**Probing Multiscale Cell-Biomaterial Interactions via Confocal/Multiphoton Imaging and X-ray μC-Tomography**

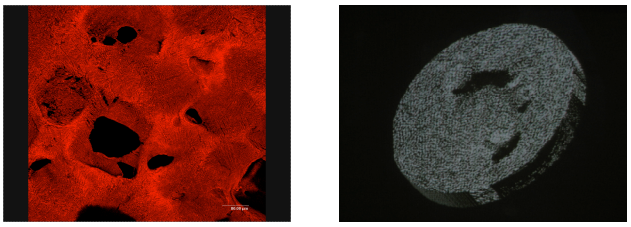
Matthew D. Treiser, Patrick Johnson, Robert A. Dubin, Aarti Rege, David Denhardt, Joachim Kohn and Prabhas Moghe.
Department of Biomedical Engineering, Department of Chemical and Biochemical Engineering, Department of Cell Biology and Neuroscience, and The New Jersey Center for Biomaterials, Rutgers University, Piscataway, NJ 08854.

**Statement of Purpose:** The underlying substrate chemistry, biomaterial architecture and mechanical properties are crucial mediators of cell-polymer interactions. Using scaffolds fabricated from tyrosine derived degradable polycarbonates copolymerized with poly(ethylene glycol) and selectively iodinated to allow radioopacity, we seek to elucidate the effect of variable chemical structure and architecture of polymeric scaffolds on the attachment, proliferation, and phenotypic expression of osteoblastic cells. The novelty of this study is its incorporation of complementary multi-scale imaging modalities such as multi-photon microscopy (MPM), confocal laser-scanning microscopy (CLSM) and Micro-Computed Tomography (MicroCT), which will allow the elucidation of the macro, meso and microstructure of scaffolds, as well as the in situ morphometric and biomineralization responses of cells.

**Methods:** Poly(DTE-DTO carbonate) blends and Poly($I_2$ DTE carbonate) scaffolds containing macropores (200-400μm) and micropores (10-20μm) were fabricated using a combination of porogen leaching and solvent phase separation. CLSM and MPM were used to image the microscale architecture of Texas-Red doped scaffolds. X-ray MicroCT (BIR©) of Poly($I_2$DTE carbonate) scaffolds was utilized to examine scaffold macroarchitecture. A manifold was used to incorporate the scaffolds and was seeded with Human SAOS-2 osteoblast cells (100,000 cells/mL) via perfusion at a rate of 1.5mL/min. Osetoblast viability was assessed as a function of time using Calcein-AM and Ethidium Homodimer. In selected studies, genetically engineered cells transfected with pGFP-paxillin (A. Horwitz, University of Virginia) or pGFP-actin (Clontech) using Lipofectamine (Invitrogen) are used to evaluate cellular cytoskeletal organization.

**Results / Discussion:** Scaffold architecture was assessed using Texas Red dyed poly(DTE-DTO Carbonate) scaffolds(Fig. 1A). Imaging of the scaffolds with multi-photon microscopy (MPM) resolved the multiscale dimensions of scaffold architecture demonstrating the presence of both macropores and micopores. MicroCT of the Poly($I_2$DTE carbonate) (Fig. 1B) scaffolds demonstrated the macroporous structure of the scaffold, including pore size and connectivity. Optical sectioning and 3D reconstruction via confocal microscopy confirmed the uniform spatial seeding of osteoblasts within the porous polymer scaffold. A majority of substrate-adherent osteoblasts was viable 96 hrs post–seeding (Fig. 2). Simultaneous imaging in fluorescence and reflectance modes allowed for in situ visualization of both osteoblasts and polymer scaffolds respectively. Preferential adhesion of osteoblasts to the macropore wall of the scaffold was observed. Using cells engineered to express green fluorescent protein fused to actin and paxillin, alterations in cytoskeletal architecture following growth on polymer films was explored. In relation to cells on polycarbonates, the actin stress fibers and paxillin organization were less prominent in cells grown on PEG-containing polymers, where cells exhibited a more rounded morphology.

**Conclusions:** Our studies exploit the complementary size scales via confocal and multi-photon imaging and MicroCT as modalities for the detailed analysis of scaffold architecture at the macroscopic, mesoscopic and microscopic levels. Analysis of living cells growing on polymer surfaces was demonstrated, providing a setup for the in situ study of cell morphometric and phenotypic responses to biomaterials. Our early data suggests that the addition of PEG in the backbone will decrease cell attachment and proliferation, of cells, but increase metabolic activity and differentiation[1], which, in turn, may be offset by the minute incorporation of iodine. In addition, the use of genetically engineered cells may provide a means of visualizing alterations in cytoskeletal elements in living, non-fixed, cells on polymer scaffold surfaces to further elucidate how polymer architecture and chemistry mediate cellular responses.



**(A)** **(B)**

**Figure 1.** (A) MPM image of Poly(DTE-DTO carbonate) blend doped with Texas Red. (200X) (B) Three-dimensional rendering of Poly($I_2$DTE carbonate) from MicroCT image. Image sample is 8mm in diameter and 1.4 mm in thickness.

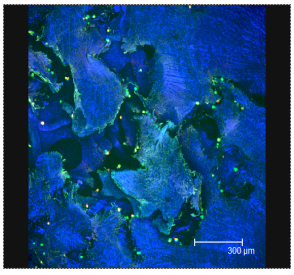


**Figure 2.** Osteoblasts seeded on poly ($I_2$DTE carbonate) and stained with calcein AM( green) and ethidium homodimer-1 (red). The scaffold (blue) was imaged in reflection mode. The image is a maximum projection image from fifty individual slices taken every ten microns (100x)

**References: (1)** Göpferich A. et al. (1999) J Biomed Mater Res. 5;46(3):390-8

**Acknowledgement:** This work is supported by RESBIO-The National Resource for Polymeric Biomaterials' funded under NIH grant EB001046 and various internal funding programs at Rutgers University.