Spatially Controlled Mechanics Modulate Stem Cells in Sequentially Crosslinked Macroporous Hydrogels

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Statement of Purpose: Utilizing matrix mechanics as a means to control stem cell behavior has received a considerable amount of attention in the past decade. This matrix property has been shown to have a profound effect on stem cell behaviors such as morphology, differentiation, and secretion of various factors.[1,2,3] Due to the heterogeneous nature of tissues, it is necessary to develop materials with the ability to spatially control mechanics and the subsequent stem cell response. To this end, we have developed a macroporous hydrogel system, based on an established sequentially crosslinked hyaluronic acid (HA) mechanism,[1] that allows for spatially controlled mechanics in 3-D. This system allows for control of human mesenchymal stem cell (hMSC) morphology, proliferation, and factor secretion in uniform and photopatterned hydrogels.

Methods: Methacrylated HA (MeHA) was synthesized with a modification efficiency of ~100%. Briefly, HA was reacted with methacrylic anhydride for 24 hours while maintaining a pH 8, followed by purification through dialysis and lyophilization. Hydrogel mechanics were determined using AFM on uniform, non-porous hydrogel slabs. In order to introduce porosity, the polymer solution was pipetted onto a PMMA microsphere template and allowed to undergo Michael Addition crosslinking (18% theoretical methacrylate consumption) using dithiothreitol (DTT) for 2 hours in 0.2 M TEA with pH 9. During the initial crosslinking step, a cell adhesion motif (GCGYG**RGDS**PG) was coupled to the HA backbone using the same reaction. The microsphere template was removed using a series of acetone, ethanol, and dIH2O washes. A range of hydrogel mechanics was achieved by diffusing in the photoinitiator I2959 (0.05 wt%) for 1 hr and exposing to UV for 0-120s to further polymerize the methacrylate groups. Photopatterned hydrogels were created with a photomask during this secondary light-initiated radical crosslinking. Incorporation of a methacrylated rhodamine dye (MeRho) allowed for visual confirmation of +UV regions. Following crosslinking, hydrogels were flash frozen and lyophilized overnight to allow for cell seeding. Cell studies were performed by seeding 10^5 hMSCs and culturing for 1 to 7 days. Cells were stained with rhodamine and FITC-phalloidin in order to visualize cell spreading, proliferation, and cytoskeletal organization under confocal microscopy and secretory factors produced in uniform scaffolds were characterized by angiogenesis and cytokine proteome profilers (R&D Systems).

Results: AFM was used to characterize the cell-scale elastic modulus of the hydrogels formed with 18% initial DTT consumption, "-UV", which could then be sequentially exposed with varying UV times, "+UV". As seen in Fig1A, a range of mechanics nearly two orders of magnitude was achieved using this sequential crosslinking method (from 2.5-65 kPa). This range spans physiologic regimes from softer myogenic-promoting substrates to stiffer bone-promoting substrates.[2]

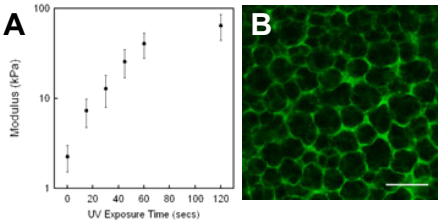

Figure 1. A) Mechanics of sequentially crosslinked gels. B) Porous gel labeled with FITC dye, scale bar=400 µm.

Differences in hMSC morphology were observed (Fig2A) in uniform soft ("-UV") and stiff ("+UV") macroporous hydrogels, namely they spread more in the stiffer +UV hydrogels (bottom row, Fig2A) compared to the softer -UV hydrogels (top row, Fig2A). When the mechanics were patterned into a single scaffold (Fig 2B), cells exhibited a highly spread morphology in the +UV regions (as indicated by red MeRho staining) and also a much higher cell density after 7 days in culture, indicating both morphological and proliferative changes based on local mechanics. Finally, nearly a dozen angiogenic/cytokine factors were shown to be mechanodependent, with six showing an optimum expression at a particular substrate stiffness (data not shown), opening up a range of therapeutic possibilities.

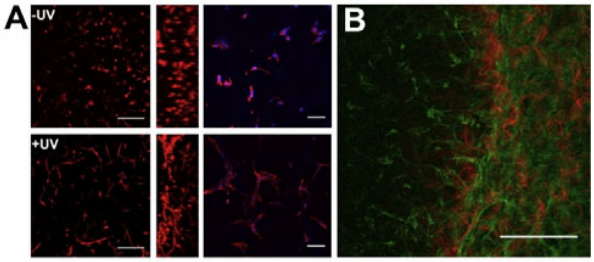

Figure 2. hMSC response to uniform (-UV or +UV, A) and patterned (B) hydrogels. Cells were uniformly seeded as evidenced by z-projection (A, middle panels) and exhibited more spread morphologies in +UV hydrogels. Patterned hMSC spreading and proliferation is shown in B, with greater spreading and proliferation shown in +UV (red) regions after 7 days.

Conclusions: We have successfully created a macroporous hydrogel with the ability to locally control matrix mechanics and direct stem cell behavior. Using this system, stem cell morphology and proliferation were controlled in both uniform and patterned hydrogels. This ability to locally control stem cell mechanics in 3-D represents a novel method to investigate stem cell responses to heterogeneous microenvironments that could prove useful to study more complex stem cell responses such as differentiation and paracrine factor secretion in certain pathologies.[3,4]

References: 1) Marklein RA. et al. Soft Matter. 2010; 6: 136-143. 2) Engler AJ. et al. Cell. 2006; 126: 677-689. 3) Seib FP. et al. Biochem Biophys Res Commun. 2009; 389:663-666. 4) Breitbach M. et al. Blood. 2007; 110:1362-1369.