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

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

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

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% CO₂
- 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.

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

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

\[ n = 0.25 \pm 0.05 \text{ for our hydrogels} \]

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.

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

\[ r \propto t^{-\alpha} \]
\[ b \propto \langle \Delta r^2 (\tau) \rangle \]

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.

Cell-Mediated Degradation

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

Untreated Group Preliminary Results:

<table>
<thead>
<tr>
<th>Day after hMSC Encapsulation</th>
<th>Average Field of View</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.02151 ± 0.02455</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.02856 ± 0.02697</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.05753 ± 0.06933</td>
<td></td>
</tr>
</tbody>
</table>

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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