Cell Migration and Contraction Behavior in Collagen-GAG Scaffolds: Influence of Microstructure and Stiffness

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Statement of Purpose: Cell migration and contraction are critical in physiological and pathological processes such as embryonic development, metastatic tumor behavior, and wound healing. While 2D systems are appropriate for studies of some aspects of cell motility, it is critical to also examine the influence of 3D construct parameters on cell motility and contractility. Quantitative study of the behavior of individual cells within a 3D ECM analog requires understanding the local environment of individual cells through accurate characterization of the scaffold chemical composition, porous microstructure, and mechanical properties. The objective of this study was to use two series of collagen-GAG (CG) scaffolds independently varying mean pore size and stiffness [1], to study their independent effect on cell motility and individual cell-generated contractile force.

Methods: CG scaffolds were fabricated via lyophilization from a suspension of type I collagen and chondroitin 6-sulfate in acetic acid [2,3]. A series of uniform scaffolds with a uniform pore microstructure and equiaxed pores, but distinct pore sizes (151, 121, 110, 96 μm) were used [2,3]. All scaffolds were crosslinked via dehydrothermal (DHT) crosslinking (105^{o}C, 24 hours, <50 mTorr) [2]. The scaffolds had a compressive modulus of 208±41Pa independent of pore size and were mechanically isotropic. Three carbodiimide crosslinking treatments were used to increase CG scaffold stiffness over an order of magnitude independent of microstructure (pore size) [4,5].

NR6 mouse fibroblasts and DU-145 prostate cancer cells were fluorescently labeled with 10 μM CMFDA Cell Tracker (Molecular Probes); CG scaffolds were fluorescently labeled with 2 μM Alexa Fluor 633 (Molecular Probes). NR6 or DU-145 cells were seeded into 6 mm dia. scaffold disks and imaged using a Perkin Elmer Ultraview Live Cell Imager at 15 minute intervals for 10 hours using a heated (37^{o}C) 25x oil-immersion objective. Scaffold disks with independently varied pore microstructure, stiffness, cell type, and cell seeding density were analyzed ($n \geq 3$ biological samples per group). A 3D image rendering software package (Imaris XT, Bitplane AG) was used to determine cell centroid displacement over time to calculate cell speed and persistence. Combining strut mechanical characterization data with the observed strut deformation during contraction allowed calculation of the contractile forces generated by individual cells within the scaffold using cellular solids modeling techniques to describe individual strut buckling within the greater scaffold microstructure.

Results/Discussion: We calculated that individual fibroblasts generated contractile forces of 11-34 nN (26 ± 13 nN). NR6 and DU-145 cells migrated through the scaffold with average speeds between 4 and 13 μm/hour; a significant effect of scaffold pore size, stiffness, cell seeding density, and cell type on cell speed was observed. A biphasic relationship was observed between scaffold stiffness and cell speed, as previously shown in other 2D and 3D experimental and computational systems. Most interesting, population motility as well as individual cell speed and directional persistence decreased significantly with increasing pore size even though the pore sizes were significantly larger than the cells. Cellular solids modeling techniques and further analysis of individual cell persistence through the scaffolds were used to investigate the influence of individual strut mechanics and the junctions between struts that define the scaffold microstructure on cell motility. This analysis suggests that strut junctions and contact guidance along the scaffold struts are critical features in regulating cell speed and persistence through the 3D scaffold microstructure.

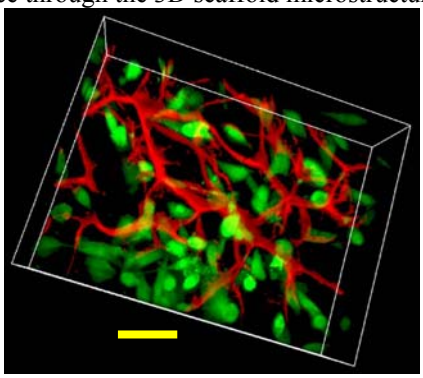

Figure 1. NR6 fibroblasts (green) in the CG scaffold (red). Scale bar: 50 μm

Conclusions: A robust system has been developed allowing analysis of the independent influence of scaffold microstructure and mechanics on cell behavior in 3D. A new technique has been introduced to measure cell contraction events in 3D constructs. Cell migration speeds in the CG scaffolds were of a similar magnitude as those reported for 2D and 3D studies of cell motility. Cell migration through the CG scaffolds is significantly influenced by scaffold stiffness and microstructure; junctions between the struts that define the scaffold microstructure appear to be particularly important in influencing cell migratory behavior and persistence.

References:

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