Engineering Hydrogels with Dual Gradients of Mechanical and Biochemical Cues to Decipher Stem Cell-Niche Interactions

Xinming Tong[1], James Jiang[2], Danqing Zhu[3], Fan Yang[1,3]
[1]Department of Orthopedic; [2]Department of Human Biology;
[3]Department of Bioengineering, Stanford University, Stanford, CA.

Statement of Purpose: Stem cells reside in a multi-factorial microenvironment including cues such as biochemical and mechanical properties. Hydrogels have been widely used as artificial cell niche to facilitate tissue growth or desirable cellular fates. Most hydrogels developed so far possess homogeneous niche cues, yet native tissues are heterogeneous with spatial gradients with niche cues. As such, biomaterials with spatiotemporal gradients would be highly desirable to help decipher cell-niche interactions in a more physiologically relevant manner. While cells niche is a multi-factorial environment, previous efforts on developing gradient materials focused largely on developing hydrogels with single gradient, such as mechanical or biochemical gradient alone. The goal of this work is to develop biomimetic hydrogels as cell niche with dual gradients of mechanical and biochemical cues to provide a platform for elucidating stem cell-niche interactions.

Methods: To create the gradient properties, we chose the photo-activated thio-ene addition for crosslinking and biochemical ligand incorporation, due to the facile control. Multi-arm polyethylene glycol (PEG) with norbornene end groups and linear PEG dithiol were used as precursors to crosslink the mechanical supporting matrix. The cysteine containing peptide CRGDS was chosen as model biochemical ligand, which is widely used to provide cell adhesion. By tuning the UV exposure time, the crosslinking density could be changed and subsequently varies the mechanical stiffness. The ligand incorporation density could also be controlled by the exposure time as well. Sliding of the photo-mask connected to a controlling syringe pump, which could change the UV exposure time on hydrogel precursors smoothly, was used to create the gradient both on mechanical stiffness and ligand density. By sequentially fabricating the mechanical supporting matrix and incorporating the ligand both in gradient manner, a dual-gradient hydrogel material could be obtained. By changing the sliding direction on each step, the two gradients could be aligned differently to maximum the screening efficiency addressing different purposes.

Results: Using cell adhesive peptide as a model biochemical ligand, we successfully developed hydrogel substrate with both mechanical and RGD gradients, which were aligned in orthogonal manner. The mechanical stiffness was characterized as from 5 kPa to 15 kPa by compressed modulus testing. And the RGD density was measured as ranging from around 0 to 14 nmol/cm^2 by using fluorometric assay. When seeded on hydrogels with dual gradients, cell spreading was influenced by both mechanical stiffness and RGD density (**Figure 1**). Keeping matrix stiffness constant while increasing RGD density promoted cell spreading. Similarly, increasing stiffness while maintaining RGD density constant also led to increased cell spreading. Human fibroblasts cultured on hydrogels with gradients also showed faster cell proliferation on side with increased hydrogel stiffness or RGD density.

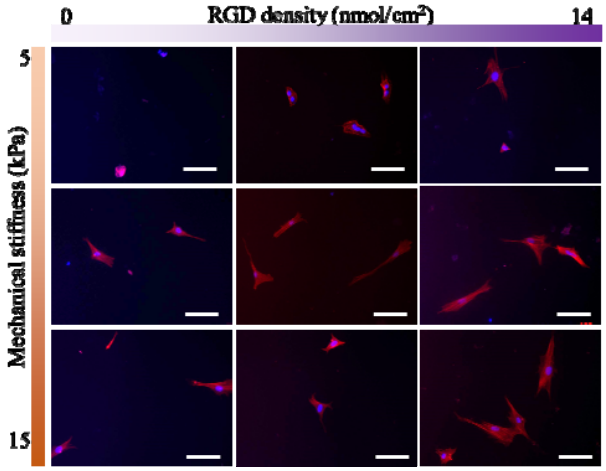

Figure 1. Representative micrographs of human fibroblast cultured on dual-gradient hydrogel substrate on day 1. (Red, F-actin; blue, nucleus; scale bar 100 μm)

Conclusions: Here we report a facile method to fabricate hydrogels with dual gradients of mechanical and biochemical signals. Our method allowed independently tunable biochemical and mechanical properties that are aligned in an orthogonal manner. This platform could provide a tool for elucidating how gradient niche properties for influencing cell fate, and may be broadly useful for both fundamental studies and translational applications.