Molecularly Engineered PEG Hydrogels with Enhanced Proteolysis

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Statement of Purpose: Proteolytically sensitive synthetic hydrogels, formed by a Michael-type addition of end-functionalized poly(ethylene glycol) (PEG) macromers with cysteine-containing peptide crosslinkers, have been explored by our lab to mimic functions of the native extracellular matrix and promote cellular migration into the polymer hydrogel [1]. These peptide crosslinkers include matrix metalloproteinase (MMP)-sensitive domains for cell-demanded proteolysis, and the hydrogels can be further functionalized with RGD sequences or growth factors to promote cell adhesion and migration.

It has been shown that physiologically normal angiogenesis can be induced by sustained delivery of vascular endothelial growth factor (VEGF) from these hydrogels [2]. However, one limitation is that the remodeling rate of the MMP-sensitive PEG may limit the rate of cellular infiltration and consequently angiogenesis. The hypothesis of this research is that increased proteolytic degradation and subsequent cellular infiltration will result in a more robust healing response. The goal of this study, therefore, is to optimize the enzymatic degradation properties of the PEG hydrogels. MMP substrates that have been optimized for degradation by combinatorial screening methods [3, 4] or that are found in other matricellular proteins [5, 6] were compared with control peptides based on the MMP substrate site within type I collagen, and the effects on peptide and hydrogel degradation were measured.

Methods: *Hydrogel Fabrication:* Peptides were synthesized on solid resin using an automated peptide synthesizer (PerSeptive Biosystems, Farmington, MA) with standard F-moc chemistry. Branched 4-arm PEG (Shearwater Polymers, Huntsville, AL) was functionalized as previously described [1]. Hydrogels were formed by mixing 10% (w/v) functionalized PEG in 0.3M triethanolamine, pH 8.0, with stoichiometric amounts of cysteine-containing peptides.

Determination of Degradation Kinetics of Soluble Peptides: The kinetic parameters of substrate degradation (K_M and k_{cat}) were measured by incubating substrates with MMP-1 or MMP-2 (VWR, Dietikon, Switzerland) at 30ºC followed by reaction with fluorescamine and detection of fluorescence, as previously described [7]. K_M and k_{cat} were determined by fitting rate *vs.* substrate concentration data to the Michaelis-Menten equation.

Biochemical Degradation of Hydrogels: Hydrogels were incubated at 37ºC in 3nM MMP-1 or 8nM MMP-2 and were checked daily for degradation.

Results: *Degradation Kinetics of Soluble Peptides:* As seen in Figure 1, faster degrading peptide substrate sequences were found for both MMP-1 and MMP-2. The fastest degrading substrates for MMP-1 (E1-E4) had k_{cat} values that were 5- to 7-fold higher than controls. Substrates E1, E2, and E4 also showed an almost 2-fold increase in k_{cat} when degraded by MMP-2. Peptides E5 and E6 had moderate increases in k_{cat} compared to controls for both MMP-1 and MMP-2. Interestingly, two peptides that were not degraded by MMP-1 (E8 and E12) showed enhanced degradation by MMP-2.

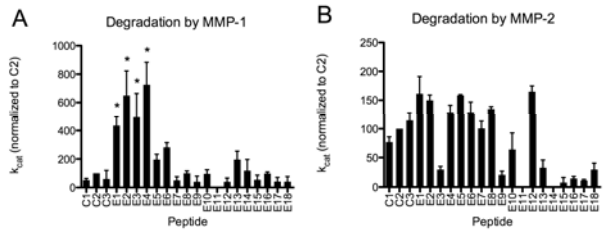

Figure 1. Kinetic parameters measured for degradation by MMP-1 (A) or MMP-2 (B). Peptides C1-C3 are controls based on type I collagen. Peptides E1-E5 are from [3], E6-E7 from [4], and E8-E16 from [5, 6]. * indicates significance at $p < 0.05$ compared to controls.

Degradation of Hydrogels: As expected from the kinetic analysis, hydrogels formed using the optimized peptides degraded faster than hydrogels with control peptides. Hydrogels formed from peptides E1, E2, and E4 degraded in 2-10 days when exposed to MMP-1 or MMP-2. Hydrogels formed from peptide E12 degraded in 8-9 days only when exposed to MMP-2. Control hydrogels and hydrogels made from non-degrading peptides remained intact for over 4 weeks when exposed to either enzyme.

Conclusions: In this study, we have identified several peptides that have significantly increased k_{cat} values compared to control peptides, including peptides with high sensitivity to MMP-1, sensitivity to MMP-1 and MMP-2, and sensitivity only to MMP-2. These optimized peptides result in hydrogels that degrade faster *in vitro*. This corroborates previous findings with hydrogels utilizing the MMP substrate sequence from collagen with various point mutations as crosslinker peptides [1]. Lutolf *et al.* further demonstrated that the kinetic parameters measured for soluble peptides ultimately determined the degradation behavior of the hydrogels *in vitro* and their ability to stimulate cellular infiltration *in vivo* [1]. This indicates that our faster degrading peptides should lead to increased cell migration in *in vitro* assays and increased cellular infiltration and ultimately more robust healing *in vivo*. Furthermore, the graded increases in k_{cat} and the differential responses for MMP-1 and MMP-2 can be used to engineer hydrogels with degradation properties tuned to promote infiltration by particular cell types. We believe that these optimized hydrogels will provide a better matrix to support angiogenesis as well as can be utilized for a range of tissue regeneration applications.

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