Characterizing Cell-Material Interactions When Chemical Cues are Presented Locally to Human Mesenchymal Stem Cells

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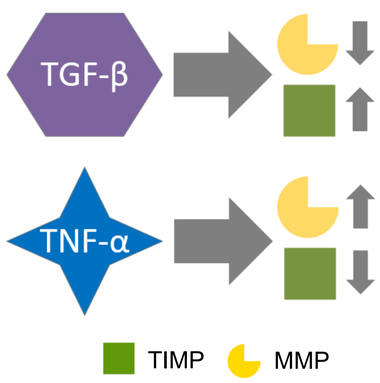
Introduction

- Cell-laden hydrogels are designed to enhance cell delivery to and create structure for damaged tissues in wound healing applications.
- Human mesenchymal stem cells (hMSCs) are chosen for cell delivery due to their importance to tissue regeneration in signaling to other cells during wound healing
- Cytokines, which are present in the native wound environment, are tethered to the hydrogel network to determine their effect on hMSC remodeling

Cytokines and Wound Healing

TNF- α and TGF-β are cytokines which signal to hMSCs during wound healing

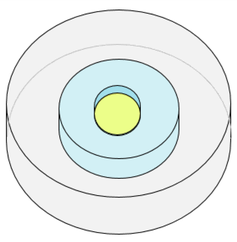


TGF-β
- Present from inflammation to remodeling
- Promotes ECM structure by increasing TIMP secretions which inhibit MMP activity

TNF- α
- Present primarily in the inflammatory stage of wound healing
- Increases network degradation by increasing hMSC secretion of MMPs and inhibiting production of TIMPs

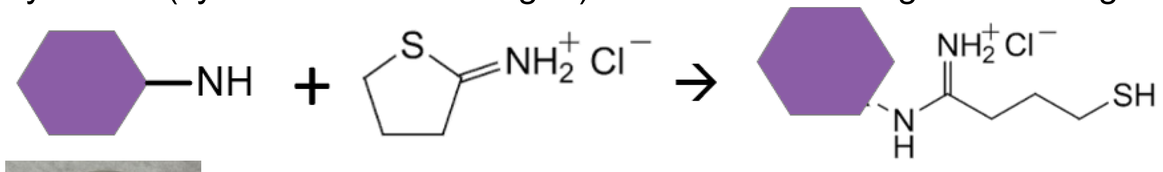
Poly(ethylene-glycol) Hydrogels

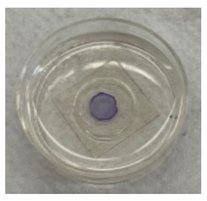
hMSCs are encapsulated into a poly(ethylene-glycol)-norbornene (PEG-N) hydrogel.
- Gels formed via a step-growth photopolymerization
- Gels are kept at 37 °C and 5% CO_2
- ☆ Hydrogels have a MMP degradable crosslinker



Cytokine Thiolation

Cytokines (symbolized with hexagon) were thiolated using Traut's reagent.

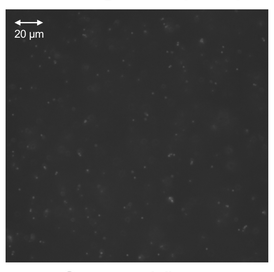




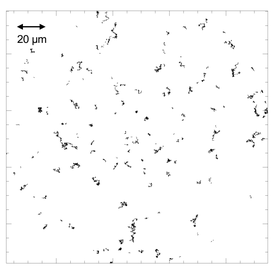
The presence of cytokines in the hydrogel is confirmed using an enzyme-linked immunosorbent substrate assay (ELISA). The blue color in the gel (left) indicates that the cytokines are successfully tethered into the network.

Multiple Particle Tracking Microrheology (MPT)

MPT is a passive microrheological technique that measures the Brownian motion of fluorescent probes embedded in the hydrogel network to obtain bulk rheological properties.



Camera View



Particle Trajectories

$\langle \Delta r^2(\tau) \rangle$

Ensemble-averaged mean-squared displacement (MSD)

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

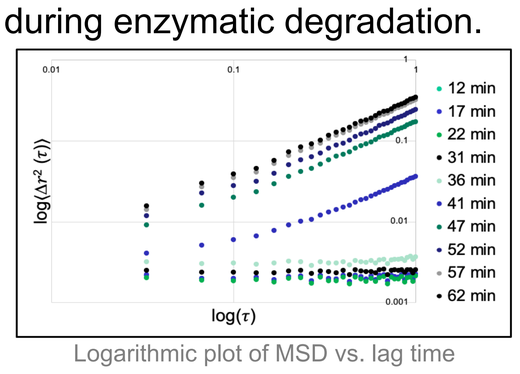
Logarithmic slope of MSD

α is the logarithmic slope of the MSD and is used to quantify the state of the material
α → 0: gel
0 < α < 1 : viscoelastic gel or liquid
α = 1: liquid

