Abstract

Genetically-encoded protein-based hydrogels have emerged as important materials for biomedical applications thanks to the precise control we have over their structural and functional properties as well as their resemblance towards native extracellular matrices. Meanwhile, design of proteins with nonlinear topologies has arisen as a new branch of protein engineering, yet of which significant applications remain to be seen. In this thesis, synthesis of new hydrogel materials using protein with uncommon architectures as building blocks are reported.

In chapter 2,3 and 4, we first demonstrate the cellular synthesis of a 4-arm star protein, (SpyCatcher)4GFP, enabled by spontaneous split-GFP reconstitution. In combination with the covalent bond-forming SpyTag/SpyCatcher chemistry, this tetra-functional protein serves as a modular building block for creating fluorescent protein networks with varied mechanics, well-suited for cell encapsulation. Conjugating (SpyCatcher)4GFP with a SpyTagged photoreceptor protein CarHc leads to another 4-arm star protein, (CarHc)4GFP, which undergoes rapid sol-gel and gel-sol transitions in response to AdoB12 and light, respectively. These chemo- and photo-induced phase transitions enables encapsulation and controlled release of protein molecules like fluorescent protein mCherry and biofilm-degrading glycosyl hydrolase PsIG, a potential agent for combatting those multidrug-resistant bacterial species such as Pseudomonas aeruginosa involved in chronic infections.

Dynamic protein-protein interactions, for instance, mechanically interlocked configuration, hold great promise in design of viscoelastic hydrogel materials. To fulfill such end, in chapter 5, we develop a series of dynamically tunable molecular networks through combined use of covalent-bond forming SpyTag / SpyCatcher chemistry and physically entangled p53 dimerization domains (Xs). The resulting networks share similar chemical composition but differs significantly in their viscoelasticity. These materials exhibit excellent compatibility towards encapsulated fibroblasts and stem cells. In addition, the impact of relaxation rates on biological processes, like cell morphology developments and bone regenerations are discussed.

Together, this thesis report creation of entirely recombinant protein-based hydrogels using uncommon protein architectures, e.g. four-arm topology and physically interlocked structure, of which the applications in therapeutic release and manipulations over cell behaviors are demonstrated.