

Table of Contents

Acknowledgements	iii
Abstract	v
1 Introduction	1
1.1 Designing functional biomaterials	2
1.2 The role of mechanical properties in biomaterials design	3
1.2.1 Elastin-like polypeptides	3
1.2.2 Crosslinking mechanisms	4
1.2.3 Controlled degradability of structural scaffolds	5
1.2.4 Effect of mechanical properties on cellular behavior	5
1.3 The role of biochemical properties in biomaterials design	7
1.3.1 Choice of adhesive proteins and peptide domains	7
1.3.2 Density and presentation of active protein and peptide domains	8
1.3.3 Temporally controlled presentation of active proteins and peptide domains	10
1.4 Outline of thesis	12
1.5 References	14
2 Design and Construction of Photoreactive Artificial Extracellular Matrix Proteins	19
2.1 Abstract	20
2.2 Introduction	22
2.3 Materials and Methods	24
2.3.1 Cloning of photoreactive aECM proteins	24
2.3.2 Protein expression and purification	28
2.3.3 aECM protein characterization	29
2.3.4 Preparation of photocrosslinked films for cell studies and mechanical testing ..	30
2.3.5 Cell maintenance	32
2.3.6 Cell spreading experiments	32
2.3.7 AFM equipment	33
2.3.8 AFM measurements: film thickness and force curves	34
2.4 Results and Discussion	35
2.4.1 Characterization of aECM proteins	35
2.4.2 PEGylated and photocrosslinked aECM films for cell studies	38
2.4.3 Cell spreading results	39
2.4.4 AFM results: film thickness and elastic moduli measurements	40
2.5 Conclusions	42
2.6 References	44
3 Lithographic Patterning of Photoreactive Cell-Adhesive Proteins	48
3.1 Abstract	49
3.2 Introduction	50
3.3 Materials and Methods	51
3.3.1 Cloning of aECM-N ₃ constructs	51
3.3.2 Protein expression and purification	52
3.3.3 Mechanical testing	53
3.3.4 Photolithographic patterning of aECM-N ₃ proteins	53
3.3.5 Cell culture	54
3.3.6 Cell patterning	55

3.3.7	Cell spreading	56
3.3.8	Atomic force microscopy	57
3.4	Results and Discussion.....	57
3.4.1	Expression and characterization of aECM-N ₃ proteins.....	57
3.4.2	Kinetics of photodecomposition	59
3.4.3	Mechanical testing.....	60
3.4.4	Cell patterning.....	60
3.4.5	Cell spreading	62
3.5	Conclusions	63
3.6	Acknowledgements.....	63
3.7	References	64
4	Towards Exploring the Proteomic Profile of Rat-1 Fibroblasts on Artificial Extracellular Matrix Proteins.....	68
4.1	Abstract	69
4.2	Introduction	70
4.3	Materials and Methods.....	75
4.3.1	Cell culture.....	75
4.3.2	Cell synchronization through contact inhibition.....	76
4.3.3	aECM protein expression and purification.....	76
4.3.4	Cell spreading experiments.....	77
4.3.5	Microarray analysis	78
4.3.6	Cell lysis and protein extraction for proteomic analysis	79
4.3.7	Affinity purification	80
4.3.8	Sample preparation for tandem mass spectrometry analysis.....	81
4.3.9	Analysis of tandem mass spectrometry data.....	83
4.4	Results and Discussion.....	84
4.4.1	Cell synchronization through contact inhibition.....	84
4.4.2	Cell spreading results	85
4.4.3	mRNA microarray results.....	87
4.4.4	BONCAT results.....	90
4.5	Conclusions and Future Work	94
4.6	References	97
5	Depth Perception: Quantifying Cellular Traction Forces in Three Dimensions.....	101
5.1	Abstract	102
5.2	Introduction	103
5.3	Materials and Methods.....	106
5.3.1	Preparation of activated coverslips	106
5.3.2	Preparation of polyacrylamide films	106
5.3.3	Functionalization of polyacrylamide films with fibronectin	107
5.3.4	Characterization of FN-modified films	108
5.3.5	Film thickness measurements	109
5.3.6	Mechanical characterization of thin films	109
5.3.7	Cell culture.....	110
5.3.8	Confocal microscopy and time-lapse imaging.....	111
5.3.9	Calculation of displacements, strains, stresses, and forces.....	112
5.3.10	Traction force inhibition using blebbistatin.....	113

