

Application of Confocal Laser Scanning Microscopy for Studying Remineralization and Demineralization Processes

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ABSTRACT

Confocal Laser Scanning Microscopy (CLSM) has been identified as a nondestructive tool for viewing the subsurface topography of enamel and dentine hard tissue. This tool is of particular interest if it allows real-time assessment of demineralization and remineralization processes within these tissues. Previously, demineralization effects on subsurface enamel have been demonstrated using CLSM (Ando et. al., 1994). It was not known, however, if CLSM could focus through a lesion and into sound enamel underneath. The purpose of this study was to determine if CLSM is capable of focusing not only into but through the depth of a lesion and into the underlying sound enamel. Lesions were prepared by cutting 4mm diameter discs of human enamel from incisors which were free of visual cracks or surface imperfections (10x). Specimens were ground with silica carbide paper (600 grit), removing approximately 50 µm of the natural surface, then polished to a high luster using AB Gamma alumina (particle size < 1.0 µm). Surface hardness of specimens after polishing (Leitz microhardness tester @ 200g load) was ~360 VHN (Vickers hardness). One-half of the specimen was covered with a clear, acid resistant nail varnish. The uncovered half of the specimen was exposed to 25ml of demineralization solution consisting of 0.1M/L lactic acid, 0.2% Carbopol 907, 50% saturated with HAP, pH 5.0 for 24h at 37°C. CLSM images were taken at the junction between the bare enamel and that covered by the nail polish. CLSM images (x,y) were made at 5µm increments from 0-40µm. Changes in the porosity of the enamel were clearly visible, and the junction between exposed/unexposed sides was well defined. Images throughout the body of the lesion were easily distinguishable. At a depth of approximately 40 µm, images on each side of the junction appeared identical. CLSM images (x,z) showed clear definition of the lesion depth as well. **Results from this study indicate CLSM is capable of visualizing not only into but through demineralized lesions into the underlying enamel. This capability is critical to the usefulness of CLSM in assessing further the processes of demineralization and remineralization. Methods to quantify images are under investigation.**

INTRODUCTION

Confocal Laser Scanning Microscopy (CLSM) is a relatively new, non-destructive technique which provides three-dimensional images by means of microscopic tomography (Engelhardt and Knebel, 1993). Initially, CLSM was applied almost exclusively to cell biology. More recently, CLSM has found potential applications in caries related studies, including investigations into the demineralization and remineralization processes.

CLSM is based on the principle of eliminating stray light from out-of-focus planes by means of confocal apertures. Images are acquired by scanning the sample with a spot-sized light source (~1 µm in diameter) and by recording the light reflected from the in-focus plane. Tomography is made possible by recording a series of consecutive images in both the x-y and x-z planes. Depth movement of the sample is made possible by a fine-focusing object table, which is moved in the z-direction. CLSM allows for the study of unsectioned, naturally moist teeth. When used to visualize the outermost surface/subsurface areas, CLSM requires no sample preparation. The sample is thus free from artifacts induced by drying, cutting, or other pre-treatments that are required by other analytical techniques such as quantitative microradiography.

MATERIALS AND METHODS

Preparation of enamel specimens

Enamel specimens were prepared by cutting 4mm cores from extracted, human incisors using a diamond core drill. The teeth were stored at room temperature in a saturated thymol solution until ready for use. Enamel cores were mounted in 1/4 inch diameter Lucite rods with dental acrylic (Dura Base, Reliance Mfg. Co.). Course polishing with 600 grit silicon carbide-water slurry was used to remove approximately 50 µm of the outer enamel. Specimens were polished with gamma alumina (Linde No. 3, AB Gamma Polishing Alumina) to a mirror finish. Enamel specimens that had surface imperfections were rejected.

One half of the surface of each specimen was covered with a clear, acid resistant nail varnish to serve as a control. Each specimen was then suspended in 25ml of a solution containing 0.5M/L lactic acid, 0.2% Carbopol 907 (B.F. Goodrich, Co.) 50% saturated with respect to hydroxyapatite, pH 5.0 for 24 hours at 37°C. After demineralization, specimens were rinsed with deionized, distilled water and stored in a cool humid environment until analysis.

