

ENDOTHELIAL CELL RESPONSE TO  
ARTIFICIAL EXTRACELLULAR MATRIX  
PROTEINS

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## ABSTRACT

ENDOTHELIAL CELL RESPONSE TO ARTIFICIAL EXTRACELLULAR  
MATRIX PROTEINS

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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 ( $pN_3phe$ ), 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  $pN_3p$ he. 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.

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## ABBREVIATIONS

<b>2×YT</b>	two times yeast/tryptone medium
<b>aECM</b>	artificial extracellular matrix
<b>AFM</b>	atomic force microscopy
<b>ATP</b>	adenosine 5' triphosphate
<b>BCA</b>	bicinchoninic acid
<b>bp</b>	base pairs
<b>BS<sup>3</sup></b>	bis(sulfosuccinimidyl) suberate
<b>BSA</b>	bovine serum albumin
<b>CAI</b>	cell adhesion index
<b>CBD</b>	cell-binding domain
<b>CHO-<math>\alpha_5</math></b>	Chinese hamster ovary cells transfected with the human $\alpha_5$ integrin subunit fused to GFP
<b>CIP</b>	calf intestinal alkaline phosphatase
<b>Cy2</b>	cyanine dye 2
<b>DAPI</b>	4',6-diamidino-2-phenylindole
<b>DIAS</b>	Dynamic Image Analysis System software
<b>DMEM</b>	Dulbecco's modified Eagle's medium
<b>DMSO</b>	dimethylsulfoxide
<b>DNA</b>	deoxyribonucleic acid
<b>dpi</b>	dots per inch
<b>ds</b>	double-stranded
<b>EBM-2</b>	endothelial basal medium-2
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b>EGM-2</b>	endothelial growth medium-2
<b>EL</b>	elastin monomer
<b>ECM</b>	extracellular matrix
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>ePTFE</b>	expanded poly(tetrafluoroethylene)

<b>FBS</b>	fetal bovine serum
<b>FTIR</b>	Fourier transform infrared spectroscopy
<b>GFP</b>	green fluorescent protein
<b>HEK 293T</b>	human embryonic kidney cells
<b><sup>1</sup>H-NMR</b>	proton nuclear magnetic resonance spectroscopy
<b>Hpg</b>	homopropargylglycine
<b>HUVEC</b>	human umbilical vein endothelial cells
<b>IC<sub>50</sub></b>	half-inhibition concentration
<b>IPTG</b>	isopropyl-1-β-D-thiogalactosidase
<b>LCST</b>	lower critical solution temperature
<b>M9 or M9AA</b>	minimal medium
<b>MALDI-MS</b>	matrix-assisted laser desorption ionization-mass spectrometry
<b>MEF-mitoGFP</b>	mouse embryonic fibroblasts transfected with Su9-GFP
<b>MetRS</b>	methionyl-tRNA synthetase
<b>OD<sub>600</sub></b>	optical density at 600 nm
<b>PBS</b>	phosphate-buffered saline
<b>PEG</b>	poly(ethylene glycol)
<b>PET</b>	poly(ethylene terephthalate)
<b>PheRS</b>	phenylalanyl-tRNA synthetase
<b>PMSF</b>	phenylmethylsulfonyl fluoride
<b><i>p</i>N<sub>3</sub>Phe</b>	<i>para</i> -azidophenylalanine
<b>RCF</b>	relative centrifugal force
<b>SDS-PAGE</b>	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
<b>SEM</b>	scanning electron microscopy
<b>SFM</b>	serum-free medium without methionine
<b>TCEP</b>	triscarboxyethylphosphine
<b>TRED</b>	trypsin in EDTA
<b>XPS</b>	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
cytosine	C
guanine	G
thymine	T