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Monitoring the Remineralization of Early Simulated Lesions using a pH Cycling Model with CP-OCT

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Abstract

If caries lesions are detected early enough they can be arrested by chemical intervention and dietary changes without the need for chemical intervention. Optical coherence tomography is ideally suited to monitor the changes that occur in caries lesions as a result of nonsurgical intervention, since OCT can nondestructively image the internal structure of the lesion. One of the most important changes that occurs in a lesion is preferential deposition of mineral in the outer surface zone. The deposition creates a highly mineralized and weakly scattering surface zone that is clearly visible in OCT images. Since this zone is near the highly reflective surface it is necessary to use cross-polarization OCT imaging to resolve this zone. Several CP-OCT studies have been conducted employing different remineralization models that produce lesions with varying mineral gradients. Previous studies have also demonstrated that automated algorithms can be used to assess the lesion depth and severity even with the presence of the weakly reflective surface zone. In this study we investigated the remineralization of lesions of varying severity using a pH cycling remineralization model and the change of the lesion was monitored using CP-OCT. Although the lesion depth and severity decreased after remineralization, there was still incomplete remineralization of the body of the lesion.

Keywords

polarization; optical coherence tomography; tooth demineralization; dental caries

1. INTRODUCTION

New caries imaging tools are needed to non-destructively assess lesion depth and severity, the efficacy of chemical intervention, and to serve as a likely surrogate end point for the testing of anti-caries agents in dental clinical trials¹. Several studies have demonstrated that polarization sensitive optical coherence tomography (PS-OCT) can be used to nondestructively measure the severity of subsurface demineralization in enamel and dentin both *in vitro* and *in vivo*^{2–15}. One of the most exciting applications of OCT is the ability to measure the internal lesion structure and monitor the changes that take place in lesions as they are repaired or remineralized with chemical intervention^{7, 10, 13, 16}. The structure of

caries lesions can vary markedly, particularly the mineral gradients and it is necessary to investigate the ability of OCT to measure the remineralization of all types of lesions.

We developed an approach to quantifying the severity of lesions by integrating the reflectivity in the orthogonal axis ($\perp$) or cross polarization (CP) image⁶. Longitudinal studies using this approach have demonstrated that PS-OCT or CP-OCT can be used for monitoring erosion, demineralization and remineralization^{6–13, 17}.

We have also demonstrated that automated algorithms can be applied successfully to calculate the depth of demineralization and the overall or integrated reflectivity from the zone of demineralization at the earliest stages of demineralization^{18–20}. This approach has significant advantages because PS-OCT can be used to rapidly acquire 2D and 3D tomographic images of areas of early demineralization on tooth surfaces. It is also important to demonstrate that the same automated methods used to assess artificial demineralization in CP-OCT images can be applied to lesions that have undergone remineralization.