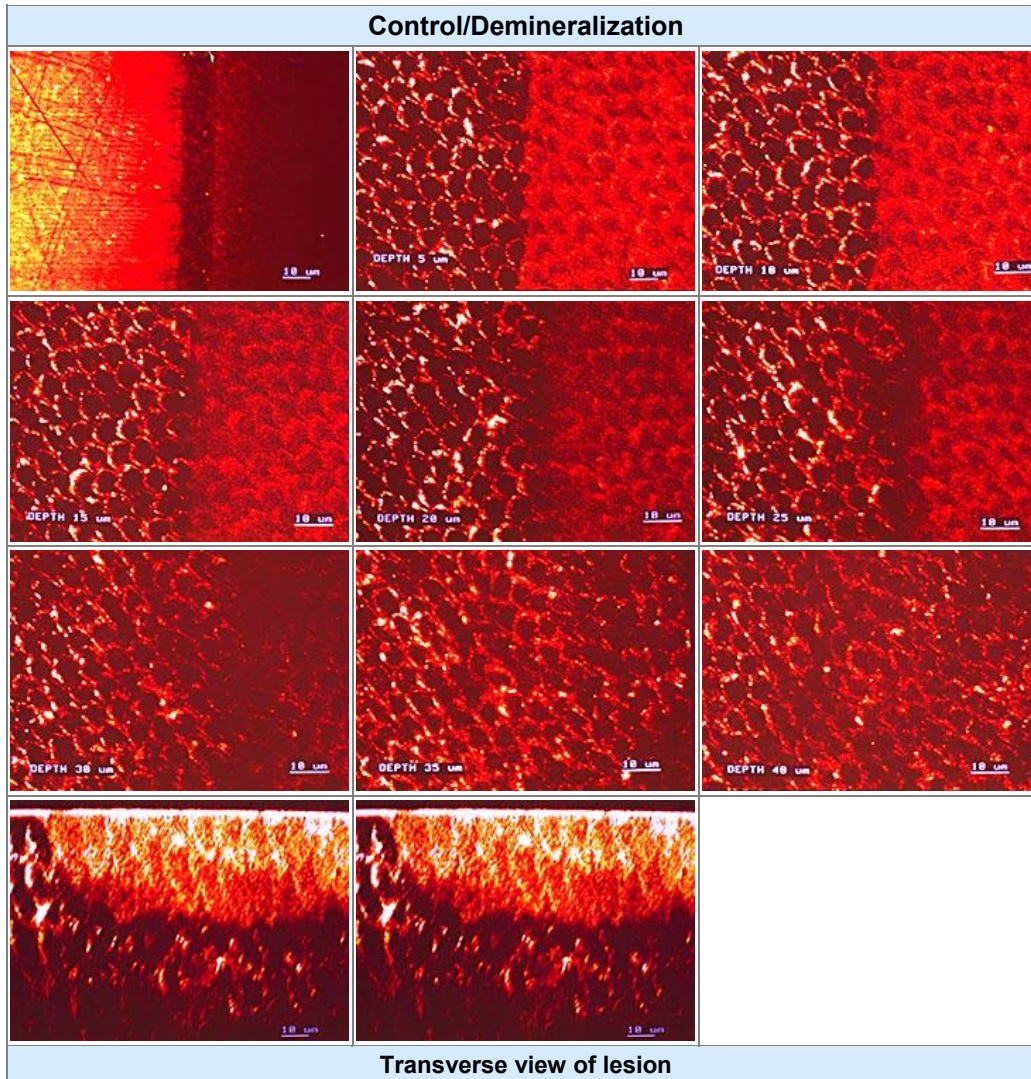
Visualization of specimens

Each specimen was mounted on a microscope slide, then placed on the microscope stage in such a way as to simultaneously visualize both control and demineralized areas of the sample. Microscopic tomographs (images parallel to the surface) of the contact zone between the demineralized and control areas were recorded to a depth of 40 µm in incremental steps of 5 µm.

RESULTS

The results of this experiment can be seen in the following series of images from a single specimen. Depth (from the anatomical surface) and scale are noted on each image. These images suggest marked differences between control (left, mineral concentrated in the interprismatic areas) and demineralized (right, mineral concentration is less and appears diffused) areas of the sample throughout the first 30µm. Beyond 30 µm the differences between control and demineralized areas are less obvious. The 40 µm image shows no difference between control and demineralization, suggesting the lesion front is located at a depth of ~35 µm. The final image is an x-z image which depicts a transverse view of the lesion.

CONFOCAL IMAGES



CONCLUSION

This experiment demonstrates that CLSM does have the ability to focus not only into but through a lesion into the underlying sound enamel. Images were taken with virtually no specimen preparation and with no tissue damage. Clearly, CLSM offers a unique perspective with respect to the process of remineralization. It's potential with regard to remineralization must still be assessed. Efforts are underway to quantify the images acquired in this experiment.