Chapter IX. Conclusion

1. Summary

The design strategy to create artificial modular proteins that combine properties of naturally occurring proteins was validated. By interspersing elastin-like structural domains with fibronectin cell-binding domains, a new family of artificial extracellular matrix proteins was designed and synthesized. Altering the primary amino acid sequence of these aECM proteins can tune the elastic modulus, the degradation properties, and cellular interactions. HUVEC grown on aECM proteins will proliferate to confluence, secrete non-activated levels of fibrinolytic regulators, form focal adhesion plaques, and resist physiologically relevant detachment shear forces. These results suggest that aECM proteins may be suitable for use as small-diameter vascular grafts.

HUVEC adhere to aECM proteins adsorbed onto tissue culture polystyrene or crosslinked into films in a sequence-specific manner. By changing the identity or density of the cell-binding domain, the rate of cell spreading and strength of cell adhesion are altered. The strength of cell adhesion and secretion and activation of MMP-2 are also affected by modifying the elastic modulus of the crosslinked film. These results suggest that aECM proteins may be ideal substrates to probe the signal crosstalk that arises when cells are presented with simultaneous biochemical and biomechanical cues.

The functionality of these aECM molecules can be further enhanced by incorporation of noncanonical amino acids with novel chemical moieties. 5TFI incorporated into an aECM protein lowers the LCST by 20°C and decreases elastase proteolysis without compromising sequence-specific HUVEC adhesion. Incorporation of a photoreactive amino acid, $p\mathrm{N_3Phe}$, allows synthesis of an aECM protein that can be

photocrosslinked into various three-dimensional shapes and two-dimensional patterns. Varying the exposure to ultraviolet light tunes the extent of crosslinking. This can be used to vary the elastic modulus of a three-dimensional substrate or to vary the amount of immobilized protein in a two-dimensional pattern. HUVEC are able to adhere and proliferate on this photoreactive aECM protein both before and after photocrosslinking. When two-dimensional aECM patterns are immobilized on a non-adhesive PEG background, stable HUVEC patterns can be created in the presence of serum proteins.

2. Future research directions

This thesis project has focused on *in vitro* analyses of endothelial cell biocompatibility with aECM proteins. Towards the goal of creating aECM-based small-diameter vascular grafts, issues of thrombogenicity and *in vivo* biocompatibility will need to be addressed. These experiments may include *in vitro* analysis of platelet and leukocyte adhesion, *in vivo* analysis of subcutaneous implantation, and eventual *in vivo* analysis of aECM-based small-diameter grafts in appropriate animal models. Due to the high degree of tunability of aECM proteins, they may be suitable for other biomedical uses beyond vascular grafts. For example, aECM films, which are transparent, are currently being evaluated for use as corneal on-lays.

Because the cell-binding domain density and elastic modulus of aECM films can be tuned independently, they may also be ideal substrates to investigate the importance of biomechanics on signal transduction mechanisms initiated by cell-matrix contacts. The experiments presented here have been limited to morphological characterization of cell spreading and adhesion and standard biochemical analyses of individual molecules, i.e.,

vinculin, tPA, PAI-1, and MMP-2. These experiments could be expanded to investigate the ability of substrate-cell interactions to exert control over more advanced cell functions. For example, experiments are currently underway to evaluate how cell migration can be modified by substrate design. It may also be interesting to probe if the expression patterns of integrins are modulated by cell-substrate contacts. Another possible line of inquiry is to quantify activation of key signal "checkpoints" (e.g., the small Rho GTPases) that result in cytoskeleton reorganization.[1-3]

Another interesting research direction is to expand the library of possible protein domains and noncanonical amino acids that can be incorporated into aECM proteins. For example, the PHSRN synergy site from fibronectin has been incorporated into an aECM protein in an attempt to enhance cell adhesion to RGD films. Inclusion of growth factors into aECM films, either by tethering to particular amino acids in the current aECM family or genetically encoding the domains into new aECM variants, may also enhance the ability to influence cell behavior. The enormous diversity of wild-type proteins suggests that this modular design approach to protein engineering could be greatly expanded to include a wide variety of bioactive and structural domains.

3. References

1. Wojciak-Stothard, B.; Potempa, S.; Eichholtz, T.; Ridley, A. J. *J. Cell Sci.* **2001**, *114*, 1343-1355.
2. Li, S.; Chen, B. P. C.; Azuma, N.; Hu, Y. L.; Wu, S. Z.; Sumpio, B. E.; Shyy, J. Y. J.; Chien, S. *J. Clin. Invest.* **1999**, *103*, 1141-1150.
3. Nobes, C. D.; Hall, A. *J. Cell Biol.* **1999**, *144*, 1235-1244.