ENGINEERING ELASTOMERIC PROTEIN-BASED BIOMATERIALS FOR TISSUE ENGINEERING AND DRUG DELIVERY APPLICATIONS

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Modular recombinant protein design allows for facile combinations of different functional domains without disrupting the original functionality of each individual domain. Furthermore, modular protein-based biomaterials have several advantages over natural and synthetic materials including precise control over molecular weight and composition, modular swapping of functional domains, and tunable physical and chemical properties.

Our lab has successfully developed a family of modular recombinant proteins, which combine a structural domain and a bioactive domain. The structural domain, which determines the mechanical properties of the proteins, consists of repetitive structural motifs derived from natural elastomeric proteins. The bioactive domain contains signaling factors to facilitate cell-protein interactions and to further guide cell behavior. This dissertation focuses on developing, characterizing, and broadening the versatilities of two elastomeric protein-based biomaterials for tissue engineering and drug delivery applications.

Abductin is an elastomeric protein found in the hinge of bivalves. An abductin-based protein with a sequence derived from the Atlantic bay scallop was produced recombinantly for the first time. Circular dichroism showed that the abductin-based protein displayed polyproline II helix (PPII) structures as the dominant structures in aqueous solution. PPII is an important structural feature for elastomeric proteins. The abductin-based protein also exhibited a reversible upper critical solution temperature (UCST) below which it formed a gel-like structure. In addition, the abductin-based protein is cytocompatible with human cells.

On the other hand, resilin is an elastomeric protein found in insect exoskeletons. It is renowned for its high resilience; resilin can be stretched or compressed many times without permanent deformation. Our lab demonstrated the potential of resilin-like proteins in cartilage engineering by showing comparable mechanical properties to natural cartilage. To expand the versatility of resilin-like proteins in tissue engineering, the tunable mechanical properties of resilin-like proteins were explored. Varying the protein concentration or stoichiometric crosslinking ratio enabled the crosslinked resilin-like proteins to achieve a wide range of mechanical moduli that spanned the stiffness of soft tissues like brain to stiffer tissues such as muscles.

To further broaden the versatility of resilin-like proteins, a “smart” resilin-like protein system, which will respond to environmental changes, was developed. A thiol-cleavable crosslinker was used to manufacture redox-responsive resilin-like hydrogels, which degrade in reducing environments. SEM images showed that the resilin-like hydrogels were highly porous structures with a pore size similar to the size of cells. This feature could facilitate cell migration within hydrogel networks for tissue engineering applications. Mouse fibroblasts also showed high viability when cultured on resilin-like hydrogels. In addition, FITC-labeled dextran, which served as a mimic of protein drugs, was encapsulated within the hydrogels. The hydrogels showed a faster release rate of

dextran in the presence of reducing agents. This feature could allow targeted drug delivery under reducing environments such as those found in tumor tissues.

This dissertation features the development and characterization of two engineered elastomeric protein-based biomaterials, abductin-based and resilin-like proteins. These two biomaterials have multiple advantages such as cytocompatibility, systematically tunable mechanical properties, and stimuli-responsive properties and thus have broadened the options for material selection in the field of biomedical engineering.