Abstract

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Human mesenchymal stem cells (hMSCs) are pivotal in the wound healing process. hMSCs regulate inflammatory cytokines and recruit elementary cell types to form new tissue. To enhance this natural healing process and restart healing in chronic wounds, additional hMSCs are delivered to wound. A common approach to this delivery is to implant an hMSC-laden scaffold in the site of the wound. Low cell migration from the hydrogel to the wound site is a current challenge with this technique. An avenue of increasing cell migration is tethering chemical cues from the native wound environment into the scaffold to direct cell migration and enhance delivery from an implantable material. In order for cell to migrate out of these scaffold, they must degrade and remodel the material. In this thesis, we characterize cell-material interactions of hMSCs in degradable poly(ethylene glycol)-norbornene scaffolds using multiple particle tracking microrheology (MPT). hMSCs are 3D encapsulated in a well-defined polymer-peptide hydrogel, providing a synthetic environment for cell migration and remodeling. The two main components of this hydrogel are 4-arm poly(ethylene glycol)-norbornene ($M_n = 20,0000\ \frac{g}{mol}$) and a matrix metalloproteinase (MMP)-degradable peptide cross-linker (KCGPQG↓IWGQCK, ↓ represents the cleavage site, $M_n = 1,346\ \frac{g}{mol}$). MPT is a passive microrheological technique that measures the Brownian motion of embedded probe particles to quantify the rheology of the material. This technique is used to characterize cell-material interactions during hMSC-mediated remodeling and motility.

We first use MPT to characterize how hMSCs remodel their pericellular regions in response to uniformly tethered pro- and anti-inflammatory cytokines. In this work we use tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, which is present in the native wound environment $24-36\ hours$ after a wound occurs and transforming growth factor-β (TGF-β), an anti-inflammatory cytokine, which is expressed $72-336\ hours$ after a wound occurs. Each of these cytokines is separately functionalized using Traut's regent, which adds additional -thiol groups to the protein surface allowing them to be clicked into the hydrogel using thiol-ene chemistry. We use MPT to characterize cellular remodeling around single encapsulated cells in three different scaffolds (control, tethered TGF-β and tethered TNF-α). These measurements show that uniformly tethering TNF-α into the hydrogel increases cellular remodeling on days 2 and 3 post-encapsulation. Tethering TGF-β into the hydrogel increases cellular remodeling ion days 3 and 4 post-encapsulation. During cell-mediated degradation, we also

measure cell morphology and motility. We measure that tethering TGF-β into the hydrogel increases cell spreading on day 4 post-encapsulation and increases encapsulated cell speed on all days post-encapsulation. Hydrogels with tethered TNF-α increase encapsulated cell speed on day 2 post-encapsulation and do not increase cell spreading. These results can be used to direct cell migration from a scaffold to wounded tissue.

The next chapter describes the development of a new technique to create hydrogels with tethered concentration gradients of polymers or proteins. This technique also enables us to measure the diffusion coefficient of solutes in hydrogels. Hydrogels with tethered concentration gradients of cell signaling molecules can be used to direct cell migration from the scaffold to the wound after implantation. A rectangular prism is the basis of our microfluidic device. Before using the microfluidic device to create hydrogels with a tethered concentration gradient of TNF-α, we make concentration gradients with a model polymer, Fluorescein poly(ethylene glycol)-thiol (FITC-PEG-SH), to validate the technique. We first make a calibration curve by measuring hydrogels with known concentrations of FITC-PEG-SH and measure their arbitrary brightness using a laser scanner. This calibration curve relates brightness with tethered molecule concentration. We form hydrogels with tethered concentration gradients of FITC-PEG-SH by allowing the molecule to diffuse for 6, 24 and 48 *hours*. Hydrogels with tethered concentration gradients of FITC-PEG-SH are placed on the laser scanner and spatial brightness is measured. The calibration curve is then used to back-calculate local concentrations every 25 *mum* along the hydrogel.Next, we curve-fit a solution of Fick's second law of diffusion to calculate the effective diffusion coefficient of FITC-PEG-SH. In the FITC-PEG-SH experiments, we hypothesize that steady state diffusion is reached when a gradient forms for 24 *hours*. We use the same method to make concentration gradients of thiolated and dyed TNF-α tethered into hydrogels. We measure an effective diffusion coefficient that is an order of magnitude faster than the predicted theoretical value. After creating multiple hydrogel replicates, we hypothesize that the more rapid diffusion is due to the electrostatic attraction between TNF-*alpha* and the hydrogels' cross-linker. This method can be scaled up or down to create hydrogels with tethered concentration gradients for cell delivery or to measure the diffusion coefficient of polymers or proteins in hydrogels.

In the final chapter of this thesis, we 3D encapsulate hMSCs in a hydrogel with a tethered concentration gradient of TNF-α. We define three distinct concentration regimes in the scaffold as a function of distance from the source of TNF-α. We use MPT and live cell imaging to measure changes in cellular remodeling and motility in different sections of the hydrogel with a tethered concentration gradient of TNF-α on days 2, 3 and 4 post-encapsulation and compare these measurements to those in hydrogels with no cytokines. We measure that the middle and low concentration regimes are more effective at increasing cell-mediated degradation. The high concentration regime does not increase cellular remodeling compared to a control scaffold. We measure increased encapsulated cell area and elongation in the scaffold with a tethered concentration gradient of TNF-α compared to control scaffolds. This work demonstrates that hMSCs

encapsulated in the hydrogel with 3D concentration gradient of tethered TNF-α change their secretions in response to the material and can degrade their local environments faster.

The work in this thesis quantifies interactions between hMSCs and hydrogels with tethered chemical cues. This thesis provides quantitative insights into how chemical cues influence individual hMSC behavior, informing the design of materials that guide basic cell functions through tethered chemical signals in hydrogels. In the future, we propose using multiple cues to create a synergistic effect on encapsulated cells or decreasing cytokine concentration in the microfluidic device's source.