Selectively Degradable Hydrogels for Investigating Cell-Cell Interactions in Co-culture

Andreia Ribeiro[1], Filipa Maia[1,2], Ozlem Yasar[3], Binil Starly[3], Jennie Leach[1]

[1]University of Maryland Baltimore County, Chemical and Biochemical Engineering Department, [2]Faculdade de Engenharia da Universidade do Porto, Department of Chemical Engineering, [3]University of Oklahoma, School of Industrial Engineering,

Statement of Purpose: Interactions between neighboring cells regulate cell behavior as well as tissue function. Tools to investigate heterologous cell-cell interactions *in vitro* are therefore critically important for the development of new diagnostic and drug screening tools. A number of methods are available to study interactions between cells on patterned substrates [1]. Among these techniques, microfabrication and stereolithography provide powerful means to pattern cells [2], but typically rely on immobilized adhesive domains that permit stronger affinity of one cell type for a specific biochemical group relative to a second cell type. While successful, these techniques generally cannot be applied to co-cultures of similar cell types (e.g., two types of neurons) that do not demonstrate selective adhesion to biochemical cues. In this work, we aim to demonstrate a new co-culture patterning approach based on the selective degradation of patterned gels thus allowing the culture of *any* two cell types for investigating cell-cell interactions *in vitro*. Using maskless projection micro-stereolithography (μSL), we developed defined multi-component micropatterned architectures of poly (ethylene glycol) dimethacrylate (PEG-DM) and carboxymethyl-cellulose-aminoethyl methacrylate (CMC-MA) on glass substrates (**Fig 1**). Since both polymer surfaces are resistant to cell and protein adsorption [2,3], the first cell type can only adhere to the free glass spaces. The degradation of the CMC-MA patterns by the enzyme cellulase provides new adhesive regions on the substrate where a second cell type can adhere. This technique allows control of cell-cell contact and spacing between two different cell types at the microscale and will be used as a new tool to investigate heterologous cell-cell interactions in co-culture systems.

Methods: PEG-DM and CMC-MA were synthesized in house [3]. Viability of NIH 3T3 fibroblasts were tested in solutions of 0.2-1.0 U/ml cellulase (Sigma) and analyzed from phase microscopy (Olympus) images captured at 48 h. CMC-MA gel degradation in 0.2-1.0 U/ml cellulase solutions were verified by measuring the weight loss of 50 μl CMC-MA gels and calculating degradation rate from the initial slope of gel weight loss versus time plots. To pattern the gels, a μSL system based on a digital micro-mirror device (DMD™, Texas Instruments) was used to create the patterns. Gel precursor solutions were formulated by dissolving PEG-DM (60-80% w/v, 700 kDa) or CMC-MA (8-12%, 90 kDa) in 0.05% photoinitiator solution (Irgacure 2959, Ciba) prepared in phosphate buffered saline. Glass coverslips were modified with trimethoxypropylsilane-methacrylate (Sigma) to allow covalent linkage of the gels to the coverslip. The gels were crosslinked under UV light and the patterns are formed in a layer-by-layer fashion by modulating the UV light according to a defined mask on the DMD chip [4]. NIH 3T3 fibroblasts were seeded in the patterned coverslips in serum-containing media and incubated for 24 h. The cell media was then replaced by fresh media containing cellulase every 1-2 h. Once the CMC-MA patterns were degraded, human SH-SY5Y neuroblastoma cells were added to the substrates and cultured in the same serum-containing media for 24 h. Images of the patterned substrates and selectively adhered cells were captured under phase microscopy.

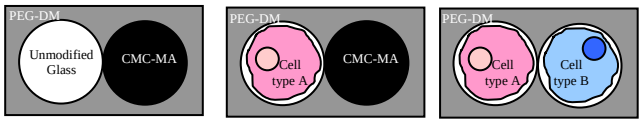

Fig 1. Schematic of approach. Step 1: PEG-DM and CMC-MA are patterned onto glass. Step 2: Cell type A is seeded onto the glass in serum-containing medium. Step 3: The CMC-MA pattern is degraded with cellulase and cell type B is seeded in serum-containing medium onto the newly exposed glass.

Results: Preliminary viability studies (**Fig 2**) revealed that the fibroblasts were sensitive to cellulase only at high concentrations: when exposed to 0.2 or 0.5 U/ml cellulose for 48 h, the fibroblasts grew to confluence, whereas at 1 U/ml the fibroblasts had low viability.

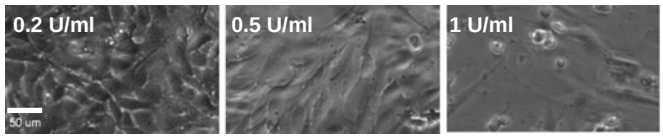

Fig 2. Phase contrast images of 3T3 fibroblasts cultured for 48 h in medium containing cellulase at 0.2-1.0 U/ml. (Scale bar, 50 μm)

Degradation studies (**Fig 3**) indicate that gel degradation rates generally increase with greater cellulase concentrations and 8% CMC-MA gels degrade faster than 12% gels.

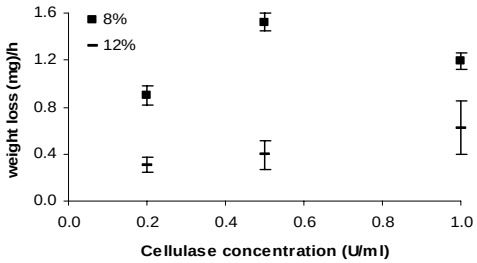

Fig 3. Degradation rates of 8 and 12% CMC-MA gels in 0.2-1.0 U/ml cellulase.

The μSL method was used to create polymer scaffolds of a variety of shapes and dimensions. **Fig 4** depicts ~100 μm PEG-DM channels (250 μm Z-thickness) alone (a) and with co-cultured cells (b).

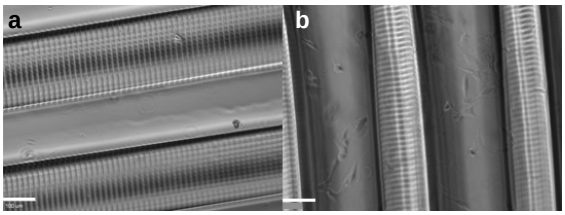

Fig 4. Phase contrast images of (a) micropatterned PEG-DM channels and (b) co-culture containing micropatterned substrates. Scale bar, 100 μm.

Conclusions: The goal of this work was to demonstrate selectively degradable gels as a new tool for investigating cell-cell interactions in co-cultures. Preliminary studies demonstrate that cells can be seeded in the presence of cellulase, CMC-MA patterns can be effectively degraded and co-cultures can be maintained in the presence of μSL-micropatterned PEG-DM gels. Ongoing work focuses on optimizing co-culture patterning using the selectively degradable architectures. Immunohistochemical staining for fibroblasts and neuroblastoma cells will be used to verify the co-culture patterning.

References:

1. Bhatia SN et al. JBMR 1997;34:189; Tsuda Y et al. BBRC 2006;348:937-44; Kidambi S et al. Macromol Biosci 2007;7:344-53; Hui EE et al. Langmuir 2007;23:4103-7. **2.** Bhatia SN and Chen CS. Biomedical Microdevices 1999;2:131-44. **3.** Reeves R, J Leach et al. in preparation. **4.** Lu Y et al. JBMR 2006;77:396-405.