Characterizing Human Mesenchymal Stem Cell Motility in Response to the Wound Environment

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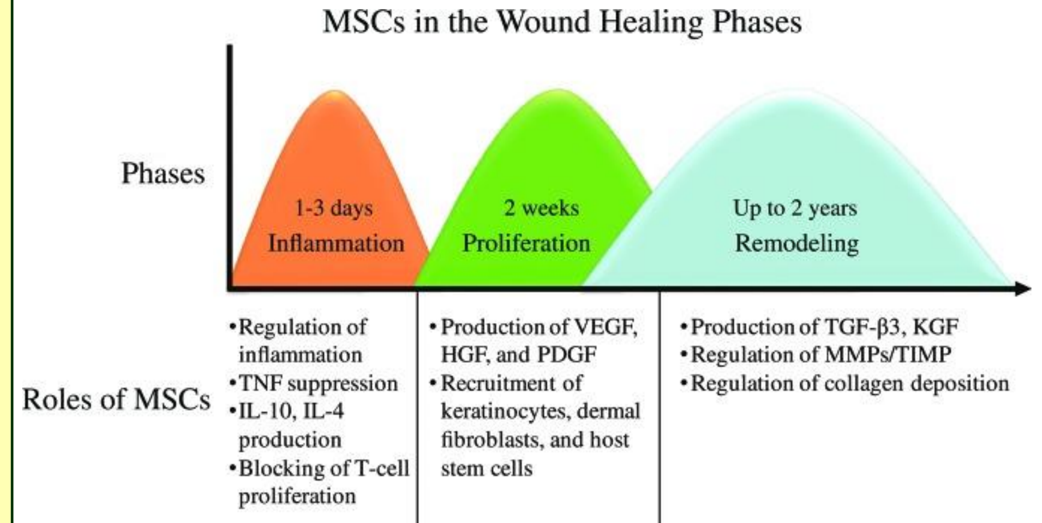
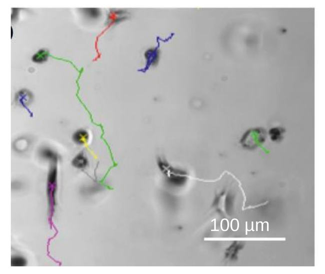
Abstract

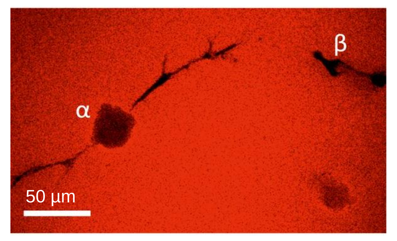
Human mesenchymal stem cells (hMSCs) provide an opportunity to treat chronic wounds by helping these wounds progress past the inflammatory phase. They perform an important regulatory function in the inflammation stage of wound healing by reducing secretion of tumor necrosis factor-alpha (TNF-α), an inflammatory cytokine, and increasing secretion of interleukin-10 (IL-10) and IL-4, which are anti-inflammatory cytokines. The ability of hMSCs to migrate through the extracellular matrix (ECM) is affected by their secretion of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs). MMPs promote hMSC migration by degrading components of the ECM to form micrometer channels to travel through to reach the wound site. TIMPs inhibit MMP degradation. Synthetic hydrogel scaffolds with encapsulated hMSCs are designed to mimic the ECM and are being developed to deliver additional hMSCs to the wound site to assist with healing. The body releases signals from the wound site during healing, including cytokines TNF-α and transforming growth factor beta (TGF-β) . TNF-α increases hMSC secretion of MMPs and TGF-β increases secretions of TIMPs. In this work, we incubate hMSC-laden synthetic scaffolds in media with cytokines to model the native environment these materials would experience after implantation in a wound. We also develop models using Michaelis-Menten enzymatic inhibition kinetics to predict how the cytokine TNF-α affects the process of hMSC-mediated remodeling of synthetic hydrogel after implantation.

