Cellular inhibition of radical-mediated polymerization of poly(ethylene glycol) hydrogels formed via thiol-norbornene click chemistry

Stanley Chu[1], Shankar Lalitha Sridhar[3], Margaret Schneider[1], Franck Vernerey[3,4], Stephanie Bryant[1,2,4]

[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder CO, [2]BioFrontiers Institute, University of Colorado, Boulder CO, [3]Department of Mechanical Engineering, University of Colorado, Boulder CO, [4]Material Science and Engineering Program, University of Colorado, Boulder CO

Statement of Purpose: Cells have been encapsulated in thiol-ene hydrogels in order to promote tissue synthesis for the regeneration of tissue. PEG hydrogels formed through thiol-ene click chemistry are considered to form relatively homogeneous and hydrophilic networks providing a suitable environment for cell culture.[1] However, a recent study has shown that radicals produced during photoencapsulation in PEG hydrogels formed from PEG diacrylate monomers can lead to lipid peroxidation and elevated intracellular reactive oxygen species.[2] This effect was reduced in a thiol-norbornene photoclickable PEG hydrogel.[3] This study aimed to investigate whether this inhibition in the polymerization would have a significant effect on hydrogel formation and subsequently on the hydrogel macroscopic properties.

Methods: Chondrocytes harvested from the femoral condyles and patellar groove of a 1-3 week old calf were encapsulated in PEG thiol-norbornene hydrogels (n=3) with a nondegradable PEG-dithiol (PEGdSH) crosslinker or an enzymatically degradable (CVPLSLYSGC) peptide crosslinker at a cell seeding density of 50 million cells/mL or 150 million cells/mL. The compressive moduli of cellular and acellular gels were measured (0.5 mm/min unconfined compression. The modulus was measured by taking the slope of the stress-strain curve from 10-15% strain). Interaction of cells and crosslinkers were investigated by fluorescently labeling PEG-thiols (PEG-monothiol (PEGmSH) and PEG-dithiol) or peptide-thiols (CVPLSLYSGC and CRGES) and incubated with freshly isolated chondrocytes in solution for 10 minutes (n=3 per condition), followed by multiple washes. Flow cytometry was used to identify populations of fluorescently labeled chondrocytes. A student's t-test assuming equal variances was used to compare significant differences in moduli. A one-way ANOVA (α=0.05) with the type of thiolated molecule as a factor was performed to compare differences in flow data.

Results: Regions of decreased crosslinking density around a cell may manifest in reduced overall hydrogel modulus at high cell seeding density. Hydrogels seeded with chondrocytes had a reduced modulus (p=0.013 for PEG crosslinkers and p=0.096 for peptide crosslinkers) compared to acellular gels of the same formulation (Fig. 1). We also investigated the possibility of crosslinker interaction with chondrocytes. Fluorescently labeled thiolated molecules were incubated with chondrocytes and their mean fluorescence was measured with flow cytometry. All groups exposed to some form of fluorescently labeled thiolated molecules showed an increase (p<0.0001) in fluorescence compared to chondrocytes receiving no treatment (Fig. 1). Additionally, there was no significant difference in fluorescence between cells exposed to PEGmSH or PEGdSH. These preliminary data suggest that crosslinkers typically used in polymerization may be immobilized by the cell, reducing the local thiol:ene ratio and that the PEG portion of nondegradable crosslinkers interact with the cell.

Radicals generated during the polymerization mechanism, such as reactive oxygen species and propagating radicals, can terminate on the cell membrane[4] and membrane proteins.[5] Additionally, PEG has been shown to interact with cell membranes due to the amphiphilic nature of PEG[6] and thiols on the cell surface can form disulfide bridges with thiolated molecules.[7] These mechanisms of cell-mediated radical termination and monomer immobilization suggest a region of reduced crosslinking density in the pericellular space. This decreased crosslinking density introduces spatial heterogeneity in the polymer network and may promote tissue deposition in early cell culture.

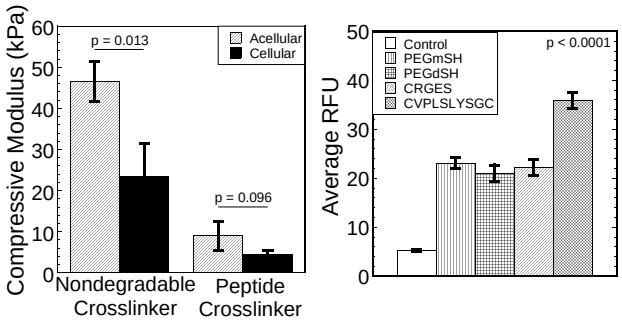

Figure 1. Left) Bulk compressive moduli of hydrogels crosslinked with a nondegradable PEG crosslinker or an enzymatically degradable peptide crosslinker (CVPLSLYSGC). Right) Average mean fluorescence of chondrocytes exposed to fluorescently labeled thiolated molecules.

Conclusions: Hydrogels densely populated with chondrocytes display a reduced bulk compressive modulus. The possible mechanisms by which this happens suggest that a region of reduced crosslinking density exists around cells. In thiol-ene hydrogels, most tissue molecules of interest are orders of magnitude larger than the mesh size of the hydrogel. Thus, localized regions of reduced crosslinking density can reach reverse gelation faster allowing for the diffusion of matrix molecules. As tissue engineers, we can utilize this phenomenon in order to produce large pieces of connected tissue.

References:

1) Kloxin, A. M., Kloxin, C. J., Bowman, C. N. & Anseth, K. S. *Adv. Mater.* **22,** 3484–3494 (2010). **2)** Farnsworth, N., Bensard, C. & Bryant, S. J. *Osteoarthritis Cartilage* **20,** 1326–1335 (2012). **3)** Roberts, J. J. & Bryant, S. J. *Biomaterials* **34,** 9969–9979 (2013). **4)** Lobo, V., Patil, A., Phatak, A. & Chandra, N. *Pharmacogn. Rev.* **4,** 118–126 (2010). **5)** Richards, D. M. C., Dean, R. T. & Jessup, W. *Biochim. Biophys. Acta BBA - Biomembr.* **946,** 281–288 (1988). **6)** Boni, L. T. *et al. Biochim. Biophys. Acta BBA - Biomembr.* **775,** 409–418 (1984). **7)** Aubry, S. *et al. FASEB J.* **23,** 2956–2967 (2009).