DESIGN AND CHARACTERIZATION OF ARTIFICIAL EXTRACELLULAR MATRIX PROTEINS FOR USE AS SMALL-DIAMETER VASCULAR GRAFTS

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ABSTRACT

Design and Characterization of Artificial Extracellular Matrix Proteins

for Use as Small-Diameter Vascular Grafts

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Synthetic small-diameter vascular grafts often fail within three years of implantation. The underlying causes of graft failure are thought to be i) a mismatch in the mechanical properties between the graft and host material and ii) an inability of the graft to support the adhesion of endothelial cells. To address these two issues, artificial extracellular matrix (aECM) proteins contain elastin-like regions to provide physical integrity and cell-binding domains derived from fibronectin to promote endothelial cell attachment. Using recombinant protein technology, a family of artificial proteins was created with differing ratios of elastin-like regions to cell-binding domains, with variable placement of amino acid crosslinking residues, and with differing identity of cell-binding domain.

Human umbilical vein endothelial cells (HUVEC) adhere in a sequence-specific manner to aECM proteins, secrete basal levels of key fibrinolytic regulators, and are capable of resisting a physiologically relevant detachment force. HUVEC spread more quickly and adhere more firmly to aECM proteins that contain the RGD versus the CS5 cell-binding domain. Decreasing the density of RGD cell-binding domains results in

decreased HUVEC adhesion. Furthermore, amino acid selection even at sites up to 16 residues away from the cell-binding domain impacts HUVEC spreading and adhesion. HUVEC also adhere more strongly to stiffer aECM films. Therefore, the identity, density, and context of the cell-binding domain as well as the elastic modulus of the substrate are all important variables in influencing cell-substrate interactions.

Proper amino acid sequence choice also influences the susceptibility of aECM proteins to elastase proteolysis; modifying 3% of the amino acid side chains results in a 7-fold reduction in degradation rate. An alternative strategy to decrease degradation involves incorporation of the noncanonical amino acid, 5,5,5-trifluoroisoleucine, into the favored proteolytic cut site, isoleucine. Replacing 82% of the isoleucines results in a twofold reduction in degradation rate without compromising sequence-specific HUVEC adhesion. Incorporation of another noncanonical amino acid, *para*-azidophenylalanine, allows synthesis of photoreactive proteins that can be patterned using photolithography. These protein patterns retain their ability to adhere HUVEC and produce stable cell patterns after 48 hours in medium supplemented with serum.

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ABBREVIATIONS

5TFI	5,5,5-trifluoroisoleucine
aECM	Artificial extracellular matrix protein
BCA	Bicinchoninic acid
BS3	Bis(sulfosuccinimidyl) suberate
BSA	Bovine serum albumin
CAI	Cell adhesion index
DPN	Dip-pen nanolithography
E	Elastic modulus
ECM	Extracellular matrix
ePTFE	expanded poly(tetrafluoroethylene)
ELISA	Enzyme-linked immunosorbent assay
HLE	Human leukocyte elastase
HMDI	Hexamethylene diisocyanate
HUVEC	Human umbilical vein endothelial cells
LAL	*Limulus* amebocyte lysate gel-clot assay
LCST	Lower critical solution temperature
LPS	Lipopolysaccharide
MALDI-MS	Matrix-assisted laser desorption ionization-mass spectrometry
M_c	Molecular weight between crosslinks
PAI-1	Plasminogen activator inhibitor
PBS	Phosphate buffered saline
PET	Poly(ethylene terephthalate)
***p*N_3Phe**	*Para*-azidophenylalanine
SDS-PAGE	Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
tPA	Tissue plasminogen activator
TSAT	Tri-succinimidyl aminotriacetate

Note: Single letter abbreviations for the natural amino acids are listed in Appendix A.