Human mesenchymal stem cells (hMSCs) role in wound healing





Motility of several hMSCs tracked for a 6-hour period. (Pathways shown by colored lines)



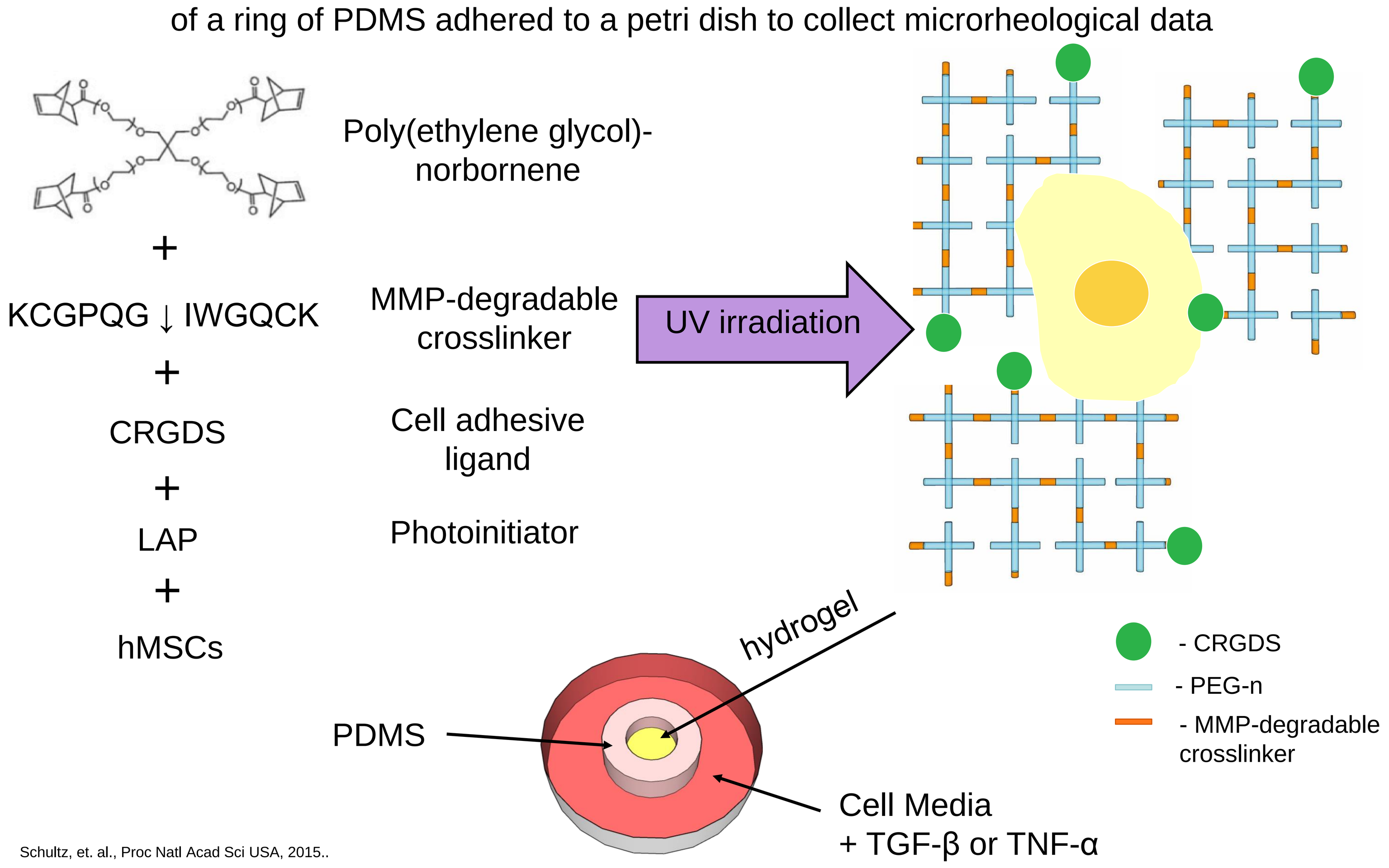
Cell migrates from point α to point β over a 48-hour period. The dark regions show the void regions of the hydrogel resulting from degradation

