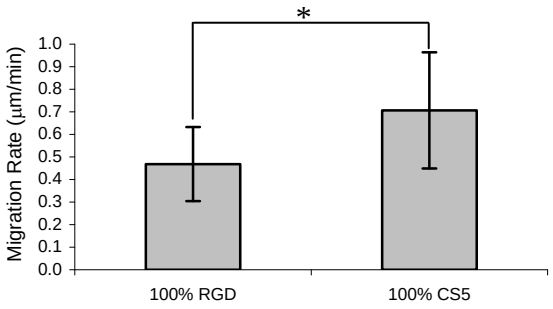
Modulating Endothelial Cell Response with Artificial Extracellular Matrix Proteins Designed for Application in Small Diameter Vascular Grafts

Julie C. Liu
Purdue University

Introduction: Current synthetic materials used for small diameter vascular grafts have a high failure rate due to thrombosis and intimal hyperplasia, which are thought to be caused by the lack of a confluent endothelial monolayer and a compliance mismatch between the graft and the surrounding tissue. We designed a family of artificial extracellular matrix (aECM) proteins that mimics the properties of natural proteins in the blood vessel and addresses the primary causes of graft failure. These proteins include elastin-based repeats to confer the needed mechanical properties and RGD and CS5 cell-binding domains from fibronectin to bind endothelial cells. The proteins can be crosslinked through lysine residues interspersed within the elastin repeats, and it has been previously shown that the mechanical properties can be tuned to the range of native elastin. Previous work has also shown that cells specifically recognize and adhere to the cell-binding domains in adsorbed, uncrosslinked proteins. This study examines endothelial cell adhesion, spreading, and migration response to the identity and density of cell-binding domains in crosslinked protein films.

Methods: *E. coli* was used as the expression host. Due to the lower critical solution temperature (LCST), the proteins were purified easily through temperature cycling. The human umbilical vein endothelial cells (HUVECs) used in all studies were purchased from Cambrex Biosciences. To create crosslinked protein films, a solution of aECM protein and crosslinker was spin-coated onto a base-cleaned coverslip. Poly(ethylene glycol) (PEG) was covalently attached to the protein films to reduce non-specific cell interactions. To assess cell attachment to aECM proteins, cells were subjected to a normal detachment force. Cell spreading and migration were assessed by recording images at 15 min intervals.

Results: The crosslinking method described herein provided uniform, coherent crosslinked films with a dry height of 8-10 nm. In the absence of PEGylation, there was a high level of non-specific adhesion to the negative control aECM-RDG-PEG containing a scrambled cell-binding sequence. Grafting one PEG molecule to every five protein strands resulted in a four-fold decrease in non-specific adhesion to aECM-RDG-PEG but did not affect specific adhesion to aECM-RGD-PEG. By mixing aECM-RGD-PEG and aECM-RDG-PEG, cell adhesion could be modulated. It was found that films containing greater than 25% aECM-RGD-PEG promoted cell adhesion levels comparable to those of the full-length fibronectin protein. The kinetics of cell spreading could also be modulated through the density of authentic cell-binding ligands, but cells on 100% aECM-RGD-PEG films spread more slowly than those on fibronectin controls. Although cell adhesion and spreading could be tuned through ligand density, migration rates were not sensitive to the concentration of the RGD cell-binding domain. The migration rates fell within the range of previously published cell speeds on fibronectin. Migration rates, however, could be modulated by changing the identity of the cell-binding ligand. Cells seeded on aECM crosslinked proteins containing the CS5 cell-binding domain derived from fibronectin had migration rates that were 1.5 times faster than those on proteins containing the RGD ligand.



Conclusions: This study provides a facile method for making crosslinked aECM protein films used for studies on cell responses. One advantage of this system is that biological ligand density can be independently changed while the physical and chemical properties of the films are kept constant. PEGylation of these crosslinked films was essential to reduce non-specific cell adhesion but did not preclude specific recognition of the cell-binding sequence. Although cell spreading kinetics on aECM proteins were slower than those on fibronectin, cell adhesion and migration responses on these proteins were similar to those on fibronectin. Varying the ligand density resulted in modulation of cell adhesion and spreading but not cell migration rates. Substituting the CS5 ligand for the RGD ligand resulted in significantly higher cell speeds. Future work will address the use of both mechanical properties and ligand presentation in modulating cell responses.

References:

Di Zio K. Macromolecules. 2003; 36: 1553-1558.
Liu JC. Biomacromolecules. 2004; 5: 497-504.
Liu JC. Biomacromolecules. 2008; 9: 2984-2988.