In previous studies we investigated the remineralization of smooth enamel surfaces employing three caries models. The first model involved pH cycling to produce lesions with a well-defined surface zone of intact enamel⁹ while the 2nd model used a different demineralization model to produce a *surface softened lesion*^{21, 22}. Both models showed markedly different outcomes after exposure to the remineralization solution. Studies have shown that remineralization requires the presence of residual partially dissolved crystals to serve as a template for growth²³. Furthermore, remineralization has been observed to proceed from the outside of the lesion towards the lesion body, therefore as the remineralization takes place in the surface zone of the lesion the diffusion pathways to the lesion body are blocked thus preventing further remineralization of the lesion body. However, the lesion does become arrested since further dissolution in the lesion body is also blocked. This is typically how lesions are arrested naturally. We observed that the *surface softened lesion* model yields the greatest change in mineral content upon remineralization since it does not contain a well-defined surface layer that inhibits diffusion²³. PS-OCT images of a *surface softened lesion* before and after remineralization have shown that there was significant growth in the thickness of a layer of remineralized enamel along with a concomitant decrease in the integrated reflectivity²¹. Last year we investigated another model, the acidic pH remineralization model of Yamazaki and Margolis²⁴ which has been shown to yield more complete remineralization of the lesion body. We found that this model produced slightly better results but did not result in the complete remineralization of the lesions^{16, 25}. It has been reported that almost complete remineralization of the lesion body can be achieved for very shallow lesions on the order of only 20- μ m versus the much deeper typically greater than 100- μ m deep lesions that we have investigated. In a study of the remineralization of shallow erosive dentin lesions by Hara et al.²⁶, almost complete remineralization was achieved. In this study, we produced *surface softened* lesions using a demineralization protocol and subsequently applied a pH cycling remineralization protocol similar to that employed by Churchley et al.²⁷, albeit without the use of pooled human saliva. The objective of this study was to produce lesions of varying depth and severity, expose those lesions to a remineralization regimen and assess the degree of remineralization.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Enamel blocks, approximately 8 to 12-mm in length with a width of ~ 3-mm and a thickness of 2-mm of bovine enamel were prepared from extracted bovine tooth incisors acquired from a slaughterhouse. Each enamel sample was partitioned into six regions or windows (two sound and 4 lesion areas) by etching small incisions 1.4-mm apart across each of the enamel blocks using a laser (see Fig. 1). Incisions were etched using a transverse excited atmospheric pressure (TEA) CO₂ laser operating at 9.3-μm, Impact 2500, GSI Lumonics (Rugby, UK). The incision area also has an increased resistance to acid dissolution that serves to more effectively isolate each group²⁸. A thin layer of acid resistant varnish in the form of red nail polish, Revlon (New York, NY) was applied to protect the sound enamel control area on each end of the block before exposure to the demineralization solution. The samples were immersed in a demineralization solution maintained at 37 °C for 1,2,3,4 days at pH 5.0 composed of a 40-mL aliquot of 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 0.075 mol/L acetate with 3 mmol/L sodium azide added to inhibit bacteria growth. This surface softened lesion model, produces subsurface demineralization without erosion of the surface²⁹. The mineral loss profiles are fairly uniform in these lesions and they emulate an active lesion. Surface softened lesions were produced on ten bovine enamel blocks. The blocks were subsequently subjected to a pH regimen in which each window was subjected to 6 hr exposure to the demin solution described above followed by 17 hrs in a remineralization solution. The remineralization solution was at pH 7.0 and it was composed of a 40-mL aliquot of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, and 150 mol/L potassium chloride and 20 mol/L cacodylate. Samples were immersed in a 4000 ppm fluoride solution once each week before cycling (once for 0,1,3 day groups and twice for 8-day group) and rinsed after 5 minutes. The samples were incubated at 37°C. Samples were 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. The all-fiber OCDR system is described in reference³⁰. The PS-OCT system is completely controlled

using Labview™ software (National Instruments, Austin, TX). Image processing was carried out using Igor Pro™, data analysis software (Wavemetrics Inc, Lake Oswego, Oregon).

PS-OCT scans acquired from PM fiber based PS-OCT systems typically contain artifacts (additional peaks) due to cross-talk and the limited extinction ratio of the fiber that may confound analysis. Automated removal of such artifacts can be carried out successfully with a few extra data alteration steps after data collection. A reference a-scan was acquired from a mirror prior to scanning the samples. The reference a-scan contains several weak artifact signals along with the primary reflection. A smaller 400-point a-scan array was extracted from the 2000-pt reference a-scan containing the principal artifacts. The reference array was normalized to the intensity of the point of interest and subtracted to selectively remove the artifacts.

2.3 Calculation of Integrated Reflectivity and Lesion Depth

The integrated reflectivity, ΔR in units of ($\text{dB} \times \mu\text{m}$) was calculated for each of the four lesion areas on the samples. Previous studies have shown that ΔR can be correlated with the integrated mineral loss (volume % mineral $\times$ microns) called ΔZ ^{8, 31}.

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 microcopy with measurements using OCT in order to avoid overestimation of lesion depth due to weak signals caused by birefringence in sound enamel¹⁸. 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. Prism from GraphPad software (San Diego, CA) was used for statistical calculations.

