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Controlled biodegradation of Self-assembling β-hairpin Peptide hydrogels by proteolysis with matrix metalloproteinase-13

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ABSTRACT

Controlled biodegradation specific to matrix metalloproteinase-13 was incorporated into the design of self-assembling β-hairpin peptide hydrogels. Degrading Peptides (DP peptides) are a series of five peptides that have varying proteolytic susceptibilities toward MMP-13. These peptides undergo environmentally triggered folding and self-assembly under physiologically relevant conditions (150 mM NaCl, pH 7.6) to form self-supporting hydrogels. In the presence of enzyme, gels prepared from distinct peptides are degraded at rates that differ according to the primary sequence of the single peptide comprising the gel. Material degradation was monitored by oscillatory shear rheology over the course of 14 days, where overall degradation of the gels vary from 5% to 70%. Degradation products were analyzed by HPLC and identified by electrospray-ionization mass spectrometry. This data shows that proteolysis of the parent peptides constituting each gel occurs at the intended sequence location. DP hydrogels show specificity to MMP-13 and are only minimally cleaved by matrix metalloproteinase-3 (MMP-3), another common enzyme present during tissue injury. *In vitro* migration assays performed with SW1353 cells show that migration rates through each gel differs according to peptide sequence, which is consistent with the proteolysis studies using exogenous MMP-13.

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1. Introduction

Hydrogels are a class of biomaterials that are finding use as scaffolds in soft tissue engineering and wound healing [1–15]. Attractive properties of hydrogels for wound healing applications include high water content, mechanical rigidity and porosity. Primarily, a hydrogel is intended to act as a provisional matrix at a site of tissue injury. Ideally it should have a degradation rate that approximates the rate of formation of new cell-secreted extracellular matrix. This results in optimal tissue integration and mechanically stability comparable to uninjured native tissue [6,8]. Thus, optimal tissue regeneration should occur when the temporary hydrogel support is degraded within an appropriate time scale.

Synthetic polymer hydrogels have been previously utilized as degradable scaffolds for tissue reconstruction therapies [16]. Common degradable functional groups incorporated into polymeric biomaterials include poly(esters), poly(anhydrides) and poly(*ortho*-esters) [17]. The rate of degradation of these polymeric materials depends on the rate of hydrolysis of their respective functional groups, which is governed by solution pH, water uptake into the hydrogel and exact composition of the network [17]. Thus, control over the degradation rate is largely limited to the action of bulk water.

Upon tissue injury, enzymes are utilized to breakdown damaged extracellular matrix components [18,19]. Typically, enzymes catalyze amide bond hydrolysis of extracellular matrix protein macromolecules, such as collagen. Degradable hydrogel networks have been designed to exploit these secreted proteases to further control degradation rates. In particular, peptide sequences corresponding to the cleavage site of trypsin [20], neutrophil elastase [21], subtilisin [22], pepsin [23], papain [24] and more broadly applicable, matrix metalloproteinases [20,25–36], have been incorporated into hydrogel networks to control material resorption.

A tissue of particular interest for regeneration purposes is cartilage, as it is avascular and thus has a poor capacity to repair itself [8,37,38]. During cartilage injury, a host of matrix degrading enzymes are expressed to breakdown the damaged ECM. In particular, matrix metalloproteinase-13 (MMP-13) expression is upregulated ~2-fold [39]. MMP-13 plays an integral role in degrading damaged extracellular matrix components, such as collagen, gelatin and fibronectin, to name a few, to make space for

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new extracellular matrix deposition [40]. For this reason, MMP-13 has been targeted in biomaterial designs to enzymatically degrade peptide-polymer based hydrogels [27,30–32]. In this manuscript, we described a class of hydrogels formed from self-assembling peptides that takes advantage of the activity of MMP-13 to control degradation.