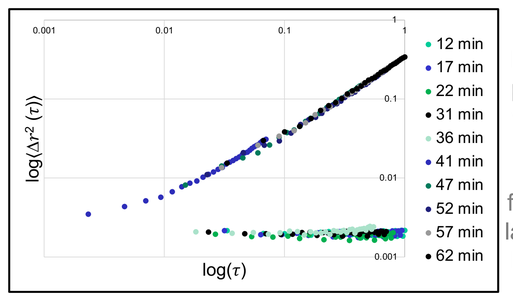
Transition from gel to sol is determined by comparing α to the critical relaxation exponent, n
α > n: sol
α < n: gel
n= 0.25 ± 0.05 for our hydrogels

Enzymatic Degradation in the Absence of Cells

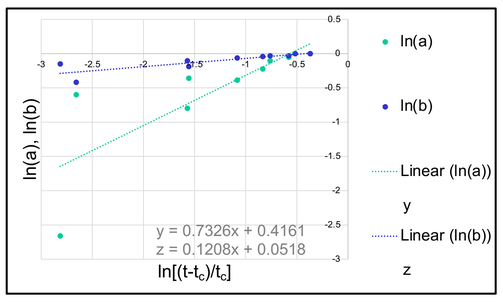
Time cure superposition (TCS) was used to determine the critical relaxation exponent of gels with tethered TGF-β, tethered TNF- α, and no cytokines during enzymatic degradation.



Logarithmic plot of MSD vs. lag time



Logarithmic plot of MSD vs. lag time applying shifting factors a (on lag time) and b (on MSD)



Logarithmic plot shifting factors vs. $(t-t_c)/t_c$ where t_c is critical time (the time at which gel-sol transition occurs)

Calculate critical relaxation exponent (n) using the following information:

Assuming

$$t \propto p \qquad a \sim {\tau_L}^{-1} \sim \left(\frac{|p - p_c|}{p_c}\right)^y \qquad b \sim \frac{1}{{J_e}^o} \sim \left(\frac{|p - p_c|}{p_c}\right)^y$$

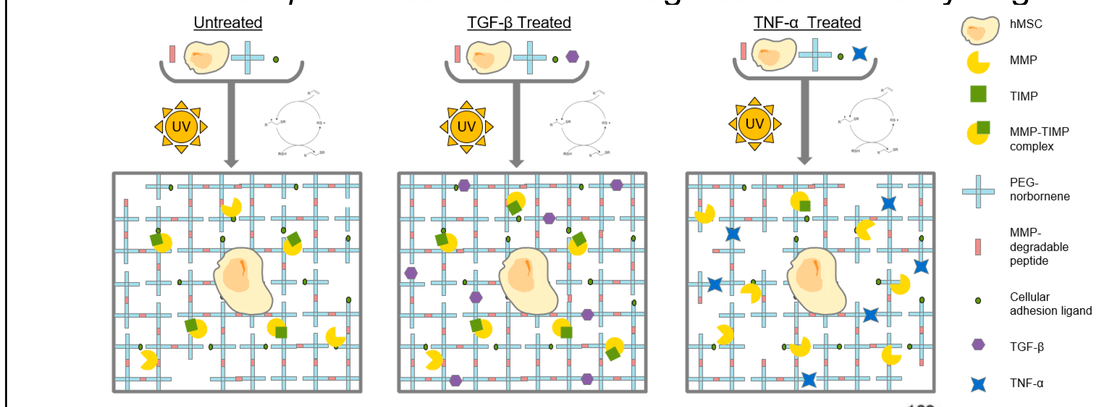
$$n = \frac{z}{y}$$

Using TCS, the following n values were determined for gels with tethered TNF- α, tethered TGF-β, and untreated PEG-N hydrogels. A t-test determined the difference in n between the 3 treatment groups is not statistically significant. This means that tethering cytokines to the network does not affect the material structure during enzymatic degradation.

Treatment	n
Untreated	0.212 ±0.11
TGF-β	0.236 ±0.18
TNF-α	0.233 ±0.12

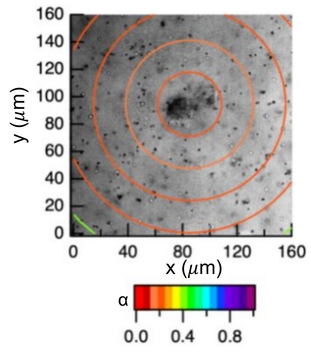
Cell-Mediated Degradation

TNF- α and TGF-β alter cell-mediated degradation of the hydrogel network



Untreated Group Preliminary Results:

Day after hMSC Encapsulation	Average Field of View α ± Standard Deviation
2	0.02151 ± 0.02455
3	0.02856 ± 0.02697
4	0.05753 ± 0.06933



Because the most activity was seen in day 4 after encapsulation, a plot is shown with radially averaged α values for a sample cell from day 4 in control treatment group (left). The circles are colored according to their respective averaged α values (see key below figure). These measurements show little remodeling directly around cells and increasing degradation as the distance from the cell increases.

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