3. RESULTS AND DISCUSSION

Figure 2 shows CP-OCT images from one of the ten samples. Linearly polarized light was incident on the sample and the reflected light was measured in the orthogonal polarization ($\perp$) to the incident light. There is little difference in the lesion severity between the 4 windows exposed to different periods of demineralization. This was unexpected and is disappointing since we anticipated lesions with increasing severity from left to right. The reflectivity of all the windows decreased after exposure to the pH cycling solution and after 3 days of cycling a distinct transparent zone was visible on many of the samples. The mean depth of the lesions ($\pm$ s.d.) measured using CP-OCT is plotted in Fig. 3 for each of the windows. All the lesions were between 50–100- μ m and there was no significant increase in lesion depth with exposure time to the demin solution. There was an apparent decrease in the lesion depth after exposure to the pH cycling but the change was small less than 25 %. Changes in the integrated reflectivity were more obvious and there was a statistically significant reduction in the integrated reflectivity for each of the four demin windows after 3 and 8-days of exposure to pH cycling. The overall decrease in the integrated reflectivity was on the order of 30%. Lesion depths and the integrated reflectivity were automatically calculated and the weakly scattering surface zone that was created after 3 and 8-days of remineralization did not interfere with those calculations.

In summary, the pH cycling regimen produced a significant reduction in the integrated reflectivity after 3 & 8 days along with the formation of a transparent surface zone of low reflectivity. However the performance did not appear to be any better than the previous remineralization models investigated.

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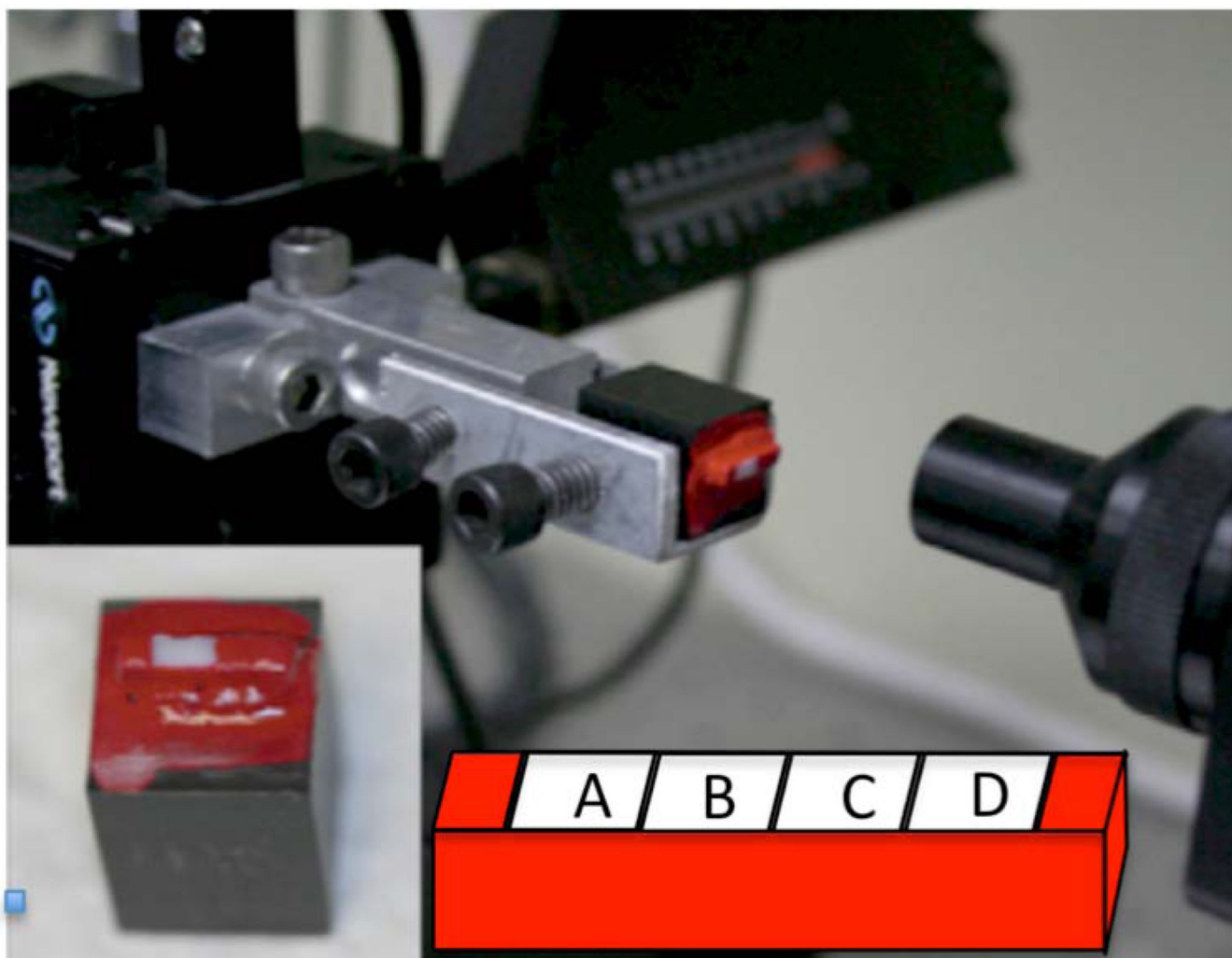


