Hydrogel Stiffness and Degradation Regulate Adipose Expansion in vitro
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Introduction
Animal studies can provide insight into the role of the extracellular matrix (ECM) in adipose function, but do not allow for precise control over environmental conditions. Historically, two-dimensional (2D) substrates have been used to investigate cell–ECM interactions. However, cells in vivo are in a 3D environment surrounded by ECM. The functional response of cells to ECM is different when cells are confined to a monolayer in a 2D versus a 3D culture system. In this study, we use poly(ethylene glycol) diacrylate (PEGDA) hydrogels containing enzymatically degradable peptide sequences to study adipose expansion in 3D culture. We are interested in the influence of ECM properties on the expansion, differentiation, and function of adipose tissue. We hypothesize that increased ECM stiffness will limit preadipocyte invasion. In this project we have investigated the role of model ECM properties on adipose tissue growth.

Materials and Methods
Peptide sequences consisting of either single (SS) or multiple (TS) collagenase-sensitive (GGL↓GPAGGK) cleavage sites were synthesized using solid phase peptide synthesis and conjugated to PEG to form SS and TS degradable PEGDA macromers. SS and TS PEG hydrogels with different crosslinker content (3-5% w/vol) were formed using free-radical photopolymerization as previously described (1, 2). Hydrogel mechanical properties were evaluated using compression testing and the degradation rates measured via collagenase enzyme incubations as previously described (Table 1) (2). 3T3-L1 preadipocyte cell spheroids were formed using methylcellulose and 20,000 cells. Spheroids were encapsulated in the hydrogels and imaged every other day from day 0 to 20 with an Axiovert 200 inverted microscope using a 5x objective (1.255 mm/pixel) (Carl Zeiss MicroImaging, Inc.). Cell invasion into the surrounding hydrogels was monitored by tracing the projected area of the spheroid using AxioVision 4.8 Image Analysis software. ANOVA was performed to compare groups (SigmaPlot 11) with p<0.05 considered statistically significant.

Results
As previously shown (1,2) for any given PEG content (or modulus) material degradation time was dependent on the hydrogel type (SS or TS) with significant decreases in degradation time in TS hydrogels. Hydrogel mechanical properties depended on PEGDA concentration. Healthy adipose tissue is ~2 kPa and lies within the range of the obtained hydrogel stiffness values 0.3-3.3 kPa (3). The 3% TS and 4% TS groups had significantly more cell invasion in 4% SS and both 5% SS and 5% TS (Figure 1). Additionally 4% TS had significantly more cell invasion than 3% SS. The 3% TS group had the most invasion (Figure 1). The invasion by 3T3-L1 decreased with increasing stiffness of the hydrogel. Interestingly, the 5% TS had an invasion area similar to the 5% SS, 4% SS, and 3% SS.

<table>
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<tr>
<th>PEGDA Concentration</th>
<th>Compressive Modulus (kPa)</th>
<th>Degradation Time (hrs)</th>
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<tbody>
<tr>
<td>SS</td>
<td>TS</td>
<td>SS</td>
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<tr>
<td>3%</td>
<td>0.38±0.04</td>
<td>0.31±0.05</td>
</tr>
<tr>
<td>4%</td>
<td>1.23±0.12</td>
<td>1.08±0.03</td>
</tr>
<tr>
<td>5%</td>
<td>3.63±0.24</td>
<td>3.33±0.28</td>
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Table 1: Compressive modulus and degradation rates as a function of PEGDA concentration and hydrogel type (SS or TS) (2).

Discussion and Conclusions
This study has shown that utilizing degradable PEGDA hydrogels it is possible to restrict the invasion of preadipocytes by modulating the stiffness and relative degradation of the hydrogels.

References
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