Synthesis, Nanostructure, and Mechanics of Thermoresponsively Tough Biomaterials from Artificial Polypeptides

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Artificial protein hydrogels have attracted interest as injectable fillers and scaffolds for tissue engineering and regeneration, but the same features that enable minimally-invasive implantation of these biomaterials typically make them susceptible to mechanical degradation in the tissue environment. Achieving a rapid and sufficiently large increase in gel toughness post-injection is a crucial challenge for developing load-bearing injectable implants that persist for the needed lifetime of the implant. To address these complex goals, the objective of this thesis has been to engineer physical hydrogels that shear-thin at low temperatures but responsively assemble into a nanostructured, reinforced state at body temperature.

For this purpose, the thermoresponsive aggregation of poly(N-isopropylacrylamide) (PNIPAM) and elastin-like polypeptides (ELPs) was leveraged to assemble nanostructured hydrogels from dual-associative block copolymers. Hybrid protein-polymers or protein fusions were formed by fusing PNIPAM or ELPs to the termini of a soluble artificial polypeptide decorated with self-associating α-helical domains. In cold solutions, these polypeptide block copolymers formed weak, injectable gels due to helix-associations alone; upon heating to physiological temperatures, the endblocks aggregated to form a reinforcing network of close-packed micelles throughout the gel, leading to over a 10-fold increase in elastic modulus and over $10^3$-fold increase in the longest stress relaxation time. Micelle packing and morphology could be tuned by endblock chemistry and block architecture, allowing for orthogonal control of gel viscoelasticity over timescales separated by four orders of magnitude.

Furthermore, through the discovery of a new gelation mechanism for ELPs, simple but tough hydrogels were engineered and explored as biocompatible substrates for tissue engineering. Unlike solutions of other ELPs that have been studied extensively for decades, ELPs that have an alanine mutation in the third position of the repeat unit (i.e. VPAVG) were found to undergo arrested macrophase separation upon heating when formulated above a critical concentration. Solidification was consistent with arrested spinodal decomposition, resulting in a bicontinuous, nanoscale network that could be manipulated by ELP design. Critically, this reversible mechanism produced extremely stiff physical gels with a relaxation time greater than $10^3$ seconds and shear moduli almost 10 MPa, nearly that of natural rubber despite consisting of 70% water. These ELPs were chain-extended via reversible coupling of terminal cysteine residues, leading to oxidatively-responsive increases in gel extensibility and overall toughness. Biofunctionalized gels maintained the viability of mesenchymal stem cells and chondrocytes in 2D and 3D, respectively, making these simple gel formulations a promising platform for biomedical applications.

Ultimately, through controlled macromolecular synthesis and functionalization, combined with a fundamental understanding of the structure and mechanics of these new materials, this thesis has led to the development of responsively tough biomaterials that are promising for long-term performance under physiological conditions.

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