Evaluation of Sensitivity and Specificity of MMP Substrates in PEG Hydrogels for Neovascularization

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Introduction
Biomaterial strategies for engineering tissues of clinically relevant size require the formation of rapid and stable neovascularization. The ability of an engineered scaffold to induce vascularization is highly dependent on its degradation rate. Matrix metalloproteinases (MMPs) play an important role in mediating cell-induced proteolytic matrix degradation, remodeling and controlled neovascularization. Poly (ethylene glycol) PEG hydrogels have been extensively investigated as scaffolds for tissue engineering applications due to their ease in tuning material properties and selective biochemical modification. In this study, the effect of proteolytic cleavage site concentration on degradation and the role of MMP-sensitive peptide PEGDA substrate specificity and sensitivity to degradation by MMPs expressed during neovascularization were investigated for degradation, neovascularization, and tissue remodeling.

Materials and Methods
Peptide substrates previously identified for cleavage by MMP-2 and MMP-14 enzymes (Turk, et al., 2001) and a collagenase-sensitive substrate were synthesized in a single (SSite) and triple (TriSite) cleavage site form. The MMP substrates were incorporated into PEG hydrogels in SSite and TriSite form and evaluated for mechanical properties, sensitivity to degradation by MMP-2 and -14 enzymes, and neovascularization and tissue remodeling in vitro and in subcutaneous implant models in vivo.

Results
The mechanical properties of the hydrogels show no statistical significant differences among all hydrogel groups investigated (Table 1). Hydrogel degradation was tested by incubation with MMP-2 and MMP-14 enzymes. Collagenase-sensitive SSite and TriSite gels had low sensitivity to degradation by both MMP-2 and MMP-14. Incubation with MMP-2 and MMP-14 enzyme showed that TriSite gels had a faster degradation time compared to SSite gels and that MMP-14 substrates had lower sensitivity to degradation by MMP-2 as compared to MMP-2 scaffolds. In vivo tissue invasion results were consistent with in vitro results (data not shown). Invasion increased from weeks 1 to 3 and with increasing cleavage site concentration (TriSite gels) (Figure 1A). Masson’s trichrome staining shows a thicker layer of collagen rich tissue in SSite gels as compared to TriSite gels. Inflammatory tissue was present in all samples but was more prominent in the TriSite gels (Figure 1B). Analysis of the microvasculature shows that all hydrogel groups supported vessel formation regardless of cleavage site concentration or the type of MMP-sensitive peptide substrate (Figure 2). There were no significant differences in vessel density between SSite and TriSite groups with decreased vessel density observed from weeks 1 to 3.

Table 1: Compressive modulus and degradation rates by MMP-2 and MMP-14 enzymes as a function of MMP substrate and cleavage site concentration (SSite or TriSite).

<table>
<thead>
<tr>
<th>Collagenase Substrate</th>
<th>MMP-2 Substrate</th>
<th>MMP-14 Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSite</td>
<td>TriSite</td>
<td>SSite</td>
</tr>
<tr>
<td>Modulus (kPa)</td>
<td>0.3±0.05</td>
<td>0.3±0.04</td>
</tr>
<tr>
<td>Degradation Time (days)</td>
<td>12±</td>
<td>12±</td>
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<tr>
<td>Degradation Time (days)</td>
<td>12±</td>
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Figure 1. A. Depth of tissue invasion.* indicates statistical significance between SSite and TriSite gels. # indicates statistical significance within the SSite hydrogel group (p<0.05) (n=5). B. Representative Masson’s trichrome stained samples at week 1(top) and week 3 (bottom).

Figure 2. A. Vessel density quantified from lectin stained tissue sections. * indicates statistical significance with p<0.05 (week1 n=5).B. Representative lectin stained samples.

Discussion and Conclusions
This is the first study involved in screening the effect of proteolytic cleavage site concentration and MMP-sensitive peptide substrates for their sensitivity to enzyme degradation by a variety of MMPs that play an important role in neovascularization and tissue remodeling inductive capabilities in vivo.