Interaction of Human Mesenchymal stem cells with synthetic scaffolds in the presence of cytokines

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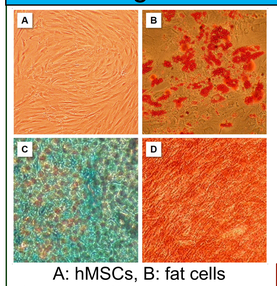
Abstract

A normal wound heals by progressing through three phases: inflammatory, proliferative and remodeling. From previous research, we know human mesenchymal stem cells (hMSCs) are key to progressing healing through all stages and can prevent and help resolve chronic wounds, which are stuck in the inflammatory phase. hMSCs directly influence the body's inflammatory response by decreasing the amount of pro-inflammatory cytokines and increasing the number of anti-inflammatory cytokines. Cytokines are proteins that act as signaling molecules to evoke biological processes, and include tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β). When secreted by a wound, these cytokines can elicit a response that regulates inflammation at the site by increasing cell-secretion of matrix metalloproteinases (MMPs) or tissue inhibitor of metalloproteinases (TIMPs). MMPs degrade the extracellular matrix (ECM) while TIMPs inhibit MMP degradation. In the Schultz Lab, we are characterizing implantable cell-laden materials that can direct cells to a wound using natural cues. We are characterizing poly(ethylene glycol)-peptide hydrogels that mimic the *in vivo* environment inside the body with multiple particle tracking microrheology (MPT). MPT measures the change in matrix degradation and cell motility of the cells after the addition of cytokines in the incubation environment. We are also developing a kinetic model for TGF- β using Michaelis-Menten enzymatic inhibition kinetics which will describe the mechanism of gel degradation. The results from MPT and the kinetic model can then be compared to determine the mechanism of matrix degradation and future scaffold design that will guide cell behavior during wound healing.

Background - Human Mesenchymal Stem Cells (hMSCs)



A: hMSCs, B: fat cells (adipocytes), C: cartilage cells (chondrocytes), and D: bone cells (osteoblasts)

Multipotent cells found in bone marrow important for making and repairing skeletal tissues (cartilage, bone and fat)

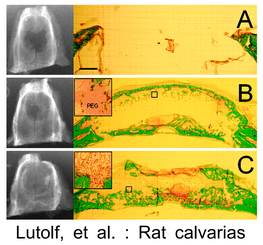
Functions:

- Repair bone and cartilage
- Form new blood vessels
- Prevent transplant rejection
- Slow the progression of autoimmune diseases
- Reduce inflammation

Most important for Schultz Lab

Mesenchymal stem cells, 2016

Lutolf et al., Synthetic matrix, 2003



Lutolf, et al. : Rat calvarias (skulls) were broken and injected with hMSCs to observe which repaired best, Figure C in this case

Background - Wound Healing Process

Normal Wound

Coagulation and hemostasis
Guided by cytokines (such as TGF-β and TNF-α)
Characteristic signs of wound

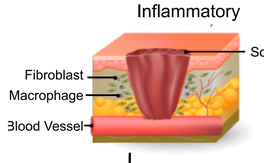
↓

TGF-β increases production of matrix components (collagen, proteoglycans and fibronectin) and begins decreasing secretion of proteases and increases production TIMPs

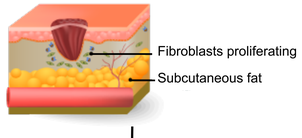
↓

Balance between degradation and synthesis, TIMPs increase while TGF-β and TNF-α regulate the process

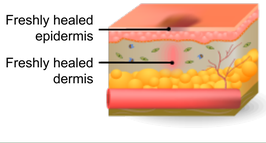
Inflammatory



Proliferative



Remodeling



Chronic Wound

Cannot be repaired within a typical time frame and becomes stuck in between the inflammatory and proliferative phases

Most commonly caused by underlying chronic conditions or disorders such as diabetes, vascular disease, limited mobility or repeated trauma

hMSCs directly influence the inflammatory response and can help enhance or restart the wound healing process

What are the Stages of Wound Healing? (2018, June). What Causes Chronic Wounds? (n.d.).

