Multifunctional thermoresponsive peptide hydrogels designed to meet the demands of biomedical applications

Review Article

The uncertainties of the long-term stability and effects of artificial materials in the human body have stimulated research into more natural materials for many biomedical applications. This search has led to the discovery of peptide hydrogels, a highly promising family of constructs that are capable of self-assembly, typically into β-sheets, and can emulate the properties of natural materials such as collagen. Fine-tuning the mechanical properties of hydrogels to solve biomedical problems is, however, a real challenge. A team headed by researchers at the University of Auckland in New Zealand has come one step further with peptide hydrogels that are reversibly thermoresponsive. Their innovative hydrogels are based on multifunctional peptides that combine a hydrogel-forming β-sheet peptide segment, an enzyme substrate that enables biodegradation, and a RGD sequence to promote cell adhesion.

Matching form to function

Nature is phenomenal at solving biomechanical problems and while self-assembling peptide hydrogels have proven to be promising emulators of nature, most hydrogels are mechanically soft at non-cytotoxic levels. Attempts to add function, for example by extending the peptide C-terminus of β-sheet peptide hydrogels, adversely affected assembly, hydrogel formation and mechanical properties. One approach to solving these problems has been to mix unmodified peptides that promote hydrogel formation together with their corresponding biofunctionalized derivatives. The resulting material heterogeneity is, however, a major drawback. Another approach involves designing hydrogels with non-disrupting functional sequences that integrate into the assembled peptide structure, but the range of possible constructs is very limited. It was these limitations that stimulated Leon-Rodriguez and colleagues to look for new methods in their efforts to construct thermoresponsive peptide hydrogels.

The need for thermoresponsivity

The self-assembly of peptides into hydrogels is triggered by external stimuli such as pH, ions, or temperature. Self-assembly results in the formation of fibrils that further entangle to form water-trapping networks. Temperature triggered (thermoresponsive) hydrogels can have upper or lower critical solution temperatures and are particularly interesting in drug delivery systems and when building scaffolds for tissue regeneration.

A tri-functional construct

The team decided to build peptide hydrogels with three basic functionalities via ‘click’ chemistry (see Figure 1).

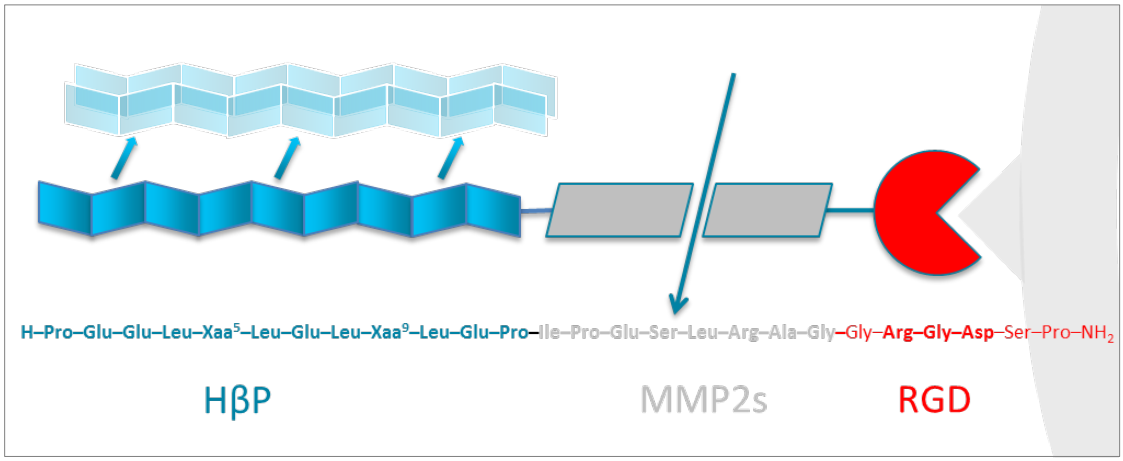

Figure 1. *A schematic representation of the multifunctional peptides.*

1. *HβP to promote formation of β-sheets*
2. *MMP2s, a sequence that can be cleaved by a matrix metalloproteinase (MMP)*
3. *RGD, tri-amino acid sequence, arginine-glycine-aspartate, a sequence that promotes cell adhesion*

The HβP sequence

H–Pro–Glu–Glu–Leu–Xaa^5–Leu–Glu–Leu–Xaa^9–Leu–Glu–Pro–

The sequence was based on the observation that anionic peptides of the type H–Pro–Y–(Z–Y)$_5$–Pro–OH, (where Y=Asp or Glu, and Z=Leu or Phe), form hydrogels close to neutral pH despite containing several glutamic acid residues. The sequence also has alternating hydrophilic/hydrophobic residues that promote β-sheet formation.

