QUANTIFYING CELL MIGRATION AND CONTRACTION BEHAVIOR IN A SERIES OF CHARACTERIZED COLLAGEN-GAG SCAFFOLDS

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Quantitative study of individual cell behavior within a three-dimensional construct 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 migration speed and individual cell contractile force.

CG scaffolds were fabricated via lyophilization from a slurry of type I collagen and chondroitin 6-sulfate in acetic acid [2,3]. A series of uniform scaffolds with homogeneous pore structure and equiaxed pores, constant composition and relative density (0.6%), but with distinct pore sizes (151, 121, 110, 96 μm) have been produced [2,3]. All scaffolds were crosslinked via dehydrothermal (DHT) crosslinking (105^{o}C, 24 hours, <50 mTorr) [2,3]. Mechanical characterization demonstrated scaffold isotropy; the hydrated scaffolds have a compressive modulus of 208±41Pa independent of pore size. Scaffold stiffness can be modified independent of pore size by modulating crosslinking density. Two carbodiimide (EDAC) crosslinking intensities were utilized to increase scaffold stiffness relative to DHT crosslinking [4]: EDAC 1:1:5 (2.0x stiffer than DHT) and EDAC 5:2:1 (7.2x stiffer).

NR6 mouse fibroblasts and DU-145 prostate cancer cells were fluorescently labeled with CMFDA CellTracker Green (Molecular Probes) and the CG scaffolds were fluorescently labeled with Alexa Fluor 633 (Molecular Probes). The cells (NR6 or DU-145) were seeded into 6 mm (dia.) scaffold disks and imaged using the Perkin Elmer Ultraview Live Cell Imager at 15 minute intervals for 10 hours with a heated (37^{o}C) 25x oil-immersion objective. A three-dimensional image rendering software package (Imaris XT, Bitplane AG) was used to determine individual cell centroid displacement over time to calculate cell migration speed and to determine individual strut deformation during cell-mediated contraction. Combining strut mechanical characterization data with strut deformation data during contraction allowed calculation of individual cell-mediated contractile forces generated in the scaffold.

A robust system has been developed allowing analysis of cell behavior within well-characterized scaffolds that allow independent manipulation of microstructure and mechanics. Individual NR6 fibroblasts generate contractile forces of 30-100 nN. Cells were observed to migrate through the scaffold with an average speed between 4 and 15 μm/hour; a significant effect on cell migration speed of scaffold mean pore size, stiffness, cell seeding density, and cell type was observed. Migration speeds were of a similar magnitude as those reported for studies of cell motility on 2D membranes [5]. Measured cell-mediated contractile forces were significantly larger than those previously reported in CG scaffolds, likely because previous studies reported average values calculated from cell populations in which not all cells were actively contracting rather than values from individual cells.

References:

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