- hMSCs help chronic wounds progress past the inflammatory phase
- hMSCs migrate through the ECM by secreting MMPs which degrade micrometer channels into the ECM and TIMPs which inhibit the action of MMPs

Maxson, et. al., Stem Cell Transl Med, 2012.
Ries, et. al., Blood, 2007.
Corrigan, et. al., Langmuir, 2009
Nuttelman, et. al., Matrix Biol., 2005.
Guvendiren, et. al., Curr. Opin. in Biotech., 2013.
Schultz,et. al., Proc Natl Acad Sci USA, 2015.

Poly(ethylene glycol)-norbornene synthetic hydrogels

hMSCs are encapsulated in a PEG-N hydrogel scaffold which is then inserted in the center of a ring of PDMS adhered to a petri dish to collect microrheological data



Schultz, et. al., Proc Natl Acad Sci USA, 2015..

Multiple particle tracking microrheology (MPT)

MPT tracks fluorescent particles to measure their Brownian motion, this allows us to determine the rheological properties and state of the hydrogel

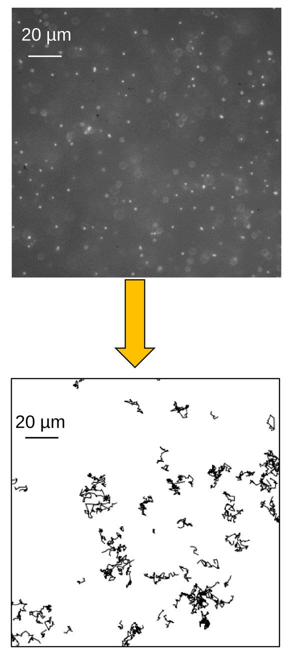
Generalized Stokes-Einstein relation

$$\langle \Delta r^2(t) \rangle = \frac{k_B T}{\pi a} J(t)$$

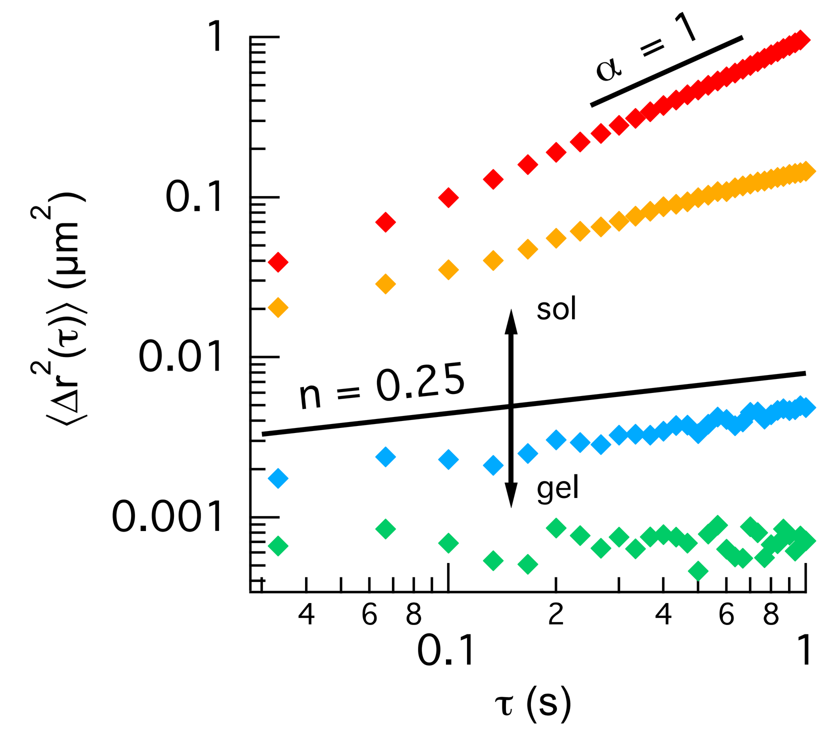
Logarithmic slope of MSD

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

$\langle \Delta r^2(t) \rangle$ – Mean-squared displacement (MSD)
J(t) – Creep Compliance
k_B – Boltzmann constant
T – Temperature
a – probe particle radius
d – dimensionality
τ – lag time



