ENDOTHELIAL CELL RESPONSE TO ARTIFICIAL EXTRACELLULAR MATRIX PROTEINS

Thesis by

Julie C. Liu

In Partial Fulfillment of the Requirements for the

Degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2006

(Defended March 21, 2006)

ACKNOWLEDGMENTS

I would like to thank my thesis advisor, Dave Tirrell, for his valuable advice and continual support. His scientific rigor and integrity will serve as a guide for me throughout my career. I appreciate especially the intellectual independence that he fostered and the sense of humor with which he approached all matters.

Thank you to my thesis committee—Anand Asthagiri, Marianne Bronner-Fraser, and Christina Smolke—for their helpful comments and support.

I am grateful to all former and current Tirrell laboratory members for their help and collaboration. In particular, I would like to acknowledge Sarah Heilshorn, Paul Nowatzki, Kathleen Di Zio, Kimberly Beatty, and Marissa Mock for their assistance and thought-provoking discussions.

On a more personal note, I thank my parents, William and Anne Liu, for providing me the opportunities that enabled me to achieve my goals. For their support and joyous companionship, I am grateful to my sister, Cindy Liu Abravanel, and her family—Mario, Megan, and Lauren Abravanel. Finally, my thanks go to Keith Alsaker for his friendship and unending encouragement.

ABSTRACT

ENDOTHELIAL CELL RESPONSE TO ARTIFICIAL EXTRACELLULAR MATRIX PROTEINS

March 2006

Julie C. Liu, B.S.E., Princeton University

M.S., California Institute of Technology

Ph.D., California Institute of Technology

Supervised by David A. Tirrell

Artificial extracellular matrix (aECM) proteins were designed originally for use in small-diameter vascular grafts. Current synthetic grafts fail primarily due to (i) a compliance mismatch between the prostheses and surrounding tissue and (ii) the inability to support the growth of an endothelial cell monolayer. To address these issues, biomimetic proteins were engineered with elastin-like repeats to confer elastomeric behavior and fibronectin cell-binding domains to promote endothelialization. Lysine residues or the non-canonical amino acid, *para*-azidophenylalanine (*p*N_3phe), serve as crosslinking sites.

Human umbilical vein endothelial cell (HUVEC) adhesion to aECM proteins was sequence-specific. Cells bind more strongly and exhibit faster spreading kinetics on aECM proteins containing the RGD versus the CS5 sequence. Furthermore, HUVECs on the former protein exhibited well-formed stress fibers and organized the $\alpha_v\beta_3$ but not the $\alpha_5\beta_1$ integrin into focal adhesions.

Although biomaterial design has focused on the sequences of cell-binding domains, elements remote to these bioactive sequences were found to affect cell response to aECM proteins. Proteins containing identical CS5 cell-binding domains differed in their placement of lysine residues that serve as crosslinking sites. Cell adhesion and spreading were more robust on proteins in which lysine residues were located at the termini versus within the elastin cassettes.

Crosslinked films of aECM proteins with RGD sequences adhered HUVECs in a sequence-specific manner. Poly(ethylene glycol) was covalently attached to films to reduce nonspecific cell interactions. Increasing the density of RGD in a film resulted in increased cell adhesion and spreading but did not have a significant effect on migration rates.

aECM proteins were made photoreactive through the incorporation of *p*N_3phe. Upon exposure to ultraviolet radiation through a patterned mask, proteins were patterned on a non-adhesive background. These two-dimensional patterns then served as templates for cell adhesion.

A new technique for studying cells on aECM proteins was developed. Cells were pulsed with homopropargylglycine. Newly synthesized proteins labeled with alkyne-containing amino acids were ligated to 3-azido-7-hydroxycoumarin. Fluorescence microscopy was used to visualize these proteins in a wide variety of mammalian cell types.

TABLE OF CONTENTS

LIST OF FIGURES, SCHEMES, AND TABLES

ABBREVIATIONS

2×YT	two times yeast/tryptone medium
aECM	artificial extracellular matrix
AFM	atomic force microscopy
ATP	adenosine 5' triphosphate
BCA	bicinchoninic acid
bp	base pairs
BS^3	bis(sulfosuccinimidyl) suberate
BSA	bovine serum albumin
CAI	cell adhesion index
CBD	cell-binding domain
CHO-α_5	Chinese hamster ovary cells transfected with the human $\alpha 5$ integrin subunit fused to GFP
CIP	calf intestinal alkaline phosphatase
Cy2	cyanine dye 2
DAPI	4',6-diamidino-2-phenylindole
DIAS	Dynamic Image Analysis System software
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dpi	dots per inch
ds	double-stranded
EBM-2	endothelial basal medium-2
EDTA	ethylenediaminetetraacetic acid
EGM-2	endothelial growth medium-2
EL	elastin monomer
ECM	extracellular matrix
E. coli	*Escherichia coli*
ePTFE	expanded poly(tetrafluoroethylene)

FBS	fetal bovine serum
FTIR	Fourier transform infrared spectroscopy
GFP	green fluorescent protein
HEK 293T	human embryonic kidney cells
^{1}H-NMR	proton nuclear magnetic resonance spectroscopy
Hpg	homopropargylglycine
HUVEC	human umbilical vein endothelial cells
IC_{50}	half-inhibition concentration
IPTG	isopropyl-1-β-D-thiogalactosidase
LCST	lower critical solution temperature
M9 or M9AA	minimal medium
MALDI-MS	matrix-assisted laser desorption ionization-mass spectrometry
MEF-mitoGFP	mouse embryonic fibroblasts transfected with Su9-GFP
MetRS	methionyl-tRNA synthetase
OD_{600}	optical density at 600 nm
PBS	phosphate-buffered saline
PEG	poly(ethylene glycol)
PET	poly(ethylene terephthalate)
PheRS	phenylalanyl-tRNA synthetase
PMSF	phenylmethylsulfonyl fluoride
***p*N_3Phe**	*para*-azidophenylalanine
RCF	relative centrifugal force
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SEM	scanning electron microscopy
SFM	serum-free medium without methionine
TCEP	triscarboxyethylphosphine
TRED	trypsin in EDTA
XPS	x-ray photoelectron spectroscopy

Amino Acid Abbreviations

Amino Acid	Three letter	One letter
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
cysteine	Cys	C
glutamine	Gln	Q
glutamic acid	Glu	E
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V

DNA Nucleotide Abbreviations

Nucleotide	One letter
adenine	A
cystosine	C
guanine	G
thymine	T