Tunable Proteolytic Degradation of Molecularly Engineered PEG Hydrogels for Enhanced Cellular Invasion

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Statement of Purpose: Synthetic hydrogels, such as those formed from Michael-type addition reactions of end-functionalized poly(ethylene glycol) (PEG) macromers with cysteine-containing peptides [1], offer several advantages as extracellular matrix mimetics and tissue engineering scaffolds including reduced risk of immune reaction and ease of material handling. To form a cell-responsive hydrogel, modified multi-arm PEG can be functionalized with cysteine-containing cell adhesion ligands, growth factor binding ligands, or growth factors and crosslinked by protease-sensitive substrates flanked by cysteine-containing domains. Previous research in our lab has shown that modifying the protease substrate in the crosslinker could render the hydrogels more or less degradable, with the more degradable hydrogels leading to more bone formation *in vivo* [1]. However, for soft tissue repair applications, the remodeling rate of the hydrogels may limit cellular infiltration and may be too slow for angiogenesis and tissue regeneration. We hypothesized that increased proteolytic degradation would lead to enhanced cellular invasion and result in a more robust healing response. Therefore, the biochemical degradation properties were examined for hydrogels formed with different protease substrates that have been optimized by combinatorial screening methods [2, 3] or that are found in matricellular proteins [4, 5]. The ability of faster degrading hydrogels to support enhanced cellular invasion was also tested in several *in vitro* models.

Methods: *Hydrogel Fabrication:* Peptides were prepared by standard solid phase synthesis. Branched 4-arm PEG (Shearwater Polymers, Huntsville, AL) was modified and hydrogels were formed as described [1].

Degradation Kinetics of Soluble Peptides and Hydrogels: Substrates were degraded at 30°C with 10nM matrix metalloproteinase (MMP)-1 or MMP-2 (VWR, Dietikon, Switzerland) or 0.1U/mL plasmin (Roche, Rotkreuz, Switzerland). The rates of degradation were quantified as described [6], and K_M and k_{cat} were determined. Hydrogels were degraded at 37°C with 10nM MMP-1, 20nM MMP-2, or 0.02U/mL plasmin and checked daily.

Cell-Induced Remodeling: Primary mouse myofibroblasts were seeded at 250,000 cells/mL in hydrogels formed with different substrate sequences that also contained RGD as a cell adhesive ligand. Cellular attachment and spreading in 3-D was visualized by confocal microscopy.

Chick Aortic Ring Assay: Segments of embryonic (day 8-10) chick aortae were embedded in the hydrogels, and cell invasion was visualized by brightfield microscopy.

Results: *Degradation of Soluble Peptides and Hydrogels:* The peptide substrates had a range of k_{cat} values for MMP-1, MMP-2, and plasmin. For example, the data from MMP-1 are shown in Figure 1A. The fastest degrading substrates for MMP-1 (E1-E4) had k_{cat} values that were 5- to 7-fold higher than controls while substrates E5, E6, and E10 had moderate increases in k_{cat}. In contrast to little degradation by MMP-1, peptide E14 was an efficient substrate for degradation by MMP-2. Peptides E1-E2, E4-E9, and E12-E13 also had small increases in k_{cat} when degraded by MMP-2. Plasmin showed a different pattern of degradation than the MMPs. The most efficient substrates were E5 and E10, which had an approximately 12-fold increase in k_{cat}, and E13-E14, which had a 5-fold increase. Hydrogels formed using the peptides with higher k_{cat} values generally degraded faster than controls for each enzyme (MMP-1 in Figure 1B).

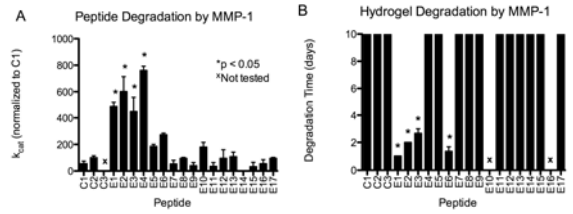

Figure 1. (A) k_{cat} of soluble peptides for MMP-1. (B) Hydrogel degradation by MMP-1. C1-C3 are controls [1]. E1-E6 are from [2]; E7-E8 from [3]; E9-E18 from [4, 5].

In Vitro Cell Invasion: Fibroblasts showed increased cell spreading compared to controls when cultured in hydrogels with selected faster degrading peptides. Additionally, cell invasion from aortic ring segments was observed for the faster degrading hydrogels while little cell invasion was observed for the controls (Figure 2).

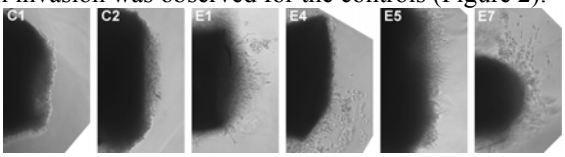

Figure 2. Aortic ring assay showing cell invasion into hydrogels. Peptides are the same as in Figure 1.

Conclusions: In this study, several protease sensitive peptides that have increased k_{cat} values compared to the MMP cleavage sequence in type I collagen were utilized to render molecularly engineered PEG hydrogels more degradable. The most efficient substrates in some cases overlap and in other cases differ among the three enzymes tested, and a range of k_{cat} values were obtained for each enzyme. The peptides result in hydrogels that degrade faster when exposed to the appropriate enzyme(s) and lead to increased cell spreading and cell invasion *in vitro*. The graded increases in k_{cat} and the differential responses for MMP-1, MMP-2, and plasmin can be used to engineer hydrogels with degradation properties tuned to the enzymes produced by particular cell types. These faster degrading hydrogels should lead to increased cellular infiltration and ultimately more robust healing *in vivo* and should provide a better matrix to support angiogenesis as well as soft tissue regeneration.

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