Modulating the Elasticity of Self-Assembled Matrices by Chemoselectively Crosslinking Distinct Fibril Populations

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Statement of Purpose: Self-assembled peptide gels are promising materials for 3D cell culture and regenerative medicine, but their limited elasticity complicates their ultimate use within these applications. While previous work has shown that intra-fibril chemical crosslinking can strengthen individual fibrils and stiffen a hydrogel,[1,2] it still does little to improve the elasticity and durability of the gel. Inter-fibril crosslinking, conversely, would result in more elastic and more cohesive gels; however, this has been a technical challenge because it is difficult to direct chemical crosslinking to occur between fibrils and not within them (Figure 1A). Here we report a novel system for creating discrete populations of fibrils with different chemical functionalities that can be utilized for specifying inter-fibril crosslinking. This will allow us to create self-assembled peptide gels with improved cohesion, elasticity, and durability.

Methods: Q11 (QQKFQFQFEQQ), Cys-Q11(CSGSG-Q11), and Lys-Q11 (GGKSGSG-Q11) peptides were synthesized with standard Fmoc protocols. An FEI Tecnai F30 transmission electron microscope (TEM) was used to investigate self-assembling behavior. Sequestration of individual peptides within distinct populations of fibrils was observed by TEM with biotinlyated peptides and avidin-gold nanoparticles. To form gels, Cys-Q11 and Lys-Q11 were mixed with Q11 but not with each other and allowed to form protofibrils in water. By allowing fibrils to form separately, Lys-terminated fibrils and Cys-terminated fibrils were formed without co-assembling the Lys- and Cys-terminated peptides within the same fibrils. Fibrillized peptides were then mixed, cast within custom templates, and overlaid with PBS to form cylindrical gels with 8 mm diameters. Gel pH was carefully equilibrated with eight PBS washes over 4h and monitored with a needle-type micro-pH probe. Finally, gels were incubated under solutions containing heterobifunctional polyethlyne glycol-based crosslinkers (Thermo Scientific 22322 and 22112). Oscillating rheometry strain sweeps were performed at 1 Hz oscillations and increasing strain values. Crosslinked species were chemically identified with HPLC and MALDI-TOF mass spectrometry.

Results: Both Cys-Q11 and Lys-Q11 peptides formed fibrils in water and in phosphate buffered saline similarly to other previously reported Q11 derivatives (Figure 1C-D).[1] Rheometry showed that the storage moduli (G′) of independently fibrillized Lys-Q11/Cys-Q11 gels reacted with an NHS-(ethylene glycol)$_{12}$-maleimide crosslinker were 2-3 times greater than unmodified Q11 or Lys-Q11/Cys-Q11 gel without crosslinker. In particular, crosslinked samples with 95% Q11/5% Lys-Q11 fibrils mixed with 95% Q11/5% Cys-Q11 fibrils (30mM total peptide) exhibited storage moduli of at least 20 kPa for strains nearly as high as 20% (Figure 1B). This result was a significant improvement over previous self-assembled

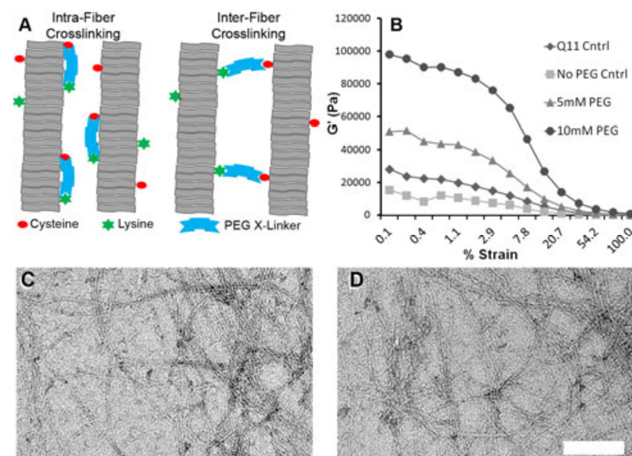

Figure 1: A) Schematic demonstrating intra-fibril and more elastic inter-fibril crosslinking. **B)** Oscillating rheometry of Q11-based gels. The 5mM and 10mM PEG samples contain 95% Q11/5% Lys-Q11 fibrils mixed with 95% Q11/5% Cys-Q11 fibrils (30mM total peptide) with either 5mM or 10mM NHS-PEG$_{12}$-maleimide crosslinker, respectively. Q11 Cntrl is self-assembled Q11 peptide only; no PEG Cntrl lacks crosslinker. **C)** Cys-Q11 and **D)** Lys-Q11 peptides both formed fibrils. Bar = 100 nm.

gels whose storage moduli fell below this value at 1-2% strain. HPLC revealed newly formed species in the crosslinked gels, and mass spectrometry identified the predicted mass (4725 Da) of the Lys-Q11-crosslinker-Cys-Q11 species. To determine whether crosslinking occurred between two distinct populations of fibrils, we prepared peptide mixtures as described that included a co-assembled biotinylated peptide in one population of fibrils. We labeled these fibrils with avidin-gold nanoparticles and observed two groups of fibrils by TEM that either bound or did not bind gold particles, strongly suggesting that we had created two distinct populations of fibrils. In addition, we are currently developing methods to visually identify and confirm the presence of two different fibril populations within a single mixture using two specific gold labels.

Conclusions: In this study we mixed combinations of self-assembling peptides into two distinct populations of fibrils. These fibrils were then mixed and allowed to form a hydrogel via the addition of buffer. Heterobifunctional chemical crosslinkers were then used to create inter-fibril crosslinks within the gel, which significantly increased both stiffness and elasticity. This approach greatly facilitates the translation of this technology into applications in 3D cell culture and regenerative medicine that demand elastic properties beyond those previously possible with self-assembled peptide matrices.

References: 1. Jung JP. Biomaterials. 2008: 13: 2143-51.
2. Paramonov SE. Macromolecules. 2005: 38: 7555-61.