Fig. 1. Sample block connected to a jig for scanning by the PSOCT system. Inset: Sample with two exposed windows surrounded by acid resistant varnish.

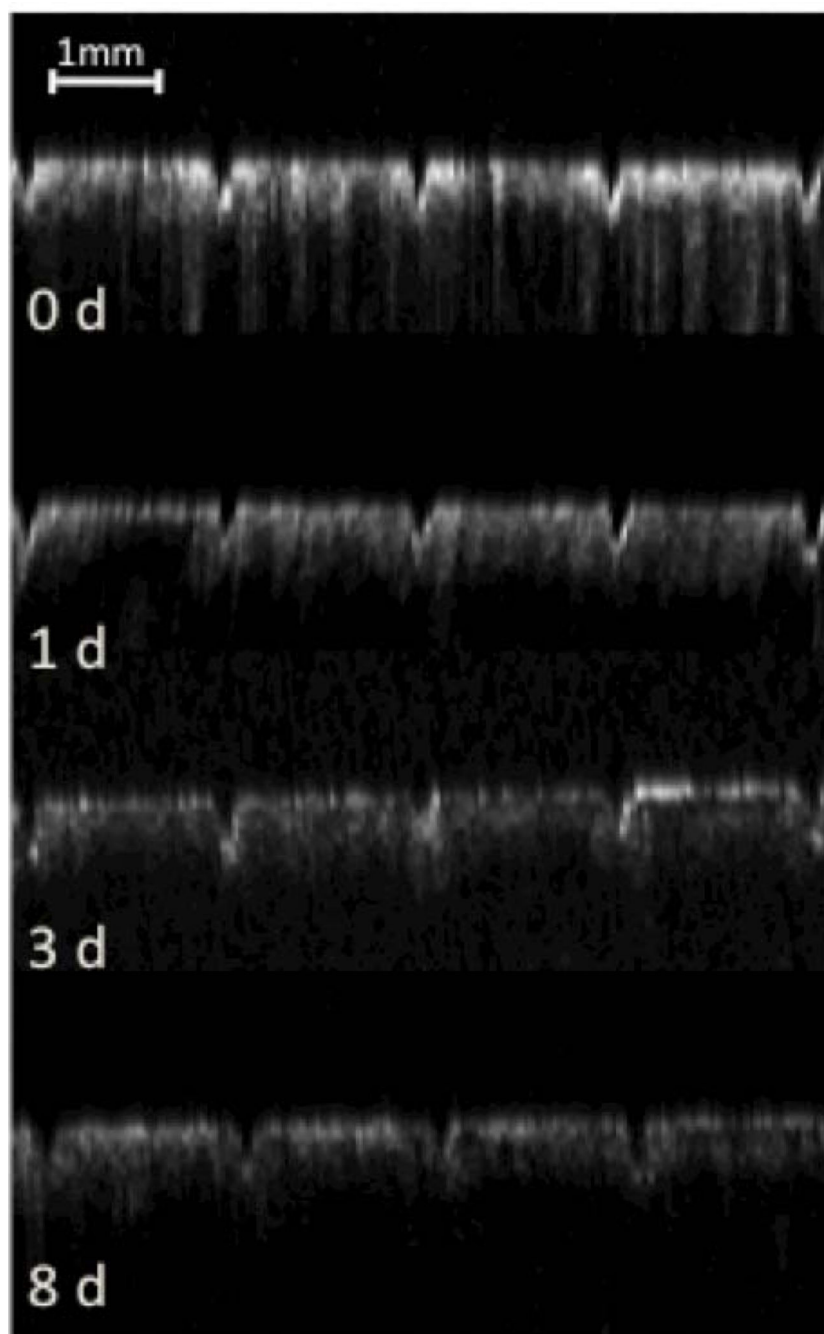


Fig. 2. CP-OCT b-scan images for one of the bovine blocks after each period of exposure to the pH cycling solution. Each of the four windows is separated by an incision and represent from left to right 1,2,3,4 days exposure to demineralization solution.

