6 CONCLUSIONS AND FUTURE WORK

There are now several examples of artificial proteins with distinctive mechanical and biological properties identified as useful for implantable biomaterials;[1-4] these illustrate the unique possibilities in architectural control and bioactivity that the engineered protein approach provides. The work presented in this thesis is an important step forward for this area of research, because it includes a successful demonstration of an artificial protein device effecting its designed function *in vivo*. Specifically, an artificial extracellular matrix (aECM) protein bearing the peptide sequence RGD in its backbone promotes the adhesion and migration of epithelial cells in a crosslinked corneal onlay film. Having been shown to epithelialize within one week, these onlay lenses will next be evaluated over several months in the same rabbit cornea model to confirm their long-term biocompatibility and their ability to support the development of mature epithelial tissue. For their ultimate application as corrective lenses, a reliable means of adhering the onlays to the corneal stroma must be engineered; this could be achieved using tissue sealants or by functionalizing their inner surface.

The success of elastin-like proteins in the ocular milieu bodes well for their use in other biomedical contexts as well. Vascular grafts are one application where there is a tremendous need for new materials, but where additional challenges are posed by dynamic mechanical stresses and direct blood contact. Here, crosslinked aECM protein films were shown to be uniform and highly-

extensible, and to mimic the elasticity of native vascular tissue. With their desirable mechanical properties and demonstrated ability to specifically and strongly adhere to endothelial cells,[5-7] the aECM materials are poised to undergo detailed *in vivo* characterizations, beginning with subdermal biocompatibility experiments and proceeding to mammalian arterial models. A requirement for these studies is the removal of the T7 tag, used for protein identification but a known antigen, from the protein. This can be accomplished enzymatically using enterokinase, or by removing the corresponding DNA from the coding sequence. The ability to control mechanical properties and degradation rates through changes in sequence and crosslinking, explored in this work, will aid the engineering of successive generations of materials to respond to the challenges of their environments.

In addition to describing promising materials for implantable devices, this thesis presents examples of how engineered artificial proteins can be used to fabricate advanced substrates to study mechanosensitive cell behavior. It was shown that incorporation of the photosensitive non-canonical amino acid *para*-azidophenylalanine (*p*N_3Phe) allowed aECM protein films of tunable modulus to be made by changes in *p*N_3Phe concentration or the degree of ultraviolet irradiation. Using variable irradiation, individual thin film substrates with patterns of mechanical properties were prepared.

Atomic force microscopy-based nanoindentation was shown to be effective in evaluating the elastic modulus of these soft thin films; the sensitivity and high resolution of this technique should make it useful for characterizing a

wide range of substrates. Automation of these or similar indentation techniques will allow the mechanical properties of finely patterned films to be rapidly mapped, facilitating their use as sophisticated cell culture substrates.

Longer photosensitive aECM proteins, of the same design as the ones described here, are expected to expand the usefulness of the materials developed in this work, since they would gel at lower *p*N_3Phe concentrations. The lower values of elastic moduli that these enable would allow a greater range of stiffness to be created in individual differentially-crosslinked films, and could soften the films from hundreds of kPa to tens of kPa, where a majority of mechanosensitive cellular responses have been observed to occur.[8]

Mechanically variant aECM protein films have an important advantage over synthetic polymer systems with the same property, because they present mechanical signals to cells in the context of biological signals. The peptide sequences need not be limited to the CS5 and RGD cell-binding domains used here. Microfluidic systems could be used to create patterns of different signal-bearing artificial proteins on a surface, which could then be subjected to variable irradiation to create films with patterns of coordinating mechanical and biological stimuli. Such substrates are likely to aid in elucidating the effects of complex environmental signals on cell behavior, which in turn will inform the future design of advanced biomaterials.

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