

CONTENTS

Title Page	i
Certificate by the Supervisors	ii
Declaration	iii
Acknowledgement	iv
List of Symbols	v
Abstract	vii
Contents	viii

Chapter-1 Introduction and Literature Review	1
1.1 Importance of Cellular Dynamics under Mechanical Stimulus	1
1.2 Importance of Microfluidics in Studying Cell Dynamics.....	5
1.3 Brief Review of Important Cellular Architecture.....	8
1.3.1. Plasma Membrane	9
1.3.1.1 Membrane Composition	10
1.3.2 Cytoskeletal Architecture.....	13
1.3.3 Focal Adhesions.....	15
1.4 Description of the Transport Phenomena Around and Inside Biological Cells.....	17
1.5 Signal Transduction in Cellular Domain.....	21
1.6 Confinement Effects: What to look for?.....	23
1.7 Literature Review.....	26
1.7.1 Microchannel Based Cell Culture System and Confinement Effect.....	26
1.7.2 Development of Traction Force Measurement.....	27
1.7.3 Mechanotransduction and the Effect of Fluid Shear Stress.....	29
1.7.3.1 Generic Principles of Mechanotransduction – Current State of the Art.....	29
1.7.3.2 Effect of Fluid Shear Stress.....	31
1.7.3.3 Mechanical Influence in Tumor Progression.....	33
1.8 Aim of the Present Thesis.....	34
1.8.1 Important inferences drawn from the literature review.....	34
1.8.2 Problem Definitions.....	36
1.8.2.1 Introduction of Microfabrication Compatible Traction Force Microscopy for Monitoring Cellular State within Microconfinement.....	36
1.8.2.2 Probing the Existence of Confinement Effect.....	37
1.8.2.3 Molecular Origin of Confinement Effect and Its Implications on Rheology of Cell Cytoplasm.....	37
1.9 Outline of the Thesis.....	38

Chapter-2 Fabrication and Characterization of Microchannel Systems for Cell Culture

2.1 Motivation	39
2.1.1 Brief Introduction to Cell Culture	39

2.1.2 Microfluidics for Cell Culture – Beneficiaries and Relevant Issues	42
2.2 Fabrication Process.....	44
2.2.1 Introduction to General Microfabrication Processes.....	45
2.2.1.1 Micromilling.....	46
2.2.1.2 Soft-lithography.....	47
2.2.2 Fabrication Protocols.....	48
2.3 Fluidic Measurements.....	49
2.3.1 Determination of Friction Factor.....	49
2.3.2 Flow Visualization	50
2.3.3 Surface Characterization.....	51
2.3.4 Friction Factor Measurement for Micro-milled Microchannels...	53
2.3.5 Correlating microfabrication characteristics with the fluid frictional behavior.....	54
2.3.6 Friction Factor Measurement for Lithographically fabricated PDMS Microchannels.....	56
2.3.7 Implications of the Surface Dependence of the Microflow Characteristics.....	57
2.4 Cell Sample Preparation and Culture: the Experimental Set up.....	57
2.4.1 Considerations for Microfluidics Cell Culture Set up.....	57
2.4.2 Cell Sample Preparation.....	59
2.4.3 Cell Survival and Growth inside the Microchannel.....	60
2.5 Summary	62

