A New Metrology for Tissue Engineering: Collinear Optical Coherence and Confocal Fluorescence Microscopies

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Abstract: We have demonstrated collinear confocal optical coherence (OCM) and confocal fluorescence (CFM) microscopies to gather simultaneous structural and functional information on tissue engineered products in a registered fashion.

OCIS codes: (110.4500) Optical coherence tomography; (180.2520) Fluorescence microscopy

Tissue engineering is an emerging interdisciplinary field that "creates devices for the study, restoration, modification and assembly of functional tissues from native or synthetic sources."[1] Tissue engineered products (TEPs) often consist of a three-dimensional scaffold that provides form and foundation for the cells as they produce the tissue of interest. Successful TEPs allow cell infiltration, and foster proliferation and differentiation within the scaffold. Cell infiltration and behavior may depend on a multitude of factors intrinsic to the scaffold, including global and local structure, surface composition, and other physical properties. While it is generally understood that a complex interaction of many variables influences the success of the TEPs, the precise nature of these interactions has yet to be worked out in many instances. A significant difficulty in furthering the understanding of the interaction between these factors and cell behavior is the lack of a high-resolution imaging technique that can penetrate deeply into the scaffold. Towards this need, we constructed a collinear optical coherence (OCM) and confocal fluorescence microscope (CFM) to non-invasively monitor both structure and function in a TEP and have volumetrically imaged a cultured tissue scaffold.

Figures a) and b) are volumetric images of a cultured tissue scaffold, and were obtained respectively with the OCM and CFM channels of the instrument. The scaffold, comprised of semi-crystalline poly(ε-caprolactone) (PCL) with about 50% porosity, was cultured with fetal chick osteoblasts for 10 weeks and then stained with nuclear fast red. Both volumetric images are composites of 70 planar images, each plane of data acquired at (4.0 ± 0.1) μm intervals through the thickness of the sample (to a maximum depth of 280 μm). We expect signal in the OCM channel from surface reflections of the scaffold, cells, and mineralized matrix deposited by the cells. In principle, signal in the CFM channel comes exclusively from fluorescence emitted by the nuclear dye.

Without the aid of the CFM image, it is difficult to ascribe reflectance levels or patterns in the OCM image to particular species in the scaffold. By comparing these two images, we can begin to discern cell-rich and matrix-rich areas within the scaffold. The osteoblasts seem to line the pore walls, as expected, and the smooth texture in image a) is probably due to reflectance from the scaffold itself. Although it is difficult to say with certainty, it appears that the matrix has higher reflectance than do the cells, as suggested by the matching pairs of arrows. In both cases, the dashed arrows point to what appears to be mineralized matrix, while the solids arrows point to regions of high cell concentration. The discrimination is made by the relative presence and lack of fluorescence signal in panel b).

We have demonstrated the use of a combined OCM / CFM for imaging tissues and cells in TEPs and have shown that by using these complimentary imaging modalities, we can detect the presence of biological tissues growing deep within the TEP. To our knowledge, this is the first application of dual mode CFM and OCM in tissue engineering. Future work will involve assignment of cell and extracellular matrix components from these images and the influence of scaffold structure or culture conditions on cell behavior.

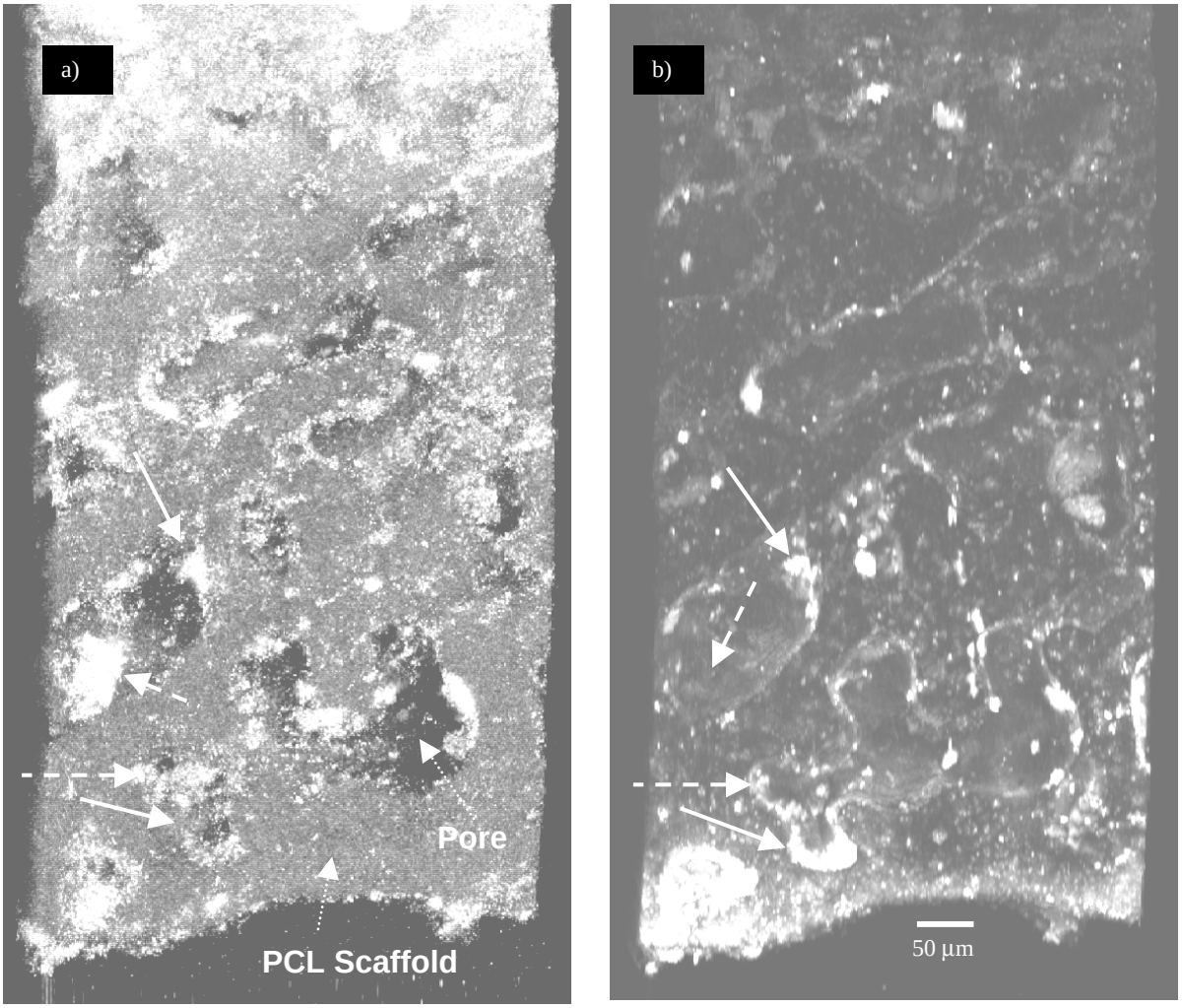

Fig. Volumetric renderings of 70 single-plane images obtained from a) OCM and b) CFM on the PCL scaffold, containing stained osteoblasts and bone matrix. Dashed and solid arrows indicate examples of suspected areas of bone matrix and cell, respectively.

Reference

1. J. M. Anderson, L. G. Cima, S. G. Eskin, et al., "Tissue Engineering in Cardiovascular Disease-A Report," J. Biomed. Mater. Res. **29**, 1473-1475 (1995).