Directing Human Mesenchymal Stem Cell Migration Through Gradient Presentation of Chemokines

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Statement of Purpose: Directed cell migration plays a critical role *in vivo* during development, immune response, and healing and is regulated through a multitude of inputs including biochemical and mechanical gradients the exact nature of which depends on the cell type and the microenvironment where the migration occurs.[1] Specifically, this work aims to elucidate important biochemical gradient parameters through the presentation of highly defined and tunable gradients of stromal derived factor 1α (SDF-1α) to direct individual human mesenchymal stem cell (hMSC) migration.

Here, we have developed a synthetic peptide-functionalized, PEG-based hydrogel that allows for the systematic tuning of crosslinking density, adhesivity, and the introduction of biochemical cues through photo-activated thiol-ene chemistry. Previously, it has been shown that within this thiol-ene hydrogel hMSC can spread, migrate and remain viable.[2] Now using photolithography, thiol-containing peptides and proteins can be covalently tethered to the hydrogel through reactions with excess –ene moieties present in the polymerized hydrogel. This provides a facile method to present distinct and spatially defined shapes and gradients to migrating hMSC within the hydrogel network to elucidate important facets of biochemical gradients such as slope and concentration and how these facets regulate important migratory characteristics such as speed and persistence. This knowledge will lead to better-engineered platforms to direct hMSC migration for wound healing applications.

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 a di-thiol functionalized MMP-degradable peptide (KCGPQ*IWGQCK, f=2) and various concentrations of a mono-thiol fibronectin peptide mimic (CRGDS, f=1). 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 videomicroscopy on a Nikon TE2000-E microscope with a Nikon environmental chamber and an external heater 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, and percent migration, were calculated using a custom Matlab (Mathworks) program.

SDF-1α was coupled with an Alexa Fluor 488-NHS (Life Technologies) at pH 7.0 and then dialyzed. The pH was then increased to 8.5 and SDF-1α-AF488 was reacted with Traut's Reagent (Thermo Scientific) following the manufacturers' protocol. Thiolated SDF-1α-AF488 was then swollen into a pre-polymerized gel along with the photoinitiator I2959 (Ciba) and UV light (365 nm, 10 mW/cm^2) exposure was manipulated using a moving photomask. Fluorescent images were taken across the gel using an LSM 710 confocal microscope (Zeiss).

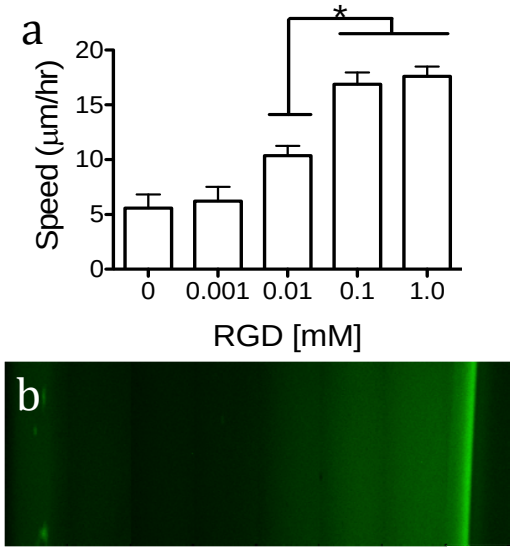

Figure 1. a) The enzyme-degradable thiol-ene hydrogels permit 3D hMSC migration that can be manipulated through the isotropic tethering of the thiol containing CRGDS. **b)** A linear gradient of thiolated SDF-1α tagged with an Alexa Fluor 488 was covalently attached to the thiol-ene network with distinct spatial control.

Results: The thiol-containing peptide CRGDS was first isotropically introduced into the hydrogel at increasing concentrations. The percent of hMSC migrating (data not shown) and the speed of migrating hMSC increased with increasing CRGDS concentration (Figure 1a). Moving to a full protein, SDF-1α was pursued to direct the otherwise isotropic 3D cell migration. Thiolated SDF-1α-AF488 was covalently tethered to the hydrogel in a tunable linear gradient (Figure 1b). Shown above is an image across the gel in the x-y plane.

Conclusions: This gradient can be adjusted by tuning the spatial and temporal exposure of UV light to the hydrogel. This system allows us to produce highly defined gradients of chemotactic proteins in a permissive hydrogel to study how gradients affect individual hMSC migration. This will allow important factors in directing hMSC migration to be elucidated.

References:

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