The amino acids at positions 5 and 9 were varied to give combinations of glutamic acid and lysine:

Peptide	Xaa^5	Xaa^9
1a	Glu	Glu
1b	Glu	Lys
1c	Lys	Lys
3b	Glu	Lys
3c	Lys	Lys

The team reasoned that the high acid level of peptide 1a would drive gel formation under acid conditions, so they exchanged the Glu at position 9 for Lys in the other variants.

The MMP2s sequence

–Ile–Pro–Glu–Ser–Leu–Arg–Ala–Gly–

When constructing a multifunctional hydrogel-generating peptide, it is important to include a sequence that will allow degradation after the hydrogel has performed its designated role. In this case, the team included a sequence that is recognized by a matrix metalloproteinase (MMP2), a protease that degrades several components of the extracellular matrix.

The RGD sequence

–Gly–Arg–Gly–Asp–Ser–Pro–NH_2

The RGD sequence (–Arg–Gly–Asp–) has been shown to promote cell-adhesion. Building in this functionality is key, for example, when designing materials that are aimed at promoting the adhesion and survival of cell types involved in wound healing or tissue regeneration.

Peptide synthesis

HβP–MMP2s and Azide–RGD peptides were synthesized using the Fmoc/tBu strategy on an aminomethyl-Chemmatrix resin (HβP–MMP2s) or an aminomethyl polystyrene resin (Azide–RGD) functionalized with a Fmoc-Rink amide linker using a PS3 Automated Solid Phase Peptide Synthesizer. After deprotection with piperidine in DMF, amino acid couplings were run with Fmoc-protected amino acid, HATU and *N*-methylmorpholine in DMF.

The HβP–MMP2s peptide was released and side-chains were deprotected by treatment with TFA/ triisopropylsilane/water. In the case of Azide–RGD synthesis, the resin was swollen in DMSO before solvent removal and treated with imidazole-sulfonyl-azide hydrochloride in DMSO, followed by addition of *N,N*-diisopropylethylamine and stirred overnight. The peptide was then cleaved after final deprotection by the same treatment described above.

Click reaction

The HβP–MMP2s and Azide–RGD were combined using a copper-catalyzed 'click' reaction. A phosphate buffer/tris(2-carboxyethyl)phosphine mixture was prepared under an argon atmosphere in the presence of $CuSO_4$. Peptides were then added in Cu(I) solution and the 'click' reaction was performed in a microwave apparatus.

Measuring the dynamics of hydrogel formation

The team started by testing the hydrogel-forming properties of the HβP–MMP2s peptides by adding to water at various pH values. Peptide 1a failed to form gels in the pH range studied (3.5–8) while peptides 1b and 1c generated gels under acidic conditions. The team therefore decided to go further with peptides 1b and 1c. They added the RGD moiety with 'click' chemistry to give peptides 3b and 3c, which also formed gels under acidic conditions (pH <5). Rheology measurements indicated that peptides 1b, 1c and 3b had similar gelation kinetics but 3c formed a hydrogel more slowly. Gel stiffness generally varied in the order 1b>1c and 3b>3c. Temperature ramp experiments in the range of 25–50 °C caused the gel stiffness of peptides 1b, 1c and 3b to decrease with increasing temperature, and vice versa for peptide 3c. All peptides could be cleaved by MMP2.

Sol-to-gel through transition from random coil to β-sheet

Measurements using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy and circular dichroism (CD) spectroscopy confirmed that these peptides formed a predominantly β-sheet structure, although the level of β-sheet assembly and random coil content could vary depending on the peptide and conditions such as temperature and concentration. For example, CD spectra showed that the level of β-sheet structure in peptide 3b increased on warming from 10 to 50 °C before decreasing at higher temperatures. By looking at the sequences of peptides 3b and 3c, the researchers suggested that the conformational variability in peptides 3b and 3c, from random coil to β-sheet, could be the result of shifts in inter-strand interactions. Such shifts would play a major role in their mechanical and thermoresponsive properties.

To summarize

This work presents a novel way of building β-sheet-forming peptides that yield hydrogels that are thermoresponsive and are stiffer than those formed by their respective peptide precursors. A key player in this project was the PS3 Automated Solid Phase Peptide Synthesizer. Distinguished Professor Margaret Brimble, who heads the Brimble Group lab at the University of Auckland, New Zealand where this work was done, added, “We can testify that our PS3 synthesizer is our robust ‘go to’ instrument! It never lets us down.” The next step in their work will be to build multifunctional peptides that can form thermoresponsive hydrogels at physiological pH.

Reference

Multifunctional thermoresponsive designer peptide hydrogels. De Leon-Rodriguez LM *et al*. Acta Biomater. 2017 Jan 1;47:40-49. doi: 10.1016/j.actbio.2016.10.014. Epub 2016 Oct 12. Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/27744067