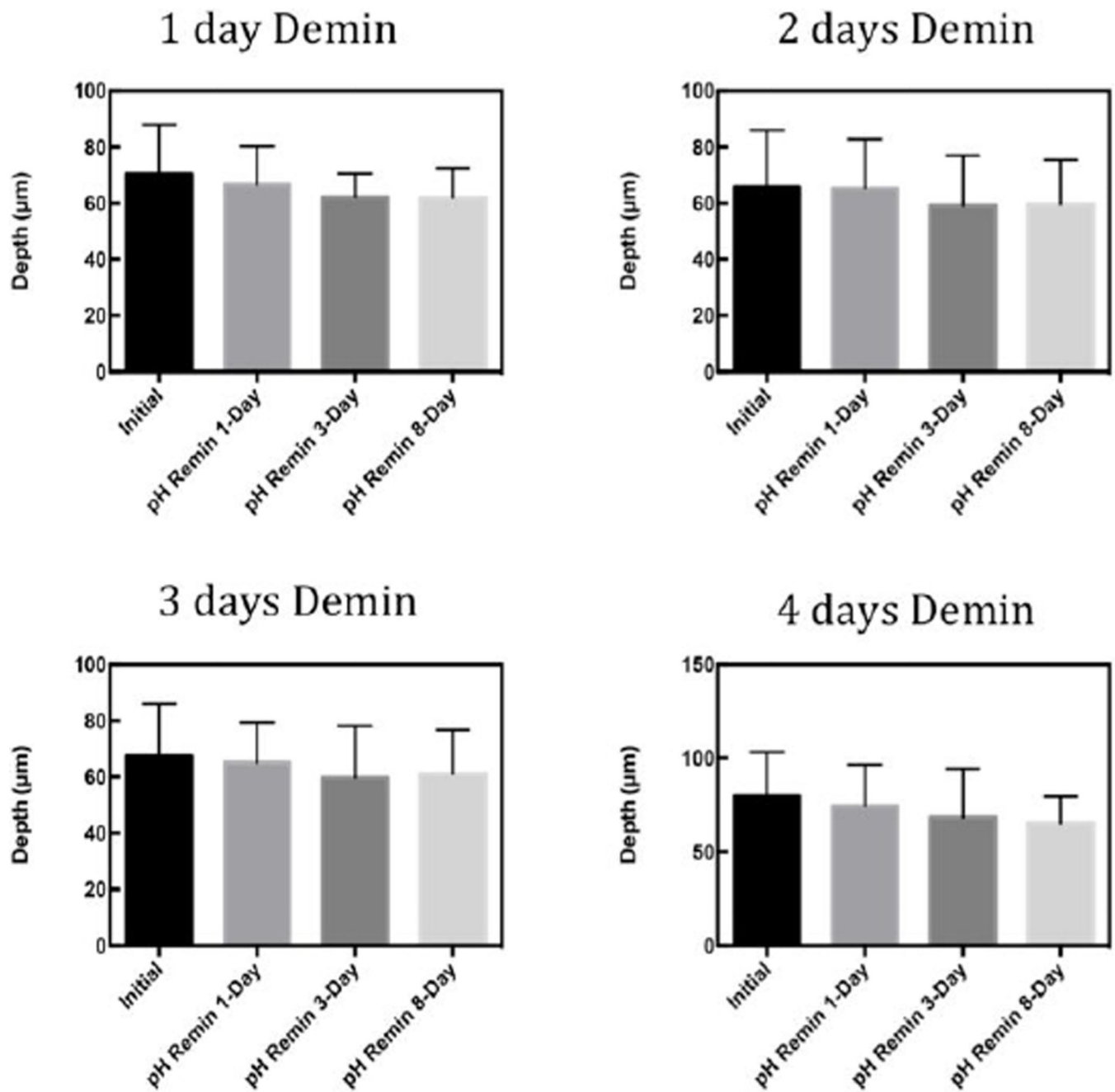


Fig. 3.

Plots of the mean lesion depth ($\pm$ s.d) for each of the four windows before and after exposure to the pH cycling regimen. Most of the groups were statistically similar and the color of the bar has no statistical significance.

