Developing Functional Biomaterials via Directed Protein Assembly

By

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Abstract

Nature has evolved an amazing toolbox that contains protein domains with great structural and functional diversity, which provides great inspiration and opens up enormous opportunities for scientists and engineers. Thanks to significant progress in bioconjugation chemistry, the past few years witnessed the development of a variety of biomaterials through direct assembly of engineered protein molecules under mild, physiological conditions, whereas the function of protein building blocks were preserved. In doing so, the researchers have been able to faithfully transfer the function at the molecular level to material properties at the macroscopic level. More often than not, the resulting materials also exhibit marked genetic programmability, thus leading to delicately controlled mechanical properties, biological activity, as well as stimuli responsiveness.

In the first part of this thesis (chapter 2), an entirely recombinant protein-based hydrogel has been synthesized through the combined use of metal coordination and oxidation-directed protein assembly. The formation of the hydrogel involved a recombinant protein, AMA, that consists of multiple domains, including two SpyTag motifs, two elastin-like polypeptides and a mutually exclusive protein (MEP) domain. The MEP domain was previously created by inserting a globularly folded Ig domain (I27w34f) into the loop region of the globular GB1 protein. The split, unstructured fragments of GB1, each harbouring a Cys residue, can assemble and reconstitute into a folded structure upon the disulphide bond formation in the presence of oxidative metal ions like Cu (II). Rheological tests revealed the formation of viscoelastic solids by simply mixing the AMA protein with Cu (II), strongly suggesting not just linear polymerization of these protein molecules but also strong inter-chain interactions likely arising from metal coordination. The hydrogels' mechanical properties are tunable by altering the concentration of metal ions or protein polymers or replacing Cu (II) with other divalent metal ions. The resulting hydrogels also exhibited excellent cytocompatibility during cell encapsulation experiments, showing their potential for biomedical applications. In the second part of the thesis (chapter 3), genetically encoded SpyTag-SpyCatcher chemistry was used to create 3D covalent protein networks as a carrier for neurotrophic factors, which may find important applications in the field of regenerative neurobiology. Our preliminary studies have demonstrated the feasibility of using entirely protein based Spy network hydrogels for 3D neuron culturing. In hope of functionally decorating the protein materials and thus promoting neurite growth, several SpyTag-fusion neurotrophic factors including nerve growth factor (NGF) were subsequently cloned and produced using Escherichia coli expression system. After purification, the resulting proteins exhibited varied solubility and yield. Decorating our protein hydrogels with these neurotrophic factors may lead to a new approach for designing biomaterials for the treatment of spinal cord injuries. Together the studies presented in this thesis demonstrate the feasibility of using directed protein assembly to create biocompatible materials and may lead to new opportunities for regenerative medicine and therapeutic delivery.