RHEOLOGICAL CHARACTERIZATION OF DYNAMIC RE-ENGINEERING OF THE PERICELLULAR REGION BY HUMAN MESENCHYMAL STEM CELL-SECRETED ENZYMES IN WELL-DEFINED HYDROGEL SCAFFOLDS

Kelly M. Schultz, Lehigh University, Department of Chemical and Biomolecular Engineering
kes513@lehigh.edu
Maryam Daviran, Lehigh University, Department of Chemical and Biomolecular Engineering
John McGlynn, Lehigh University, Department of Chemical and Biomolecular Engineering

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Human mesenchymal stem cells (hMSCs) are critical for wound healing. hMSCs are active in each phase of wound healing regulating inflammation. Inflammatory regulation also enables hMSCs to restart stalled healing in chronic wounds. Before hMSCs reach a wound, they migrate out of their niche and across several rheologically distinctive microenvironments. The aim of synthetic implantable wound healing scaffolds is to mimic these *in vivo* microenvironments to enhance delivery of hMSCs to the wound while providing structure to the surrounding tissue. To use these materials as implantable wound healing scaffolds, we must determine how to leverage the material microenvironment to encourage cell migration and cell delivery to the wound. These materials mimic both physical and chemical aspects of the native extracellular matrix and enable precise engineering of the cues initially presented in the microenvironment to 3D encapsulated cells. Although these scaffolds are initially well-defined, they are designed to allow cellular remodeling and degradation, which constantly presents new environmental cues to the cell.

In our work, we encapsulate human mesenchymal stem cells (hMSCs) into a synthetic poly(ethylene glycol (PEG)-peptide hydrogel scaffold. This scaffold consists of a 4-arm star PEG backbone end-functionalized with norbornene which is cross-linked with a matrix metalloproteinase (MMP) degradable peptide sequence. The encapsulated hMSCs secrete MMPs, enabling them to degrade the hydrogel scaffold prior to and during motility. To characterize cellular re-engineering of the pericellular region we use multiple particle tracking microrheology (MPT) to measure the dynamic changes in the material temporally and spatially. In MPT, 1 micron fluorescently labeled probe particles are embedded in the material and the Brownian motion of these particles is measured and used to determine material properties. Our work measures hMSC-mediated degradation in the pericellular region has a microenvironment where the cross-link density decreases as distance from the cell increases. This is called a reverse reaction-diffusion degradation profile. The hMSC is keeping the scaffold stiff directly around it to spread and attach prior to motility. To do this, the cell simultaneously secretes tissue inhibitors of metalloproteinases (TIMPs), which bind to the catalytic part of the MMP making it inactive and unable to degrade the scaffold. This MMP-TIMP complex then diffuses through the scaffold and unbinds, making the MMP active and degrading the scaffold. This is modeled using Michaelis-Menten competitive inhibition.

We then inhibit TIMPs to determine how this changes the rheological properties in the pericellular region and hMSC motility. After TIMP inhibition, we measure a reaction-diffusion degradation profile in the pericellular, which is the opposite of the degradation profile created by untreated hMSCs. Additionally, cell motility is greatly enhanced in the scaffold. This could be due to durotaxis, the migration along a stiffness gradient to a higher moduli material, or due to a decreased material barrier in migration. This simple chemical treatment has the potential to enhance hMSC delivery to wounds from this hydrogel scaffold but it is unclear if the change in the bulk properties of the scaffold could increase degradation to the point where the scaffold can no longer support the wounded tissue if used for implantation.