Chapter 8

CONCLUSION

8.1 Summary

The artificial extracellular matrix (aECM) protein family was engineered originally for use in small-diameter vascular grafts. These proteins contain fibronectin domains for cell adhesion and elastin-like repeats for elastomeric behavior. This thesis describes the development of new aECM proteins containing the RGD and PHSRN sequences derived from fibronectin.

Endothelial cells recognize specifically the cell-binding sequences in adsorbed and crosslinked aECM proteins. Cell adhesion and spreading is altered through the choice of cell-binding domain, the identity of amino acids distant from the cell-binding domain, and the density of the RGD sequence. aECM proteins also can be photopatterned through introduction of the non-canonical amino acid, *para*-azidophenylalanine (*p*N_3phe). These protein patterns serve as a template for cell adhesion.

A fluorescent imaging method developed in this thesis could be used for visualizing newly synthesized proteins of cells on aECM substrates. Newly synthesized proteins incorporating an alkyne-containing amino acid were ligated to an azide-containing dye. Fluorescent imaging of these proteins indicated that a subset was localized to the nucleoli.

8.2 Future Directions

Endothelial cell migration rate was not affected significantly by density of the RGD domain in crosslinked aECM films. Preliminary results, however, indicate that cells migrate more quickly on aECM proteins with the CS5 rather than the RGD sequence (Appendix B.2). Further work is necessary to understand the mechanism by which cells are moving on these substrates. The effect of crosslinked films of variable ligand composition (mix the RGD, CS5, and PHSRN sequences together) also could be explored. Another way to increase migration rates may be to use soluble or tethered forms of angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF).

aECM proteins are being currently investigated for use in other tissue engineering applications. Crosslinked aECM proteins containing the RGD sequence have been examined for use in corneal onlays.[1] Variants of aECM proteins that contain domains from the DSL family of ligands for the Notch receptor are being developed currently for use in neurorestoration.[2] Although the aECM protein family shows promise for many tissue engineering applications, more rigorous biocompatibility tests need to be performed. Inflammatory and thrombogenic properties first should be monitored *in vitro* by using platelet adhesion assays and monitoring inflammatory and thrombogenic markers. Animal studies must also be done to determine the immunogenicity and performance of these materials.

Mechanical properties of aECM films can be modulated through the extent of crosslinking and the molecular weight between crosslinks.[3-5] Cells respond to the mechanical cues of their environment,[6-8] and preliminary results in the Tirrell laboratory

indicate that cell adhesion and matrix metalloproteinase-2 activation is modulated by the mechanical properties of crosslinked aECM films.[9] Furthermore, by changing the *p*N_3phe incorporation level or the irradiation time, protein films with a gradient in mechanical properties can be produced.[10] Microfluidic channels are being used now with aECM proteins containing *p*N_3phe to form gradients of mechanical properties and ligand densities. These gradients will then be used to study directed migration of endothelial cells.

8.3 References

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