5.4	Results and Discussion.....	113
5.4.1	Characterization of FN-modified films	113
5.4.2	Mechanical Testing	113
5.4.3	Analysis of cellular traction forces in 3D: in-plane and normal components ..	116
5.4.4	Analysis of cellular traction forces in 3D: a time evolution of the force components.....	119
5.4.5	Analysis of cellular traction forces in 3D: “push-pull” phenomena	121
5.4.6	Traction force inhibition with blebbistatin	124
5.5	Conclusions	125
5.6	References	125
6	Conclusion.....	129
6.1	Summary	130
6.2	Future Directions	131
6.3	References	132
	Appendix A	134
	Appendix B	167
	Appendix C	206

List of Figures

Figure 2-1. Photoreactive aECM protein sequences	24
Figure 2-2. Schematic of the protocol for cloning aECM proteins	25
Figure 2-3. SDS-PAGE analysis of the purification of aECM proteins	35
Figure 2-4. LC-MS spectra of purified (A) RGD-N ₃ and (B) RDG-N ₃ proteins	37
Figure 2-5. HUVEC spread areas on (A) fibronectin; positive control, (B) BSA; negative control, (C) RGD-N ₃ , and (D) RDG-N ₃	39
Figure 2-6. AFM topography scans of photocrosslinked RGD-N ₃ protein	40
Figure 2-7. Representative loading indentation profiles for thin films of photocrosslinked RGD-N ₃ protein	42
Figure 3-1. Characteristics of aECM-N ₃ proteins	51
Figure 3-2. Representative ¹ H NMR spectra of an aECM-N ₃ construct expressed in media supplemented with phenylalanine or with <i>p</i> N ₃ Phe	58
Figure 3-3. Incorporation of <i>p</i> N ₃ Phe into the CS5-N ₃ protein as a function of concentration in the expression medium	59
Figure 3-4. AFM images of patterned RGD-N ₃	61
Figure 3-5. Confocal microscopy of Rat-1 fibroblasts attached to photopatterned RGD-N ₃	62
Figure 3-6. Rat-1 fibroblast cell spread areas on (A) fibronectin, (B) BSA, (C) RGD-N ₃ , and (D) RDG-N ₃	63
Figure 4-1. aECM protein sequences	73
Figure 4-2. Schematic of BONCAT methodology	74
Figure 4-3. Chemical structures of methionine (Met) and azidohomoalanine (Aha)	75
Figure 4-4. FACS analysis of Rat-1 fibroblasts before (A) and after (B) cell synchronization by contact inhibition	85
Figure 4-5. Images of cells captured after 2 hours of spreading	86
Figure 4-6. Rat-1 cell spread areas on (A) FN, (B) BSA, (C) RGD-N ₃ , (D) RDG-N ₃	87
Figure 4-7. Gene ontology analysis of newly synthesized proteins in Aha-treated samples using DAVID	93
Figure 4-8. Pathway analysis of the imported list from Aha-treated samples using DAVID	94
Figure 5-1. Schematic of a representative gel sample with microscope objective	105
Figure 5-2. Detailed schematic of the compression tests	110
Figure 5-3. Incremental loading tests using polyacrylamide samples	114
Figure 5-4. Representative stress-strain curves of loading and unloading cycles on cylindrical hydrated polyacrylamide samples	114
Figure 5-5. Displacement contour slices along the long axis of the cell and corresponding line plots as a function of depth for samples of varying heights	118
Figure 5-6. Time evolution of displacement and traction force contours during cell migration for a single cell	120
Figure 5-7. 3-D “push-pull” phenomenon observed in the z-plane displacement profiles of tracked cells	123
Figure 5-8. Traction force inhibition using blebbistatin	124

List of Tables

Table 2-1. Theoretical and measured amino acid compositions of RGD-N ₃ and RDG-N ₃ proteins.....	38
Table 4-1. Selected list of differentially expressed mRNA transcripts from ratio experiments with FN, RGD-N ₃ , and RDG-N ₃ samples	90
Table 4-2. List of identified newly synthesized proteins shown to play a role in cell-ECM interactions, focal adhesion formation, and cytoskeletal reorganization.....	92
Table 5-1. Mechanical characterization results for polyacrylamide substrates from unconfined uniaxial compression experiments	115