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: Although living cells reside in a multifactorial environment that provides a variety of biochemical and physical cues to direct cell behavior and fate, the complex interplay among niche cues largely remains an unaddressed challenge due to our traditional reliance on single-cue investigations. Combinatorial screening of cell behaviors with material libraries has emerged as a promising approach to help elucidate cell-niche interactions and to identify optimal niche properties that lead to desired cellular responses. However, current approaches often use individual samples with homogenous niche properties; and variations in biochemical or mechanical cues were applied discretely to study groups. This strategy renders investigations less efficient and low throughput within a narrow range of niche cues, and requires many samples and tedious work. To overcome this limitation, scaffolds with gradient niche properties can maximize the screening efficiency and better mimicking native tissues. However, previous efforts toward developing gradient materials have focused largely on developing biomaterials with single gradients, such as a mechanical or biochemical gradient alone, which still cannot capture the multifactorial physiological niche. Thus, the goal of this study is to address this critical need by developing biomaterials with spatiotemporal gradients in multiple properties to decipher cell-niche interactions in a high-throughput and physiologically relevant manner.

Methods: We chose the photo-activated thio-ene addition for hydrogel crosslinking and biochemical ligand incorporation. Multi-arm polyethylene glycol (PEG) with norbornene end groups and linear PEG dithiol were used as precursors to crosslink the mechanical supporting matrix. A cell adhesive peptide CRGDS was chosen as a model biochemical ligand. To introduce a mechanical gradient, a gradient of UV exposure was achieved by sliding a photomask over the precursor solution, using a syringe pump. The exposure time window, which determines the stiffness range of the gradient, was tuned by adjusting the sliding speed and total exposure time. To introduce a biochemical gradient, CRGDS solution were dropped on top of the hydrogel. Sliding a photomask over the system yielded ligand incorporation in a gradient, while adjusting the sliding speed and total exposure time affected the range of ligand density. By changing the sliding direction on each step, the two gradients could be aligned differently to maximum the screening efficiency addressing different purposes.

Results: Here, we reported a facile method to fabricate hydrogels with dual gradients in mechanical and biochemical properties. We tuned the degree of photoactivated thiol-ene radical addition, for both hydrogel crosslinking (mechanical stiffness) and the incorporation of biochemical ligands, by sliding a photomask over the substrate to control the duration of UV exposure (**Fig. 1A-F**). Hence, we were able to sequentially and independently create simultaneous gradients in mechanical stiffness and ligand density. We obtained gradient hydrogels with tunable mechanical stiffness (**Fig. 2A**) by adjusting UV exposure time . To visualize biochemical ligand gradient, FITC-conjugated peptide was used, which confirmed increased biochemical ligand density with fluorescent imaging (**Fig. 2B**). Increasing exposure time for varying duration led to increasing RGD gradient (**Fig. 2C**). When seeded on hydrogels with dual gradients, cell spreading was influenced by both mechanical stiffness and RGD density (data not shown). Our sequential fabrication strategy allowed two gradients to be applied to a single hydrogel with variable alignment (orthogonal in this study). We anticipate that this method will empower fast screening to determine how individual niche cues influence cell fate and to identify optimal conditions for the evaluation of cell responses of interest. Our design strategy also serves as a high-throughput tool for mechanistic studies of how cells respond to environmental signals, which we anticipate will critically empower a broad spectrum of tissue-engineering applications to improve human health.

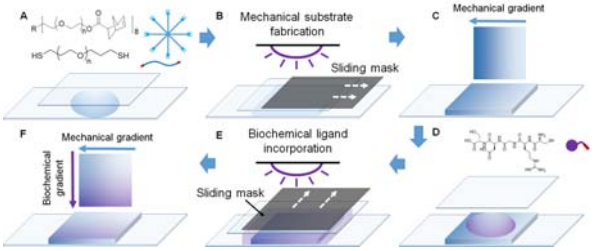

Figure 1. Fabrication schematics of dual gradient hydrogels.

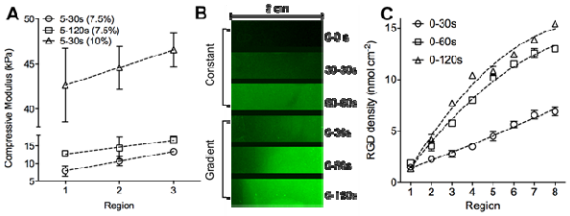

Figure 2. Characterization of dual gradient hydrogels.

Conclusions: Here, we report the construction of polyethylene glycol-based hydrogels that simultaneously harbor separate, orthogonal gradients in mechanical stiffness and biochemical cues. Tuning these gradients by controlling the extent of UV-based photocrosslinking resulted in differential adhesion, density, elongation, and spreading of live human fibroblasts. We anticipate that these parameters can be further manipulated to faithfully recapitulate biologically relevant microenvironments to lay the groundwork for advances in tissue engineering.