Optical Coherence Microscopy for *in-situ* Monitoring of Cell Growth in Scaffold Constructs

Cicerone, M.T. , Dunkers, J.P., Washburn, N.R., Landis, F.A., Cooper, J.A.
National Institute of Standards and Technology, Polymers Division, Gaithersburg, MD, USA

It is generally understood that a complex interaction of many variables influences the success of cell infiltration, proliferation, and differentiation within a tissue scaffold. One characteristic that has a large influence on the development of functioning tissue is the three-dimensional morphology of the scaffold itself. Quantification of morphological characteristics of the pores, such as volume, size distribution, connectivity, tortuosity, local curvature and composition, and comparison of those quantities with local cell viability will undoubtedly play an important role understanding the rich interaction between scaffold and cell.

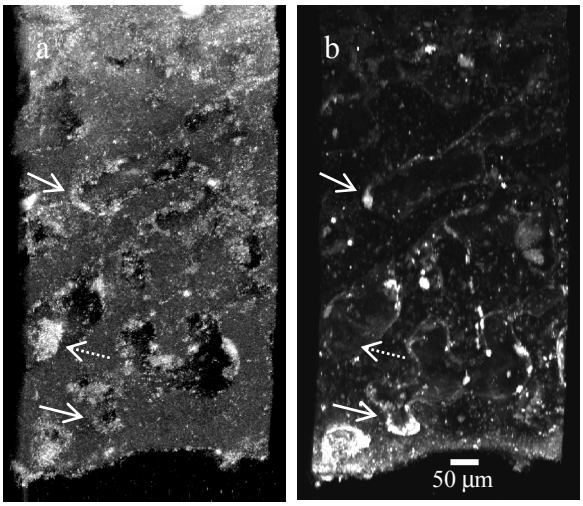
Traditional endpoint measurements of scaffold – cell interactions make it difficult to establish causal relationships because the history and future of cells in a particular region of a scaffold must be assumed; there is no temporal record. Non-invasive, *in situ*, imaging of tissue and scaffold would allow temporal correlations to be made between cell behaviour and local scaffold parameters such as geometry.

A non-invasive method for scaffold characterization and, ideally, simultaneous cell monitoring, is needed. Nuclear magnetic resonance (NMR) has been used for 3D imaging of biomaterials and tissues, but spatial resolution attainable by NMR methods is typically about 10 μm; insufficient for imaging at the cellular level. Some work has been reported on scaffold characterization using laser scanning confocal microscopy (LSCM) [1]. This method gives sufficient resolution but the image depth is limited to ≈ 80 μm in more opaque systems, due to large background signals from scattered light.

Optical coherence tomography (OCT) is a photon time-of-flight method that has both excellent background rejection capability against light scattered out-of-plane, good dynamic range (> 100 dB), and good sensitivity (≈ 1 pW reflected light), making it ideal for obtaining image data deep in highly scattering media. Izatt et al. [2] demonstrated that when an OCT instrument is configured with confocal optics, the resulting optical coherence microscope (OCM) gives the background rejection, sensitivity, and dynamic range of an optical coherence tomography instrument, and also excellent spatial resolution inherent in a confocal microscope.

We demonstrate that an OCM, coupled with confocal fluorescence (CFM) detection channel can be used to perform non-destructive imaging of a polymer TE scaffold containing osteoblasts to depths of 250 μm. We demonstrate that the combination of the techniques shows promise for *in situ* measurements of cell growth in a bioreactor, even for highly opaque TE scaffolds.

The micrographs below are volumetric renderings of 70 single-plane images obtained from a) OCM and b) CFM on a cross-sectioned poly (ε-caprolactone) tissue scaffold, containing stained osteoblasts and bone matrix. Only stained cells show up in micrograph b, and comparison of the two images reveals that scaffold, cell, and bone material each appear to scatter near-IR light differently, which results in distinct signal levels as seen by OCM. Dashed and solid arrows indicate examples of suspected areas of bone matrix and cells respectively.



In cases where the cells under investigation have not been programmed to express fluorescent protein, or cannot be fluorescently tagged without altering their function, an *a priori* knowledge of characteristic OCM response levels from extracellular matrix (ECM), cells, and scaffold makes non-invasive imaging possible.

We demonstrate use of the OCM / CFM instrument to investigate the relationship between local pore geometry and cell growth and ECM deposition. The scaffolds we use are prepared by blending equal weights of poly(ε-caprolactone) (PCL) with poly(ethylene oxide) (PEO) in a twin-screw extruder. This forms a bicontinuous, two-phase material with micrometer-sized domains, and selective dissolution of the PEO with water results in a porous material. We vary the average pore size of the TE scaffolds in the range of (50 μm to 200 μm) by annealing them for differing amounts of time. A more detailed description of scaffold preparation is provided elsewhere [3].

1. M. Attawia, J. Devin, abd C. Laurencin, J. Biomed. Mat. Res, **29**, 843 (1995).
2. J. Izatt, M. Kulkarni, H.-S. Wang, K. Kobayashi, and M. Sivak, IEEE J. Sel. Top. Quant. Elect. **2**, 1017-1028 (1996).
3. N. Washburn, C. Simon, A. Tona, H. Elgendy, A. Karim, and E. Amis, J. Biomed. Mat. Res. **60**, 20-29 (2002).