Maxson et al., Concise review: role of mesenchymal stem cells in wound repair, 2012

Background - Microrheology

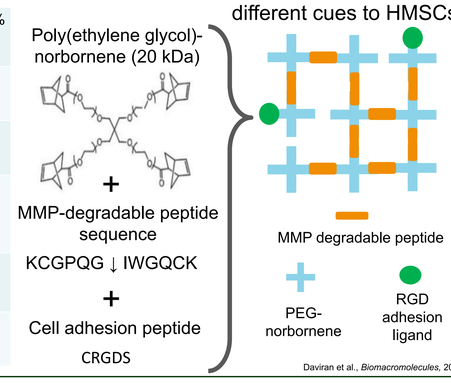
- Encompasses a set of rheometric methods or techniques with unique capabilities that are part of the toolbox for characterizing the rheological properties of materials to aid understanding or help design new materials
- Limited to fairly soft materials
  - •Moduli no more than four pascals
  - •Fluids with viscosities lower than honey
- New capabilities
  - Small sample volumes
  - Short acquisition times
  - Sensitivity
  - Extended range of frequencies
  - Local Rheology
  - Simple Experiments

Microrheology. Eric M. Furst and Todd M. Squires, Oxford University Press (2017).© Eric M. Furst and Todd M. Squires.

Setup - Synthetic Scaffolds and Hydrogel

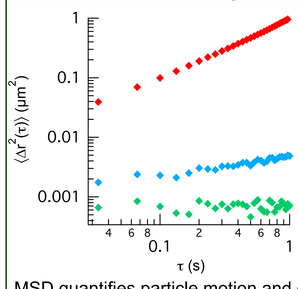
Used as a drug delivery vehicle, provide structural integrity and mimic the material properties of the native tissue as well as a system that can be modified to provide different cues to HMSCs

Poly(ethylene glycol)-norbornene (20 kDa)	PEG-N	3 mM, 6 wt%
MMP-degradable peptide (1,305 Da)	KCGPQG ↓IWGQCK	3.9 mM
Cellular adhesion peptide CRGDS	CRGDS	1 mM
Lithium phenyl-2,4,6-trimethylbenzoylphosphinate	LAP	2.2 mM
Human mesenchymal stem cells	hMSCs	2.0 x 10^5 cells/mL
1 µm probe particle		0.13% solids/vol



Daviran et al., *Biomacromolecules*, 2020

Analysis – Mean Square Displacement



α = 1: freely diffusing probe in fluid — Fluid

0 < α < 1: sub-diffusive probe motion — Viscoelastic fluid/gel

α → 0: probe is arrested in gel scaffold — Gel

2 µm

MSD quantifies particle motion and can help us get information about the state of the material and its rheology. An MSD curve is produced (above) for all the particles in the video at each different lag time. The slope of this curve is alpha which is used to measure how the state of the material and scaffold changes during the degradation of the hydrogel. Some values that can be used to further analyze this data are below.

$$\langle \Delta r^2(\tau) \rangle \longrightarrow \alpha = \frac{d \log \langle \Delta r^2(\tau) \rangle}{d \log \tau},$$

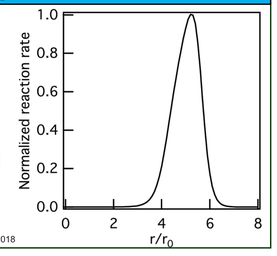
Mean Square Displacement — Logarithmic slope of MSD

McGlynn, J. A., Length scale, 2020 *Savin et al., Static and Dynamic*, 2005

Future Work - Kinetics

$$R = \left(\frac{\frac{k_{off}}{k_{on}}[I_0][S_0][E]}{1 + \left(\frac{k_{off}}{k_{on}} + \frac{k_{des}}{k_{ads}}\right)[E] + \frac{k_{off}}{k_{on}}\frac{k_{des}}{k_{ads}}[E]^2} \right) \times \frac{e^{(-\phi(\frac{r}{r_0}-1))}}{\frac{r}{r_0}}$$

Daviran et al. produced the above rate equation and graph to predict where the cell degradation will begin from its center and compared it to MPT measurements. Future work will focus on fitting this model to our MPT data to determine the mechanism of matrix degradation.

