Development of a Collagen-GAG Scaffold Microarray for High-Throughput Analysis of Cell Fate Decisions

Emily A. Gonnerman, Lisa M. McGregor, Brendan A. Harley.
Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana IL 61801

Statement of Purpose: Recent advances in genomics, proteomics, and systems biology have been enabled by combinatorial methods that permit high-throughput, multiscale analyses; however, no equivalent 3D, combinatorial biomaterials exist to probe the relationship between extrinsic signals and cell fate. We are developing homologous series of scaffolds, "scaffold microarrays," in order to systematically investigate the microenvironmental regulation of cell behavior. Each node of the array is a scaffold that presents a microenvironment with defined pore size/geometry, stiffness, and chemistry. The purpose of this investigation was to validate the array methodology by varying the scaffold microstructure within a single array chip and analyzing the effects on cell attachment rates for varied seeding densities.

Methods: Scaffolds were fabricated from an acidic suspension of type I collagen (0.5 wt.%) and chondroitin sulfate via a freeze-drying process[1]. The suspension was placed in a prototype array mold consisting of a single chip with holes of 96 well plate geometry and spacing; the mold's removable base was divided into two areas with differing thermal conductivities ($k_1/k_2 \approx 870$). The disparate local heat transfer during freezing resulted in the creation of two distinct microstructures with maximum average pore sizes of 98±6 μm. Osteoblastic MC3T3-E1 cells were seeded onto the scaffolds at constant volume/varied cell number and cultured in the array chip for 48 hours.

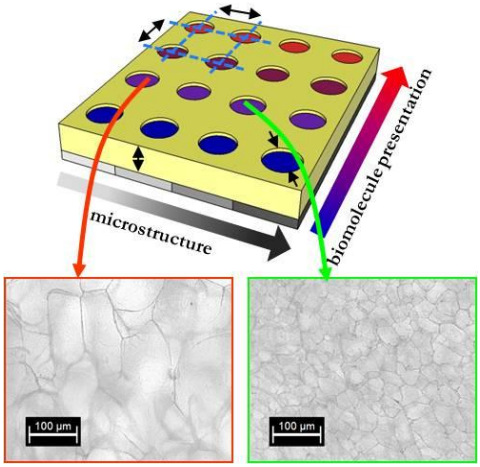

Figure 1. Schematic of array mold with varied microstructure; representative contrast phase images of resultant scaffold microstructure.

Scaffolds were rinsed briefly in PBS to remove non-adhered cells. Total cell number and percent cell attachment were determined by incubating each scaffold in 450 μL culture medium (α-MEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin) and 50 μL of alamarBlue labeling solution (Molecular Probes, Eugene, OR) on a shaker at 37˚C for 4 hours; the absorbance values were measured at 570 nm using a plate reader and related to calibration curves performed with cells of the same passage number as the samples. In addition, cellular attachment to the scaffolds in the array chip was examined in situ via multiphoton confocal microscopy; viable cells were labeled with calcein, while scaffold struts were labeled with AlexaFluor 633 (Molecular Probes).

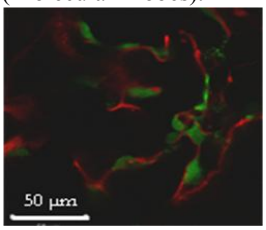

Figure 2. Confocal image of HL-1 cell (calcein, *green*) attachment to collagen-glycosaminoglycan scaffold (*red*).

Results: For the seeding densities examined on scaffolds of the same average pore size, significant differences in total cell number were apparent after 48 hours, but there were no significant differences in the cell attachment rates. This is consistent with previous cell attachment studies performed with macroscale scaffolds[2].

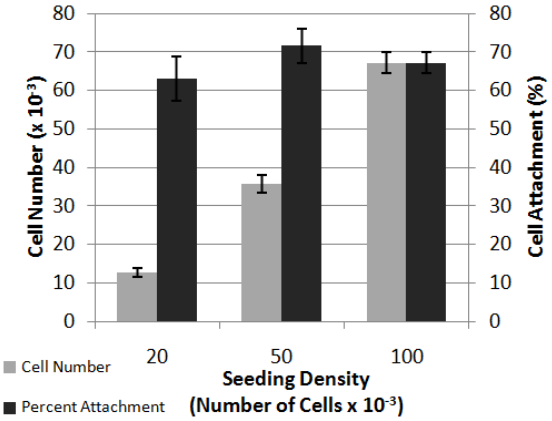

Figure 3. MC3T3-E1 cell number and attachment after 48 hours of culture in scaffold array with constant pore size.

Conclusions: Overall, the initial results show promise in developing scaffold microarrays with systemically varied microenvironments. Thus far, we are able to produce scaffolds with varied average pore sizes within a single array chip. We have also demonstrated that while the total number of cells that attach to the scaffolds varies with initial seeding density, the cell attachment rate is constant for scaffolds with the same average pore size.

References:

1) O'Brien et al. Biomaterials. 2004;25:1077-1086.
2) O'Brien et al. Biomaterials. 2005; 26:433-441.