Quantifying Individual Cell Migration and Contraction Behavior in a Series of Well-Characterized Collagen-Glycosaminoglycan Scaffolds

B.A. Harley[1], H.-D. Kim[1], M.H. Zaman[1], I.V. Yannas[1], L.J. Gibson[1]
[1]Massachusetts Institute of Technology, USA

Statement of Purpose: Porous, tissue engineering scaffolds serve as an analog of the extracellular matrix; the scaffold acts as an insoluble regulator affecting cell processes such as motility and contraction. Previous quantitative study of individual cell migration speeds and traction forces on flat substrates has shown that substrate stiffness significantly modifies cell behavior [1]. The average cell contraction force generated within collagen-glycosaminoglycan (CG) scaffolds, calculated by measuring gross morphological changes in scaffolds seeded with millions of cells, has previously been reported to be 1 nN [2]. Quantitative study of individual cell behavior within a three-dimensional construct requires understanding the structural and mechanical environment of individual cells through accurate characterization of the scaffold compositional, microstructural, and mechanical properties. The objective of this study was to use two series of CG scaffolds independently varying mean pore size and stiffness [3], to study the effect of scaffold mean pore size and stiffness on individual cell migration speed and contractile force.

Methods: *Scaffold Fabrication:* CG scaffolds were fabricated via lyophilization from a slurry of type I collagen and chondroitin-sulfate in acetic acid. The CG suspension was frozen and the ice content sublimated using a technique developed to produce homogeneous pore structure with equiaxed pores [4,5]. The final freezing temperature was varied to produce a series of uniform scaffolds with equiaxed pores, constant composition and relative density (0.6%), but with distinct pore sizes (151, 121, 110, 96 μm) [4,5]. All scaffolds were then crosslinked via a standardized dehydrothermal (DHT) crosslinking procedure (Temp.: 105^{o}C, Time: 24 hours, Pressure: <50 mTorr) [4,5]. Mechanical characterization of the scaffolds demonstrated mechanical isotropy; the hydrated scaffolds have a compressive modulus of 208±41Pa independent of pore size and individual struts have a modulus of 5.8±1.1MPa. Scaffold stiffness can be modified independent of pore size by modulating crosslinking density. Carbodiimide (EDAC) crosslinking was utilized to increase scaffold stiffness relative to DHT crosslinking [6]. Two EDAC intensities were utilized: EDAC 1:1:5 (2.0x stiffer than DHT) and EDAC 5:2:1 (7.2x stiffer).

Cell Tracking: Time-lapsed 3D confocal microscopy was used to track individual cell migration within the scaffold and to measure individual strut deformation during cell-mediated contraction. NR6 mouse fibroblasts were fluorescently labeled for 20 minutes using a 1:1000 dilution of CMFDA CellTracker Green (Molecular Probes). Scaffolds were fluorescently labeled using a 1:2000 dilution of Alexa Fluor 633 (Molecular Probes). 1x10^{5} cells were seeded into 6 mm (dia.) scaffold disks. The scaffold was then imaged using the Perkin Elmer Ultraview Live Cell Imager at 15 minute intervals for 10 hours using a heated (37^{o}C), 25x oil-immersion objective. A three-dimensional image rendering software package (Imaris XT, Bitplane AG) was used to calculate individual cell centroid displacement over time and individual strut deformation. Combining strut mechanical characterization data with strut deformation data during contraction allowed quantification of individual cell-mediated contractile forces generated in the scaffold.

Results / Discussion:

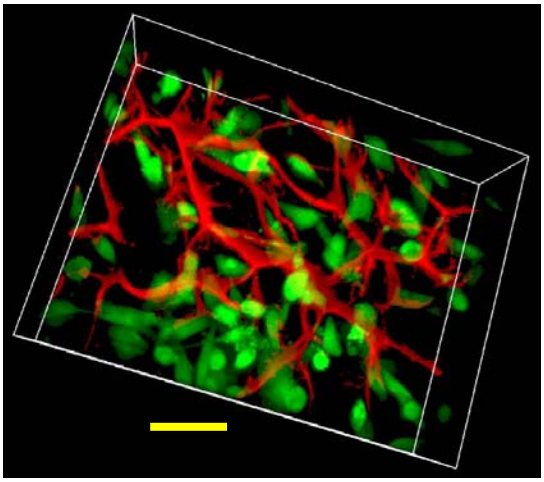

Fig. 1. Three-dimensional imaging of NR6 fibroblasts (green) within the CG scaffold (red). Scale bar: 50 μm

Time-lapsed images of individual cell position and behavior as well as scaffold geometry was observed (Fig. 1). Individual NR6 fibroblasts generate contractile forces of 30-100 nN in CG scaffolds. NR6 fibroblast were observed to migrate through the scaffold with an average migration speed of 0.34 μm/min. Continuing work is quantifying the effect of scaffold pore size independent of stiffness and scaffold stiffness independent of pore size on cell-mediated contraction and migration.

Conclusions: Experimental procedures have been developed allowing quantification of individual cell behavior within a series of well-characterized CG scaffolds. Migration speeds were observed to be of similar magnitude as those reported for studies of cell motility on 2D membranes [1]. Measured cell-mediated contractile forces were significantly larger than those previously reported in CG scaffolds [2], predominantly due to the fact that previous studies reported average values calculated using the assumption that all cells contracted at the same time and in a single direction.

References: [1] Lo CM, Wang HB, Dembo M, Wang YL. Biophys J, 79, 2000. [2] Freyman TF, Yannas IV, Yokoo R, Gibson LJ. Biomaterials 22, 2001. [3] B.A. Harley, F.J. O'Brien, I.V. Yannas, and L.J. Gibson, Trans. Soc. Biomater. 30, 2004. [4] F.J. O'Brien, B.A. Harley, I.V. Yannas, L.J. Gibson, Biomaterials, 26, 2005. [5] O'Brien FJ, Harley BA, Yannas IV, Gibson LJ. Biomaterials 25, 2004. [6] Lee CR, Grodzinsky AJ, Spector M. Biomaterials 22, 2002.