ABSTRACT

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Cellular activities are composite responses to stimuli from the surroundings. Materials for biological applications, therefore, must be designed with care such that undesired interactions between cells and the materials will not be elicited while cellular responses that are beneficial to the dedicated applications are promoted. Efforts have been made to construct such materials based on both synthetic polymers and natural polymers including poly(ethylene glycol) (PEG) and proteins. In particular, recombinant proteins have drawn great interest for their biocompatibility and the uniformity of material properties that is found in manufacturing of synthetic polymers. Recombinant proteins are designed at the DNA level, which allows precise control over the translated protein sequence. By assembling DNA sequences that encode amino acids with desired functional groups or protein domains conferring desired functionalities, we can tailor recombinant protein-based materials. In this dissertation, work toward developing functional biomaterials based on both synthetic polymers and recombinant proteins are presented.

The first part of this thesis encompasses the development of a new thiol-based crosslinking approach to achieve independent control over degradability and mechanical properties of a hydrogel system. Thiol chemistry was chosen as the focus here because it can easily be incorporated into recombinant protein designs by inserting cysteine residues. In addition, the low frequency of cysteine residues in natural proteins can reduce unwanted reactions between the hydrogel material and encapsulated biomolecules or cells. We utilized divinyl sulfone (DVS) to form thioether crosslinking through thiol-ene addition and ferric ethylenediaminetetraacetic acid (ferric EDTA) to make disulfide crosslinks via thiol oxidation. By controlling the ratio between the non-reducible thioether bonds to reducible disulfide bonds, hydrogels with similar mechanical properties can be made with different degradability in reducing conditions. Accelerated degradation and increased

release of encapsulated dextran was observed in response to an extracellular reducing condition. High viability of encapsulated fibroblasts also suggested that the crosslinking approach was cytocompatible. This work demonstrated the potential of thiol crosslinking by DVS and ferric EDTA for making redox-responsive drug delivery vehicles and tissue engineering scaffolds.

In the second part, we developed protein adhesives using thiol- or catechol-based adhesion. Every year more than 310 million surgeries are performed around the world, and more than 50% of these surgeries use sutures or staples for wound closure.[1-2] Surgical sealants or adhesive can be applied together with sutures and staples to mitigate the risk of infection. Protein-based adhesives could be more biocompatible than synthetic polymer-based adhesives and have the potential to provide biochemical cues for cellular responses. Many adhesive proteins have been found in nature. Among them, mussel adhesive proteins have been actively studied for their outstanding underwater adhesion. The capability of being able to cure in a wet environment is critical for an ideal surgical sealant and adhesive. Mussels use both thiols and a catechol, 3, 4-dihydroxyphenylalanine (DOPA), to achieve underwater adhesion. Inspired by mussel adhesive proteins and modular recombinant design, we developed two proteins with highly similar amino acid sequences that harbor either thiol or DOPA groups. The adhesion performance, including curing kinetics and adhesion strength, and cytocompatibility were compared between the two proteins. The similarity in the protein sequences allows us to focus on the performance difference between thiol- and DOPA-based adhesion. We also showed that there was an increase in the adhesion strength when the two proteins were combined. This increase could be a result of a cross-reaction between thiol and DOPA groups. Our work provides insight into selecting the chemistry for designing adhesives based on the needs of the application.

In the last part, we studied the lower critical solution temperature (LCST) behavior of elastin-like polypeptides (ELPs) by using a series of ELPs with rationally-designed charge distributions and chain lengths. The LCST behavior of ELPs are controlled by multiple factors including the amino acid composition, ELP chain length, protein concentration, salt identity, salt concentration, and pH of the solution. Fusion of other non-ELP recombinant protein domains to ELPs have also been shown to influence the LCST behavior of the ELP fusion protein. Inspired by this effect, we explored the use of short non-ELP sequences as

a new way to tailor the LCST behavior of ELP-based proteins. We designed the non-ELP and the ELP sequences with different pH-dependent charge states and showed that pH sensitivity was introduced to the LCST behavior by electrostatic and hydrophobic interactions between the non-ELP and ELP sequences. The electrostatic interactions were shielded by the ionic strength in the protein solution. The pH sensitivity was introduced by the non-ELP sequences, and this sensitivity decreased when the relative length of the ELP domain increased. We also found that the hydrophobicity of the non-ELP sequences changed the interactions between the proteins and Hofmeister ions in solution. Our results demonstrated the potential of using non-ELP sequences as a new factor in controlling the LCST behavior of ELP proteins.

References:

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