Chapter-3 Development of Microfluidics-Compatible Traction Force Microscopy

3.1 Basics of Traction Force Microscopy.....	63
3.1.1 Background	63
3.1.2 Mechanistic Origin of Cellular Traction Force	65
3.2 Analysis	66
3.2.1 Determination of Displacement Field.....	66
3.2.2 Calculation of Traction Field.....	67
3.3 Adaptation to Bio-microfluidic Substrates	68
3.3.1 Substrate Preparation for Traction Force Microscopy.....	69
3.3.2 Related Cell Culture.....	70
3.3.3 Cytocompatibility Assay of UPTFM substrate.....	71
3.3.4 Microchannel fabrication.....	72
3.3.5 Cell adhesion and detachment kinetics by Trypsin treatment – Validation with Existing Technologies.....	73
3.4 Variation of Traction Force during Static Incubation	76
3.5 Experiment with Varying Flows.....	78
3.5.1 Cell adhesion inside microchannel and detachment.....	78
3.5.2 Ca^{2+} Signals in Shear-stress activated HeLa Cells.....	82
3.5.3 Shear activated Ca^{2+} release as function of local traction force.....	82
3.6 Assessment in Perspective of the Vesicle Lift-off Models.....	83
3.6.1. Detailed Description of the Physical Model and Simulation.....	84

3.6.2. Model Simulation.....	86
3.6.3. Modified considerations for cell deformation and simulation predictions.....	88
3.6.4. Theoretical parametric variation of critical shear rate for detachment.....	91
3.7 Summary.....	92

Chapter-4 Shear Adaptation of Cancer Cells in Microfluidic Confinements: The Confinement Effect

4.1 Motivation	95
4.1.1 Relevance to Cancer Progression.....	96
4.1.2 Need for Quantification of Response Kinetics.....	97
4.2 Sample Preparation	100
4.2.1 Substrate Preparation for UPTFM	100
4.2.2 Fabrication of Microfluidic Set-up	101
4.2.3 Cell culture within microchannel.....	101
4.3 Simultaneous Fluorescence Recovery After Photobleaching (FRAP) and Traction Force Microscopy (TFM) Experiments	102
4.3.1 FRAP Protocol	102
4.3.2 Traction Force Microscopy	104
4.3.3 Results and Discussion.....	104
4.3.3.1 Immediate Responses.....	105
4.3.3.2 Late Responses.....	107
4.3.3.3 Role of Cytoskeleton.....	108
4.4 An Exclusive Confinement Effect for Cancer Cells.....	114
4.5 Lipid Raft Recycling Phenomenon – Diffusion-Reaction Kinetics.....	117
4.6 Metastatic Potential and Cellular Response.....	120
4.7 Summary.....	122

Chapter-5 Mechano-Biomolecular Perspective of The Confinement Effect

5.1 Epidermal Growth Factor Receptor (EGFR) Activation – Molecular Origin of Confinement Effect	125
5.1.1 Background	125
5.1.2 Immunofluorescence and Western Blot Analysis	128
5.1.2.1 Immunofluorescence or Immunocytochemistry.....	129
5.1.2.2 Western Blot.....	129
5.1.3 Confinement Specific EGFR Activation.....	131
5.1.4 T_R Related Confinement Effect can be abolished by EGFR specific Inhibitor Treatment.....	133
5.2 Mechanism of Stress Induced EGFR Activation and Its Downstream Signaling.....	135
5.2.1 Motivation.....	135
5.2.2 Fluid Shear Stress mediates EGFR Clustering.....	138
5.2.2.1 Förster or Fluorescence Resonance Energy Transfer (FRET) Experiments.....	138
5.2.2.2 Results and Discussion.....	139

5.2.3 EGFR Autophosphorylation Activates STAT-1 in JAK independent manner.....	142
5.2.3.1 Immunofluorescence Analysis.....	142
5.2.3.2 Results and Discussion.....	142
5.2.4 Ratio of p-STAT1 to p-ERK Dictates the Cytosolic Viscosity.....	146
5.2.4.1 Measurement of Rheological Properties of Cytoplasm – Limitations of Existing Technologies.....	146
5.2.4.2 Monitoring Intracellular Rheology with Julolidine Dye.....	147
5.2.4.3 Results and Discussion.....	148
5.2.4.4 Inferences.....	151
5.3 Summary.....	152
Chapter-6 Conclusions and Scope of Future Research	
6.1 Summary and Outlook	155
6.2 Scope of Future Research	157
References	159
List of Journal Publications from the Present Thesis	175