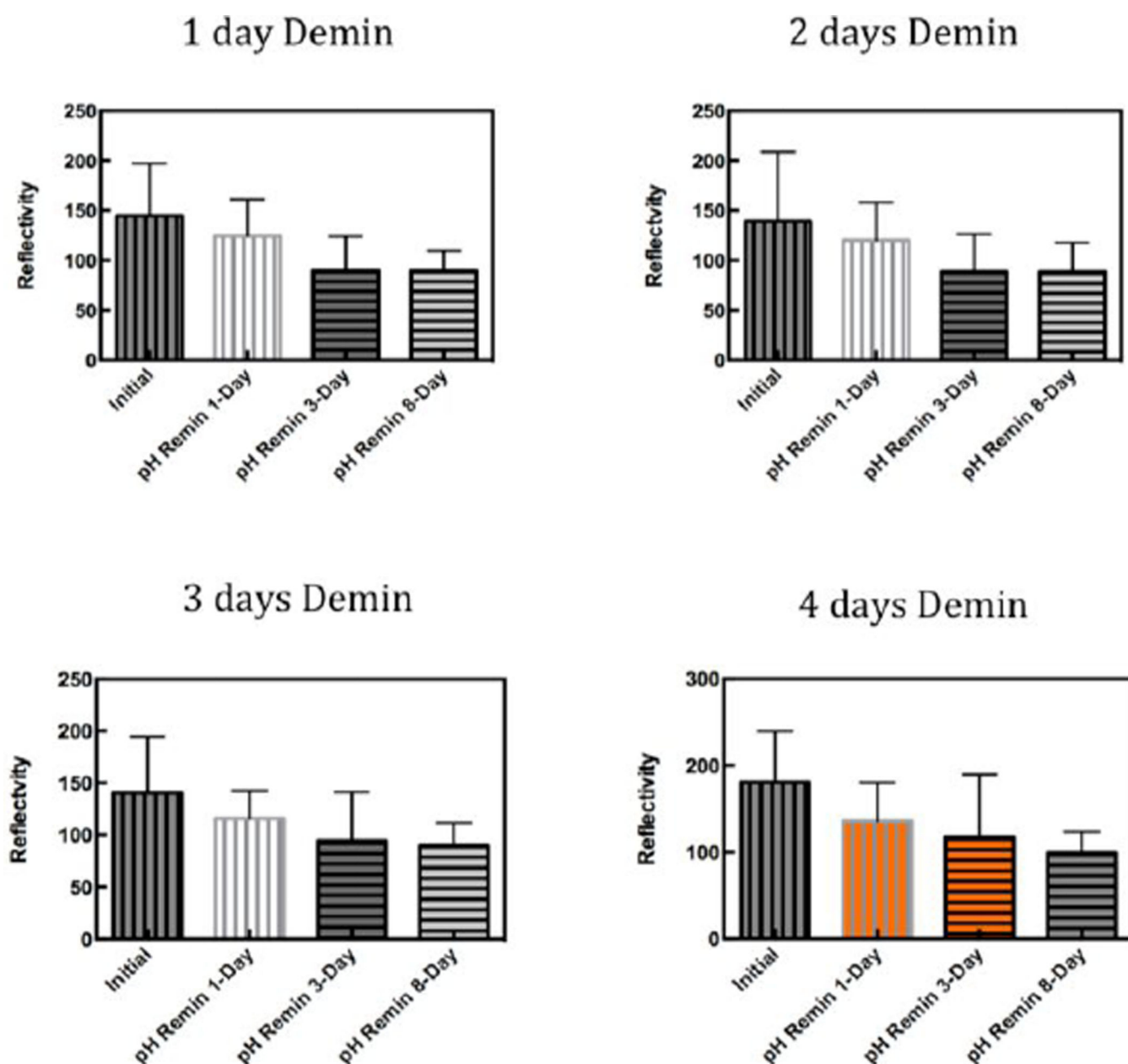


Fig. 4.

Plots of the mean integrated reflectivity ($\pm$ s.d) for each of the four windows before and after exposure to the pH cycling regimen. Groups in each plot with either the same color or pattern are statistically similar ($P > 0.05$). The pH 1 & 3 day groups in the 4 day demin plot (colored orange) are statistically similar.