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Statement of Purpose: Cell migration is a critical process *in vivo* for development, healing, and immune response and is regulated by a myriad of inputs including structural, biochemical, and cell-cell contacts, the exact nature of which depends on the cell type and the microenvironment where the migration occurs.[1] This work aims to elucidate important factors in isotropic and directed, 3D hMSC migration and provide valuable engineering principles to design materials for applications as cell carrier platforms and/or substrates for recruitment of endogenous hMSCs to a bone defect site. We are developing a tunable 3D microenvironment that recapitulates specific structural and biochemical facets of the extracellular matrix that regulate cell migration such as adhesivity and cellular degradability.

Here, we have developed a synthetic peptide-functionalized, PEG-based hydrogel that allows for tuning of the crosslinking density and adhesivity to regulate cell mediated degradation and subsequent 3D cell migration. We have shown that hMSC spread and migrate throughout this system efficiently at low crosslinking densities and high adhesivity. Continuing this work, we are looking at directed hMSC migration through the gradient presentation of specific chemotactic cues. This is motivated by their importance in spatially directing hMSC homing to fracture sites and hMSCs primary role in the bone fracture healing process. The combination of this hydrogel network with a microfluidic device allows for the presentation of a tunable gradient of chemotactic factors, such as stromal-cell derived factor-1α (SDF-1α), within a user-defined 3D microenvironment.

Methods: hMSCs were encapsulated within a tetra-functionalized PEG-norbornene (f=4) based hydrogel that was photopolymerized (180 seconds at 10 mW/cm^2) through a radical, step growth polymerization with varying off-stoichiometry ratios of (thiol/ene = 0.65, 0.725, 0.85) di-thiol functionalized MMP-degradable peptide (KCGPQ*IWGQCK, f=2) and a mono-thiol fibronectin peptide mimic (CRGDS, f=1). These monomers were synthesized as previously described by Fairbanks *et al*.[2] For isotropic migration studies, 30 µL of pre-polymerized solutions were photopolymerized and allowed to swell for 24 hours in Experimental Media (low-glucose DMEM, 10% fetal bovine serum, 50 U mL-1 each penicillin/streptomycin, 1 µg mL-1 fungizone antimycotic) and this allowed for cell recovery from trypsinization. After 24 hours of culture, 3D cell migration was analyzed using live cell video microscopy on a Nikon TE2000-E microscope with a Nikon environmental chamber and an external heater (In vivo Scientific) and CO_2 regulator (In vivo Scientific) for 6 hours using Metamorph software (Molecular Devices). Using positional information cell migration metrics, such as speed, persistence, mean free path, and percent migration, were calculated using a user-developed Matlab (Mathworks) program. For anisotropic migration studies, 6 uL of pre-polymerized solution was introduced to a µ-Slide Chemotaxis 3D `(Ibidi) following the manufacturer's protocol and photopolymerized in place. Experimental Media was introduced to the two source chambers for 24 hours and then replaced with or without a specific concentration of SDF-1α to form the gradient across the hydrogel. Cell migration was then followed for 12 hours and analyzed as discussed previously.

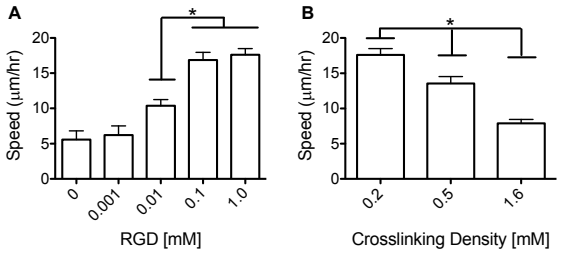

Figure 1. Isotropic migration speed of hMSCs encapsulated within a 3D peptide-functionalized PEG-based hydrogel with varying (A) adhesivity and (B) crosslinking density. (*P <0.05)

Results and Conclusions: As shown in Figure 1, isotropic hMSC migration within the 3D peptide-functionalized, PEG-based hydrogels is regulated by the adhesivity and crosslinking density of the network. Cell speed was shown to increase with increasing RGD concentration and decreasing crosslinking density. The percent of cells migrating followed a similar trend as cell speed whereas persistence was not significantly different between the various systems. The development of this tunable 3D system allows for further complexity to be introduced through the presentation of chemoattractant gradients. Hydrogels with 1.0 mM RGD and 0.5 mM crosslinking density were used to study the gradient concentration effects of SDF-1α. Preliminary work shows directed hMSC migration with SDF-1α concentration gradient presentation of 0.1, 1, 10, and 100 nM/mm with positive velocities towards the gradient of 1.13±0.18, 1.11±0.10, 1.41±0.14, and 1.11±0.21 µm/min, respectively. No migration was observed in the blank control. Future work will include comparisons of the effect of the gradient slope and initial concentrations of the sink and source chambers, and potentially other chemotactic factors such as platelet-derived growth factor can be easily introduced. Understanding these important regulators of directed hMSC migration will help in the engineering of more effective materials to recruit endogenous hMSCs and promote colonization of the material at bone fracture sites.

References: [1] Friedl PJ Cell Biol 2010;188:11-9.
[2] Fairbanks, B.D. Adv. Mater. **2009**; 21:5005–5010.