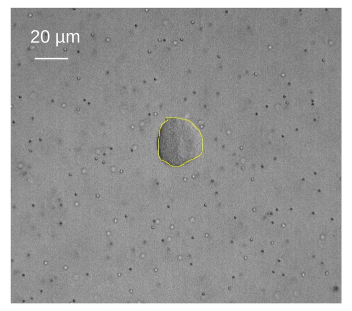
Florescent probe particle image converted into particle trajectories



Schultz, et. al., Proc Natl Acad Sci USA, 2015.
Corrigan, et. al., Langmuir, 2009.
Daviran et. al., *ACS Biomater. Sci. Eng.*, 2018.

MATLAB code development

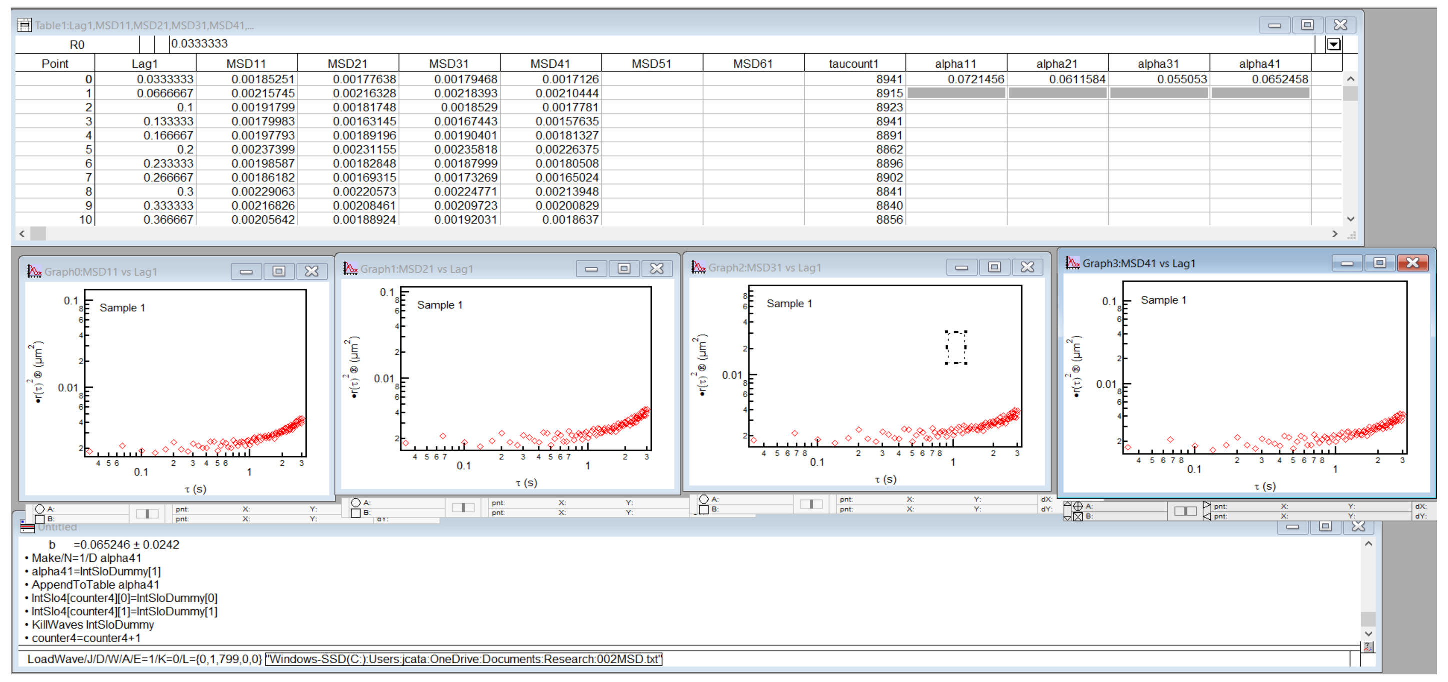
We measure cell speed by finding cell centers in ImageJ. I developed a MATLAB code that both speeds up and increases the accuracy of this process. The user circles the cell for Image J to calculate the center of the cell (x,y). MATLAB automatically iterates through all the cell samples.



