PROGRAMMING MOLECULAR ASSOCIATION AND VISCOELASTIC BEHAVIOR IN PROTEIN HYDROGELS

Thesis by

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ABSTRACT

Recombinant artificial proteins contain genetically encoded information that specifies their assembly into higher order structures by physical or chemical cross-linking as well as elastic behavior and biological or chemical function. This thesis describes the use of artificial proteins to construct molecular networks containing covalent cross-links involving the thiol side chain of cysteine residues and physical cross-links involving the association of helical domains as coiled coils. The goal of this work was to demonstrate how the viscoelastic properties of protein hydrogels could be encoded within an artificial protein sequence.

Using genetic engineering methods, a telechelic protein denoted ERE was designed from elastin- and fibronectin-derived repeating units and expressed in *Escherichia coli*. ERE was end-linked by the reaction of terminal cysteine residues with tetrakis-vinyl sulfone-functionalized 4-arm star PEG to form hydrogel networks. The effects of varying the precursor concentration and cross-linker stoichiometry on the swelling ratio and mechanical properties of the hydrogels were studied in detail in Chapter 2. The capacity for ERE hydrogels to serve as an artificial extracellular matrix was also assessed by the encapsulation of mouse fibroblasts, which survived the cross-linking reaction and exhibited a spread morphology within the gel.

Chapter 3 describes a set of recombinant artificial proteins that can be cross-linked by covalent bonds, by association of helical domains, or by both mechanisms. These proteins were used to construct chemical, physical, and chemical-physical hydrogel networks in which the mechanism of cross-linking determines whether the material response to mechanical deformation is elastic or viscoelastic. In viscoelastic networks, stress relaxation and energy dissipation could be tuned by controlling the ratio of physical cross-linking to chemical cross-linking, and the physical cross-links could be disrupted either by protein denaturation or by mutation of the primary sequence.

Network dynamics control the viscoelasticity and erosion rate of materials and influence biological processes at multiple length scales. In Chapter 4, variation of the protein sequence was explored as a strategy to tune the characteristic relaxation timescale of protein networks. Single point mutations to coiled-coil physical cross-linking domains in chemical-physical hydrogels altered the characteristic relaxation time over five orders of magnitude. Using a pair of orthogonal coiled-coil physical cross-linking domains, networks with two distinct relaxation timescales were also engineered.

The dynamic properties of protein hydrogels can also be controlled by interactions between protein domains and small molecule ligands. In Chapter 5, the viscoelastic behavior of chemical-physical protein gels was tuned by swelling the gels with small hydrophobic molecules including vitamin D3 and fatty acids. The proposed mechanism for this effect involves binding of the ligands within the hydrophobic pore or channel created by a coiled-coil physical cross-link. Exploiting natural and designed protein-ligand interactions represents a new approach to developing hydrogel "formulations" in which the viscoelastic properties of the material can be engineered to meet specific design criteria.

In addition to exhibiting interesting dynamic properties, polymeric hydrogels containing permanent covalent cross-links and reversible physical cross-links often display enhanced toughness and extensibility. Protein hydrogels cross-linked by covalent thioether bonds and physical coiled coils could be extended further than control covalent hydrogels and exhibited a greater work of extension, which is considered a measure of material toughness. These results demonstrate progress toward engineering tougher, more extensible protein hydrogels by the incorporation of physical cross-linking by coiled-coil protein domains.

PUBLISHED CONTENT AND CONTRIBUTIONS

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