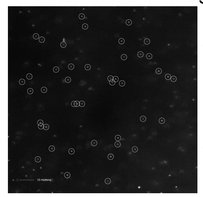


Daviran M et al., Role of Cell-Mediated, 2018

Method - Multiple Particle Tracking Microrheology (MPT)

MPT uses Brownian motion to extract bulk rheological properties

1. Filter noise in images



Neighboring pixels will be averaged together and background gradients are removed to achieve uniform illumination which will remove potential bright spots that would have previously been identified as a particle.

$$A_w(x,y) = \frac{1}{(2w+1)^2} \sum_{i,j=-w}^{w} A(x+i, y+j).$$

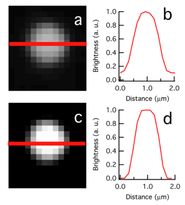
Boxcar Average

particle separation distance > W > particle radius

$$A_{\xi_n}(x,y) = \frac{\sum_{i,j=-w}^{w} A(x+i,y+j) \exp\left(-\frac{i^2+j^2}{4\xi^2}\right)}{\left[\sum_{i=-w}^{w} \exp\left(-i^2/4\xi^2\right)\right]^2}.$$

Gaussian Average

2. Locating particles in images



Particles are identified based on brightness and distance between others. Only the top 30-40% of particles are selected to avoid darker pixels being selected just because they are bright. Eccentricity and radius of gyration are calculated to exclude particles that may not be the correct size or not particularly circular.

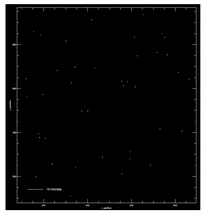
$$\begin{pmatrix} \varepsilon_x \\ \varepsilon_y \end{pmatrix} = \frac{1}{m_0} \sum_{i+j^2 \le w^2} \begin{pmatrix} i \\ j \end{pmatrix} A(x+i, y+j),$$

Offset Distance

$$R_g^2 = \frac{1}{m_0} \sum_{i^2+j^2 \le w^2} (i^2+j^2) A(x+i, y+j).$$

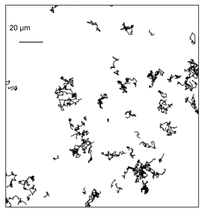
Squared Radius of Gyration

3. Refining particle positions



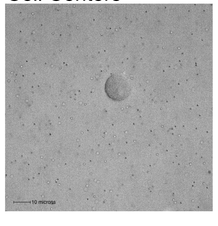
Positions connected to form trajectories using a probability distribution function that describes Brownian motion. To limit the particles that swap positions, the particle separation distance should be greater than the distance the particle moves between each frame. Particles that disappear and reappear between frames are taken into account by specifying the max amount of frames in a row that a particle will be considered different if it reappears. Trajectories of particles can also be rejected if the particle does not appear for enough frames in a row.

4. Linking positions into trajectories



The algorithm creates a text file with the particles x and y coordinates, brightness, radius of gyration squared, eccentricity, frame number and particle ID.

5. Cell Centers

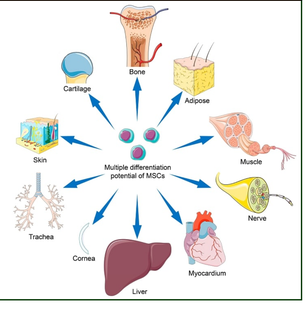


The cell centers from each experiment were measured by tracing the outline of the cell and used to calculate the cell center.

Conclusions

1. Able to understand how cells degrade our material and change the way the cells move
2. Able establish design rules so hMSC scaffolds can last longer in the body and correctly deliver cells to a wound

This research is important in the field of biomaterials to evolve biological systems by mimicking the body's environment and characterizing the necessary design rules. hMSCs play an important role in each phase of the wound healing process and implantable synthetic hMSCs would prevent wounds from becoming chronic.



Louis and Cona, What are Mesenchymal Stem Cells (MSCs)?, 2020

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