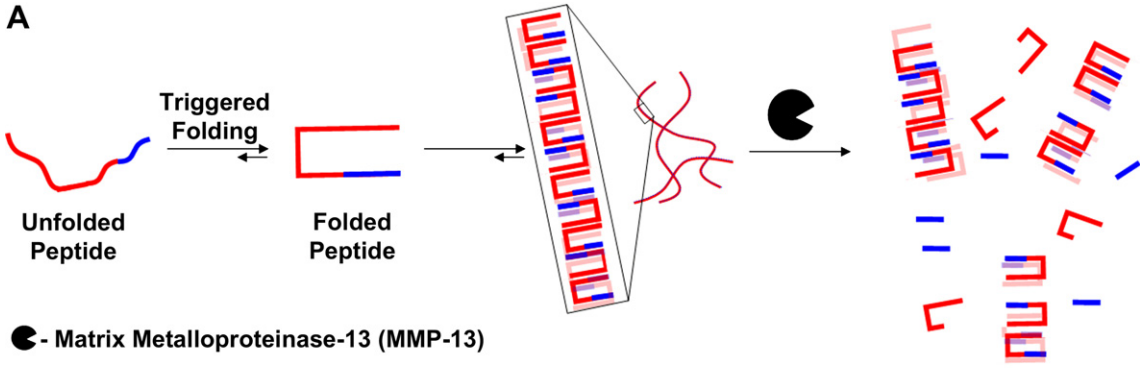
Degrading Peptides (DP) are a new series of *de novo* designed, 20-amino acid containing sequences that incorporate MMP-13 specific cleavage sites that vary in their respective MMP-13 susceptibilities. These peptides are designed to undergo triggered intramolecular folding into a conformation capable of rapid self-assembly affording fibril networks with a spectrum of degradation profiles, Fig. 1A. Individual DP peptides are composed of N- and C-terminal strand regions that have alternating hydrophobic (isoleucine or valine) and hydrophilic lysine residues. A central four residue sequence (-V^{D}PPT-) connects the two strand regions and is designed to adopt a type II' β-turn when folding is triggered, Fig. 1B. Hydrogel formation is initiated with temporal resolution by controlling the folded state of the peptide. At neutral pH and low ionic strength, electrostatic repulsion between protonated lysine side chains keeps the peptide unfolded, disfavoring self-assembly, Fig. 1A. Increasing the ionic strength with NaCl to 150 mM screens the positive charge, allowing the peptide to fold into a facially amphiphilic β-hairpin. Once folded, these peptides are designed to self assemble into a β-sheet rich network of fibrils where each fibril is composed of a bilayer of folded hairpins that have hydrogen-bonded along the fibril long axis, Fig. 1A. The resulting network of fibrils constitutes a self-supporting hydrogel. Detailed investigations on other self-assembling β-hairpin peptides support this mechanism [41–43].

To impart susceptibility to MMP-13, DP peptides were designed with an MMP-13 cleavable, six residue sequence, PTG-**X**KV, at the C-terminus of the peptide, Fig. 1B. The sequence includes a proline at the P3 position, a small amino acid at the P1 (glycine) position, a basic amino acid at the P2′ (lysine) position and a hydrophobic residue at the P3′ (valine) position of the substrate [44,45]. To vary the biodegradation rates, the amino acid at the P1′ position was varied to include isoleucine (Ile), leucine (Leu), phenylalanine (Phe) or alanine (Ala). MMP-13 has a large hydrophobic binding pocket in the S1′ subsite, that can accommodate large hydrophobic amino acids at the P1′ position [46]. Based on previous enzymatic studies [47], the ease by which MMP13 should degrade a given hydrogel network should follow DP2 (X = Leu) > DP3 (X = Ile) ~ DP1 (X = Phe) > DP4 (X = Ala). In addition to these gels, a control gel, ^{D}DP3, was prepared from the enantiomer of DP3, which primarily consists of residues of D-chirality. Gels prepared from the ^{D}DP3 peptide should not be degraded. The degradation of the DP peptide family was assessed over a 14 day time period by monitoring the mechanical properties of the hydrogels exposed to MMP-13 by oscillatory rheology. Gel degradation products were characterized by liquid chromatography coupled with mass spectrometry. Lastly, *in vitro* migration assays were performed for selected gels employing SW1353 cells induced to secrete MMP-13; these migration studies investigate the possibility of controlling migration rates by manipulating the networks' susceptibility to MMP-13 degradation.

2. Materials and methods

2.1. Materials

PL-Rink Amide resin was purchased from Polymer Laboratories. 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was purchased from Peptides International. Piperidine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was purchased from Sigma–Aldrich. All Fmoc-protected amino acids were purchased from NovaBiochem. Diisopropylethylamine (DIEA), trifluoroacetic acid, ethanedithiol, anisole and thioanisole were purchased from Acros. Diethyl ether, acetonitrile (MeCN), methanol, Tris (tris(hydroxymethyl)aminomethane), sodium chloride, calcium chloride, zinc sulfate and hydrochloric acid were purchased through Fisher. Proteolytic enzymes, matrix metalloproteinase-13 (Leinco Technologies, M1242) and matrix metalloproteinase-3 (Enzo Life Sciences, ALX-201-042-C005), were utilized in the rheological proteolysis experiments. SW1353 cells (primary grade II chondrosarcoma cells) were purchased from ATCC® (Cat. HTB-94™). Cells were grown in DMEM (Sigma D6546) supplemented with 10% heat inactivated FBS



B

Names	Sequence
DP1	IKVKIKVKV^{D}PPTGFKVKIKV-NH$_2$
DP2	IKVKIKVKV^{D}PPTGLKVKIKV-NH$_2$
DP3	IKVKIKVKV^{D}PPTGIKVKIKV-NH$_2$
DP4	IKVKIKVKV^{D}PPTGAKVKIKV-NH$_2$
^{D}DP3	^{D}I^{D}K^{D}V^{D}K^{D}I^{D}K^{D}V^{D}K^{D}V^{L}P^{D}P^{D}TG^{D}I^{D}K^{D}V^{D}K^{D}I^{D}K^{D}V-NH$_2$

Fig. 1. (A) Environmentally triggered folding and self-assembly leading to hydrogelation. Subsequent biodegradation of β-hairpin hydrogels. (B) Sequences of MMP-13 susceptible β-hairpin peptides.