 2.0 mmol/L calcium, 2.0 mmol/L phosphate and 0.075 mol/L acetate. After the demineralization period, the acid resistant varnish was removed by immersion in acetone in an ultrasonic bath for 15 minutes and the acid-resistant varnish was applied again to the sound and lesion windows. Sample windows were subsequently exposed to an acidic remineralization solution for 4, 8 or 12-days by covering appropriate windows with acid resistant varnish at each time point. It has been hypothesized that the lower pH reduces preferential deposition of mineral in the outer enamel, enhancing remineralization of the lesion body [14]. The acidic remineralization solution was composed of 4.1 mmol/L calcium, 15 mmol/L phosphate, 50 mmol/L lactic acid, 20 mmol/L HEPES buffer and 2 ppm F^- maintained at 37°C and a pH of 4.8 [14]. The remineralization control window was exposed to the acidic remineralization solution for 12 days in order to examine its effect on sound enamel. After the 12 days of remineralization, the acid resistant varnish was removed and the samples were stored in 0.1 % thymol solution to prevent fungal and bacterial growth.

2.2. Dehydration and Near-IR Reflectance Imaging

Each sample was placed in a mount connected to a high-speed XY-scanning system, Newport ESP 300 controller and 850G-HS stages, coupled with an air nozzle and a light source. All surfaces excluding the windows were covered with black nail polish, OPI (North Hollywood, CA) in order to confine water loss to the exposed surface and prevent the transmission of light through the sides of the sample. Each sample was immersed in the water bath for 30 seconds while being vigorously shaken to enhance water diffusion. After the sample was removed from the water bath, an image was captured as an initial reference image and the air spray was activated. The air pressure was set to 15 psi and the computer controlled air nozzle was positioned 2 cm away from the sample.

An Indigo Alpha near-IR camera (FLIR Systems, Wilsonville, OR) with an InGaAs focal plane array, a spectral sensitivity range from 900 nm to 1750 nm, a resolution of 320×256 pixels and an InfiniMite™ lens (Infinity, Boulder, CO) was used to acquire all the images during the dehydration process. The area per pixel was approximately 0.003 mm^2 . Light from a 150 W fiber-optic illuminator FOI-1 (E Licht Company, Denver, CO) was directed at the sample at an incident angle of approximately 60° in order to reduce specular reflection and the source to sample distance was fixed at 5 cm. Several band-pass (BP) and long-pass (LP) filters were used to provide different spectral distributions of near-IR light. Band-pass filters centered at 1300 nm with 90 nm bandwidth (1260–1340 nm), 1460 nm with 85 nm bandwidth (1420–1500 nm) (Spectrogon, Parsippany, NJ) and long-pass filters at 1400 nm (1400–1700 nm) and 1500 nm (1500–1700 nm) (FEL LP series from Thorlabs, Newton, NJ) were used. The illuminating light intensity was set to its maximum for 1300 nm BP, 1460 nm BP and 1500 nm BP filters and to 80% of the maximum for the 1400 nm LP filter in order to avoid oversaturation. Each measurement consisted of capturing a sequence of images at 4 frames per second for 30 seconds. For each measurement, the air nozzle and the light source were centered on the ROI, and this process was repeated for each window (6 times per sample). The dehydration setup was completely automated using LabView™ software.

2.3 Near-IR Reflectance Analysis

Near-IR reflectance images were processed and automatically analyzed using a dedicated program constructed with Lab View™ software. A 5×5 pixel ROI was specified for each window and an average measurement was recorded for each time point. The image contrast was calculated using $(I_L - I_S)/I_L$ for the final image ($t = 30$), where I_S is the mean intensity of the sound enamel and I_L is the mean intensity of the lesion. In addition to lesion contrast, the intensity difference between the final and initial images, $I(t=30)$, was calculated using $I(t=30) - I_I$, where $I(t=30)$ is the mean intensity at $t = 30$ seconds and I_I is the mean intensity prior to turning on the air nozzle.

2.4 Polarized Light Microscopy

After sample imaging was completed, 200 μm thick serial sections were cut using an Isomet 5000 saw (Buehler, IL), for polarized light microscopy (PLM) and transverse microradiography (TMR). PLM was carried out using a Meiji Techno Model 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 imbibed in water and examined in the brightfield mode with crossed polarizers and a red I plate with 500 nm retardation. PLM images were acquired at 40× magnification.

3. Results and Discussion

Figure 1 shows the visible (A) and PLM cross-sectional (B) images of one of the samples after exposure to remineralization. Windows exposed to the demineralization solution could be discriminated from sound and remineralization control windows by visual examination as shown in Fig. 1A, but the lesion, 4, 8, or 12-day remineralization windows could not be differentiated from one another.

Near-IR reflectance images acquired with the 1400 nm long-pass filter of the sample after 30 seconds of dehydration are shown in Fig. 1C. The intensity difference was recorded instead of raw intensity data due to differences in anatomical structure of the sample, i.e. variation in depth to DEJ, presence of cracks, enamel tufts and birefringence due to orientation of the enamel spindles. In addition, 10 of the 15 lesion windows exhibited a “bump” in the intensity profile as shown in Fig 2. This phenomenon was confined only to the lesion windows with the most severe lesions and can potentially be exploited to distinguish an active enamel lesion from an arrested lesion. We explored this phenomenon further by imaging with crossed polarizers, which reduced specular reflection and successfully eliminated the bump from the profile. Therefore, it is likely that the excursion in the intensity profile is due to specular reflection from the surface of the lesion. Although the light source was directed at the sample at an incident angle of approximately 60°, the highly roughened and porous surface of the demineralized enamel during the dehydration process may have deflected some specular reflection to the camera.

The 1300 nm BP filter yielded the lowest lesion contrast and the three other filters, 1460 nm BP, 1400 nm LP and 1500 nm LP, showed higher lesion contrast. It was anticipated that the wavelengths between 1400 nm and 1700 nm would yield the largest differences between windows due to increased absorption by water [11]. However, the lesion contrast measurements were highly variable due to structural variations and the intensity difference measurements were calculated to minimize this discrepancy. There was a 4-fold difference in magnitude between the sound and lesion windows for the intensity difference measurements. We plan to submit a full publication with the statistical analysis on all 15 samples in the future. In a previous study, it was shown that the state of hydration of the tooth had a profound effect on the optical transparency of sound enamel; empty pores act as scattering sites at near-IR wavelengths [11]. The highly mineralized surface layer inhibits free diffusion from the lesion body to the outer surface and the rate of loss of mobile water from the lesion is likely to be highly dependent on the thickness and mineral content of that highly mineralized surface layer.

Acknowledgments

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Fig. 1.

Visible, cross-sectional PLM and near-infrared reflectance imaging of a sample. The red dotted line in the visible light reflectance image (A) represents the position of section shown in (B). Near-infrared reflectance images with the 1400 nm long-pass filter (C) of the sample after 30 seconds of dehydration.

Fig. 2.

Typical time-near-IR reflectance intensity difference profiles over 30 seconds of the sample shown in Fig. 1 when the 1400 nm long-pass filter was used.