Microfabricated Polymer Scaffolds for Spatially Patterned Multi-lineage Differentiation of Stem Cells

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Statement of Purpose: A major goal in the field of tissue engineering is to create microenvironments mimicking the niche in which cells differentiate *in-vivo*. Current challenges include the lack of methods to incorporate complex spatial-patterning of extracellular matrix (ECM) components and growth factors within a single scaffold that would direct a single stem cell population to differentiate into multiple lineages and create complex tissue structures. We have recently developed a digital micro-mirror device (DMD)-based scaffold fabrication technique[1] that allows precise, pre-designed patterning of multiple molecules and the generation of complex architectures in a high-throughput, layer-by-layer fashion using functionalized poly(ethylene glycol) diacrylate (PEGDA)-based macromers. ECM components have been successfully conjugated to PEG and spatially patterned along with growth factors within the 3D scaffolds. Marrow stromal cells (MSCs) remain viable during the microfabrication process. We are currently evaluating the effects of various scaffold and culture properties in multi-lineage differentiation of MSCs inside such patterned 3D structures.

Methods: Spatially-patterned scaffolds were fabricated using a previously reported layer-by-layer, stereolithography process that couples a digital micromirror (DMD) chip to a conventional computer projector and an ultra-violet (UV) light source[1]. Each layer of a scaffold was spatially patterned using a dynamic mask produced on a Microsoft PowerPoint slide. A cell adhesive peptide, RGD, and a glycosaminoglycan, heparan sulfate, were covalently conjugated to acrylate PEG-succinimide esters and patterned within DMD-fabricated 3D scaffolds. A murine MSC cell line, D1, was encapsulated. Osteogenic medium was used to culture the scaffolds and gene analysis was performed using RT-PCR.

Results / Discussion: Spatially-patterned polymer scaffolds with pores of precise size and shapes were successfully fabricated in a layer-by-layer method using the DMD system (Figure 1)[1]. Encapsulated D1 cells remain viable, as shown through calcein staining, after an 8 min irradiation with a maximum UV intensity of ~115mW/cm^2, as shown in figure 2A. Furthermore, heparin-PEG-acrylate was incorporated in pre-determined regions and stained using alcian blue as depicted in figure 2B. Heparin has been shown to bind to certain growth factors, specifically FGF-2, and therefore sequestering FGF-2 into pre-determined regions efficiently mediates localized osteogenic differentiation, leading to more complex tissue structures. Scaffolds were paraffin-embedded and stained for matrix calcification and collagen I, markers for osteoblasts. Gene expression analysis also confirms these spatially patterned scaffolds can effectively support D1 MSCs differentiation into the bone lineage. Optimization of osteogenic and chondrogenic differentiations are currently underway.

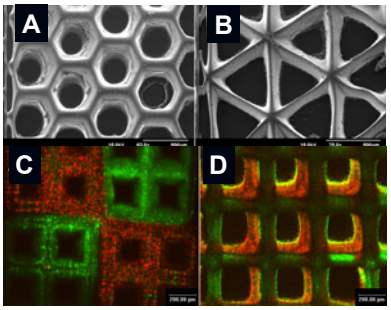

Figure 1: *DMD-fabricated scaffolds with various pore size and shapes (A, B) and pre-designed spatial patterning (C, D).*

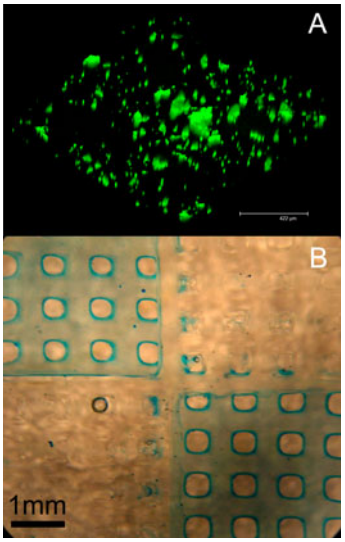

Figure 2: *A shows encapsulated D1 cells remain viable (green fluorescence) after a 48-hour incubation time. **B** shows alican blue staining of heparin in a quadrant-specific geometry. Heparin-PEG-acryl was added to the PEGDA monomer solutions prior to photo-polymerization. Structures can be washed between polymerization to pattern multiple solutions.*

Conclusions: We have developed a novel scaffold microfabrication system to spatially pattern ECM components and growth factors and generate precise internal architectures. Heparin- and RGD peptides were successfully incorporated within these scaffolds. Progenitor cells can be efficiently encapsulated or seeded on these scaffolds and successful differentiation into the osteogenic lineage has been demonstrated. These spatially patterned scaffolds could ultimately consist of intricate architectures that combine both spatial and controlled-release kinetics of biochemical factors, thus creating an ideal environment for studying hybrid tissue formation from a single progenitor cell population.

References:

1. Lu Y, Mapili G, Suhali G, Chen S, Roy K. *J Biomed Mater Res A*. 2006 May;77(2):396-405.

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