Brightfield cell image with cell circled by user in ImageJ

```
while 1
    MIJ.run("Select None");
    MIJ.run("Flip Vertically");
    ij.IJ.runMacro('setTool("freehand");');
    ij.IJ.runMacro('waitForUser("Waiting for user to draw. Press Okay to continue");');
    MIJ.run("Measure");
    MIJ.run("Open Next");
    if(strcmp(MIJ.getCurrentTitle,'cell_001.tif'))
        break;
```

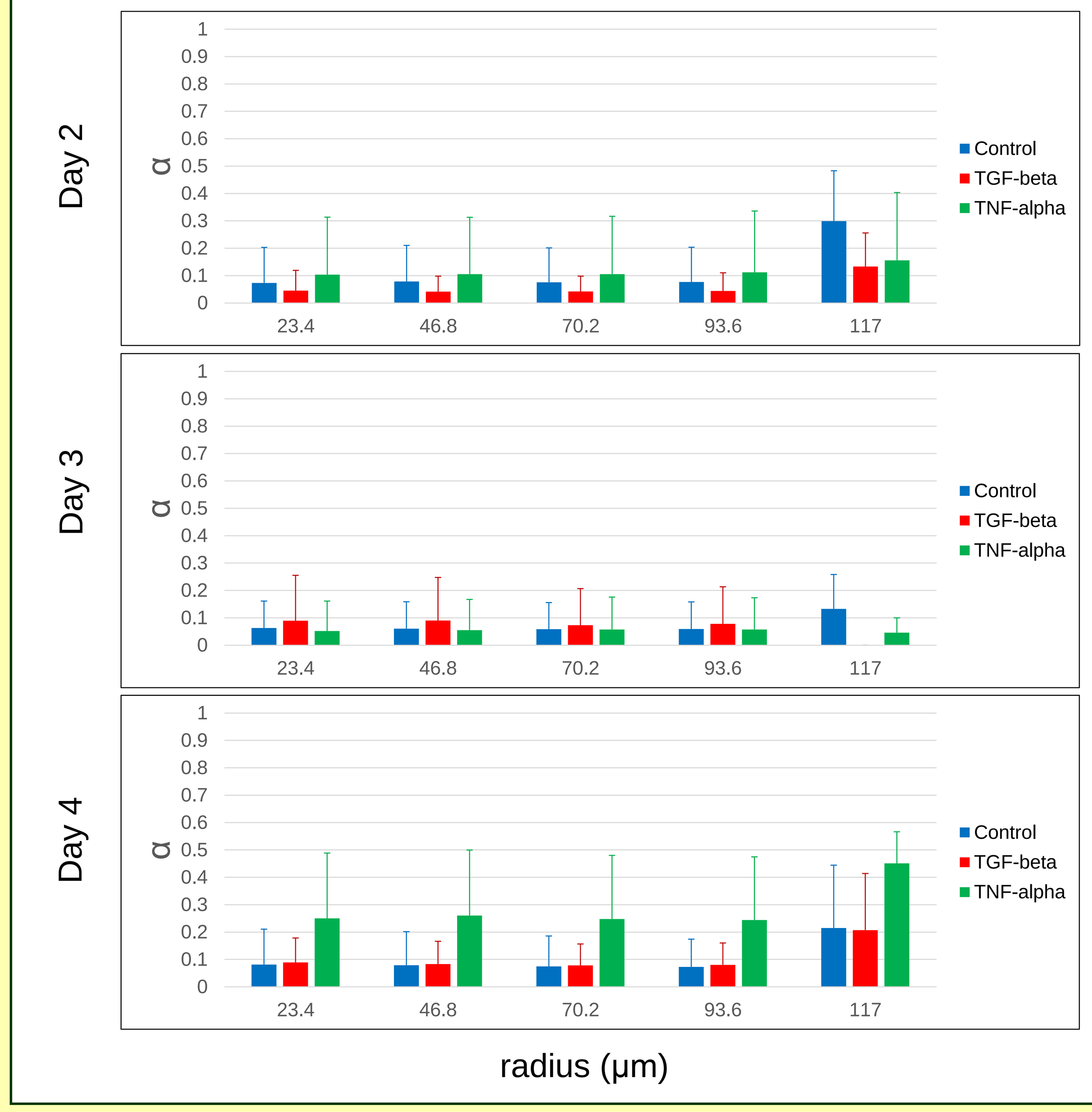
MATLAB code relies heavily on Fiji's Miji program's (mij.jar) ability to take MATLAB strings and convert to Image J Macros



