Making Synthetic Hydrogels Inspired by Spider Silk

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Statement of Purpose: Spider silk exhibits exceptional mechanical properties, including strength, toughness, and elasticity, surpassing those of man-made polymers and natural materials like Kevlar and steel. These properties are attributed to silk's unique hierarchical structure, featuring alternating nanoscale β-sheet-rich crystalline and amorphous domains. The crystalline regions provide strength, while the amorphous regions contribute flexibility. Combined with its biodegradability, silk presents promising opportunities for developing high-performance biomimetic materials.

Large-scale natural harvesting of silk is impractical due to the cannibalistic behavior of spiders, while recombinant production faces challenges such as low yield and genetic engineering difficulties. While synthetic methods allow for better control, they often face issues like low coupling efficiency and suboptimal mechanical properties.

Our study addresses these challenges by developing biocompatible silk mimics that replicate the molecular arrangements of natural silk through the conjugation of β-sheet forming peptides with hydrophilic polymers (**Figure 1A**). This approach allows for precise tuning of peptide length and sequence to control self-assembly, as well as polymer modifications to manage amorphous and crystalline domains, offering a deeper understanding of silk and its properties.

Methods: Peptides with varying lengths and sequences of amino acids were synthesized using standard solid-phase peptide synthesis. The peptides were coupled to a hydrophilic amorphous polymer, polyethylene glycol (PEG), via a click reaction between an azide on the peptide and the dibenzocyclooctyne (DBCO) functional group on the PEG. This approach enabled precise control over the resulting material's properties, facilitating the design of silk mimics with tailored mechanical and structural characteristics.

Results: We used a systematic approach to understanding how we can modify the molecular structure of peptide-polymer hybrids to control the mechanical properties of silk-mimetic hdyrogels. influences the properties of hydrogels by systematically modifying four different variables within our peptide-polymer system: peptide length, peptide composition, polymer architecture, and hydrogel concentration (**Figure 1 B-D**). Rheometry showed that our best hydrogel, which is an 8-arm PEG functionalized with Val sequences, had a stiffness of above 200 kPa and most of its mechanical

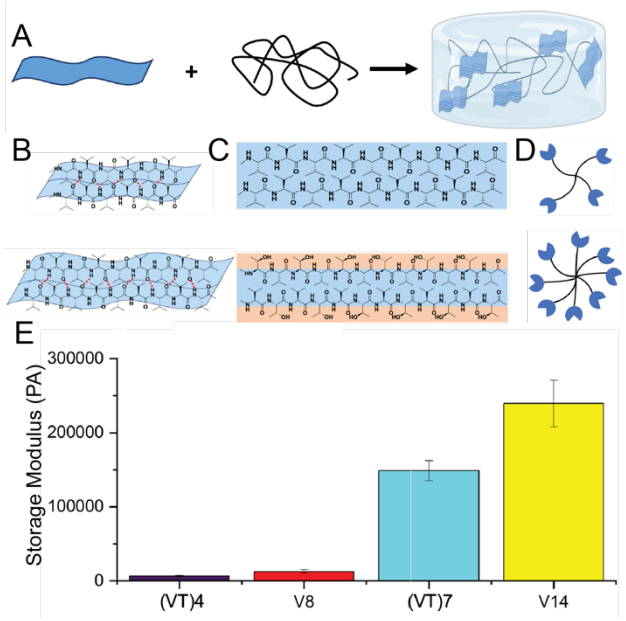

Figure 1 We have developed synthetic silk-mimetic mimetic hydrogels in which (**A**) β-sheet crystals are crosslinked with amorphous polymer domains. We can tune the properties of the hydrogel by adjusting (**B**) the length of the peptide sequence, (**C**) peptide sequence, where valine (V) sequences have beta-sheets with hydrophobicity on both faces of the beta-sheet, and Val-Thr (VT) peptides have a hydrophilic and hydrophobic face. We can modulate the amorphous polymer crosslinking by (**D**) using 4-arm or 8-arm PEG. (**E**) We find that hydrogels with 14 amino acid Val sequences formed hydrogels with high storage moduli and resilience under strain.

properties at ~10% strain, which is notably high for self-assembled peptide hydrogels. X-ray scattering, circular dichroism, FTIR, and NMR analyses confirmed the successful formation of self-assemblies and provided insights into the characteristics of the crystalline domains.

The results demonstrate that modifying the β-sheet peptide significantly tunes the mechanical properties of these gels, and that increasing the number of hydrogen bonds between peptides via their length was most effective at improving mechanical performance.

Conclusions: This study highlights the successful development of self-assembled peptide hydrogels with remarkable and highly adjustable mechanical properties. The observed properties are attributed to nanoscale structural modifications. These findings advance our understanding of designing silk-inspired biomaterials with customized mechanical attributes, enhancing their potential applications in tissue engineering, drug delivery, and biocompatible coatings.