A second part of the code takes the cell centers and corresponding probe text files as an input to create Igor Pro code that can be used to calculate, graph, and tabulate the alpha values for a set of 4 distances (r) away from each cell.

TGF-β & TNF-α effects on cell degradation profiles

Data taken over 4 days of degradation of hMSC-mediated scaffold remodeling when incubated in TGF-β, TNF-α, and cell media (control).



- Cells begin degrading the hydrogel at larger distances first.
- We expect TNF-α samples to be the most degraded, followed by Control and then TGF-β samples.
- At day three we see very little difference in the samples' level of degradation
- At day four TNF-α samples are significantly more degraded than other samples at every distance

Michaelis-Menten kinetic model

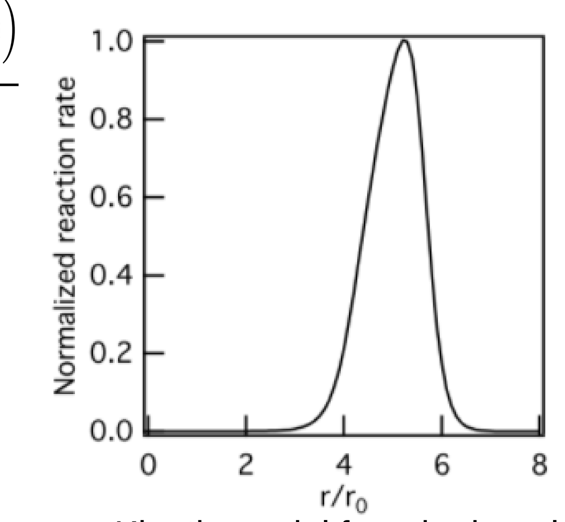
hMSC degradation of a PEG-N hydrogel can be modeled using Michaelis-Menten kinetics.

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

$$\phi = r \sqrt{\frac{k_{cat}}{D}}$$

R – rate of MMP-TIMP unbinding
r – distance from the cell
$[S_0]$ – initial MMP (substrate) concentration
$[I_0]$ – initial TIMP (inhibitor) concentration
k_{off} & k_{on} – rate of unbinding and binding of TIMPs and MMPs
k_{ads} & k_{des} – rate of adsorption and desorption
r_0 – radius of cell
k_{cat} – catalytic rate constant
D – diffusivity

Daviran et. al., *ACS Biomater. Sci. Eng.*, 2018.



- Kinetic model for a hydrogel without cytokines
- To model TNF-α sample kinetics, initial MMP concentration can be modified to fit experimental data

Conclusions

- We can add cytokines TGF-β and TNF-α into cell media to test their effects on hMSC degradation.
- TNF-α increases hMSC degradation by increasing MMP secretion, effects are especially prevalent at a larger time scale.
- Although TGF-β increases TIMP secretion, additional TIMP secretion doesn't slow down degradation any further, meaning TIMPs are already present in excess.
- Cell-mediated degradation kinetics when incubated with TNF-α can be found by altering MMP concentration in a Michaelis-Menten kinetic model

Acknowledgements

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