Study of the mechanics modeling of red blood cells deformation and mechanical properties

Liu, Yongping

2007

Liu, Y. (2007). Study of the mechanics modeling of red blood cells deformation and mechanical properties. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/35993>

<https://doi.org/10.32657/10356/35993>

a530289

Study of the Mechanics Modeling of Red Blood Cells Deformation and Mechanical Properties

Liu Yongping

School of Mechanical and Aerospace Engineering

A thesis submitted to the Nanyang Technological University
in FULFILMENT of the requirement for the degree of
Doctor of Philosophy

2007

ACKNOWLEDGEMENTS

I would like to express my wholehearted appreciation to my supervisors Dr. Li Chuan and Dr. Lai Chi Keung for their constructive academic advice, guidance and patience throughout this three-year hard work.

Special thanks are also given to Dr. Liao Kin and Dr. Vincent Chan for their provided convenience and assistance to conduct the experimental work in their laboratory.

I would like to thank my parents too. Without their encouragement and support, I would not be able to accomplish this difficult task.

Table of Contents

Acknowledgements	i
Table of Contents	ii
Abstract	vii
List of Publications	ix
List of Figures	x
List of Tables	xv
Nomenclatures	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Background of Cell Mechanics	1
1.2 Structure of Erythrocytes	4
1.3 Rationale and Premise Of Cellular Mechanics Of RBCs	6
1.4 Constitutive Laws of RBC Membrane Mechanics..	9
1.5 Reviews of Previous Experimental Works on RBCs.....	16
1.5.1 The Principle of Optical Trapping Techniques.....	17
1.5.2 Experimental Works on RBC by Optical Trapping Techniques	18
1.5.3 Experimental Testing on RBC Deformation in Fluid Flow.....	21
1.5.4 Summary.....	23
1.6 Objectives and Scopes	24
1.7 Layout of This Thesis	25

CHAPTER 2	THE DEFORMATION OF AN ERYTHROCYTE UNDER THE RADIATION PRESSURE BY OPTICAL STRETCH	29
2.1	Introduction	29
2.2	Mechanical Modeling	30
2.2.1	Geometry Of Cell Membrane	30
2.2.2	Kinematic Relation For Deformation	31
2.2.3	Constitutive Law	32
2.2.4	Equilibrium and Governing Equations	34
2.2.5	Boundary Conditions	36
2.2.6	Radiation Pressure From The Optical Field	38
2.3	Numerical Method	39
2.4	Results And Discussion	41
2.4.1	Profile Of Deformed Cell	41
2.4.2	Critical Pressure, Transverse And Longitudinal Strains	42
2.4.3	Optimal Values Of Membrane Properties	43
2.5	Conclusions	46
CHAPTER 3	AN EXPERIMENTAL STUDY ON THE DEFORMATION OF ERYTHROCYTES UNDER OPTICALLY TRAPPING AND STRETCHING	57
3.1	Introduction	57
3.2	Experiment	58
3.2.1	Cell And Microbead Preparation	58
3.2.2	Optical Tweezers	59

Contents

3.2.3 Force Calibrations	59
3.2.4 Image Processing	60
3.2.5 Experimental Results	61
3.3 A Simple MECHANICAL Model ANALYSIS	62
3.3.1 Assumption and Simplification For Mechanical Analysis	62
3.3.2 Geometry And Kinematic Relation	63
3.3.3 Constitutive Relations	65
3.3.4 Governing Equations	65
3.3.5 Boundary Conditions And Numerical Calculations	66
3.3.6 Numerical Solutions	68
3.4 Comparison Of Numerical And Experimental Results	69
3.5 Conclusions	70
CHAPTER 4 NUMERICAL ANALYSES ON THE MECHANICAL	81
PROPERTIES OF ERYTHROCYTE BY LASER	
STRETCHING	
4.1 Introduction	81
4.2 Mechanical Modeling	83
4.2.1 Geometry Of Cell Membrane	83
4.2.2 Kinematic Relation For Deformation	84
4.2.3 Equilibrium Equations	84
4.2.4 Governing Equations	85
4.2.5 Boundary Conditions (Bcs)	89
4.3 Numerical Method	90

4.4 Numerical Results	92
4.4.1 Profile Of Cell Deformation	92
4.4.2 Transverse Strains	93
4.5 Comparison Between Numerical And Experimental Results	94
4.6 Conclusions	96
 CHAPTER 5 FINITE ELEMENT ANALYSIS OF A FLOW-DEFORMED	110
 BIOMIMETIC VESICLE MANIPULATED BY OPTICAL	
 TRAP: A PARAMETER STUDY	
5.1 Introduction	110
5.2 Statement Of The Problem And Assumptions	112
5.3 The Hyperelastic Solid Model Of Cells	114
5.4 Numerical Analysis	115
5.4.1 Numerical Setup For Finite Element Analysis	115
5.4.2 Parameters For Analysis	117
5.5 Numerical Results	119
5.5.1 Benchmark Check For The Size Of Computational Domain	119
5.5.2 Numerical Results For The Vesicle	120
5.5.3 Numerical Results From The Fluid Field	121
5.6 Discussion	122
5.6.1 Deformation And Drag Force	122
5.6.2 Influence Of Mooney-Rivlin Coefficients	124
5.7 Conclusion	124

Contents

CHAPTER 6	CONCLUSIONS	140
6.1	Conclusions	140
6.1.1	General Summary.....	140
6.1.2	Contributions.....	141
6.2	Recommendations	142
Appendix I-1		144
Appendix I-2		145
Appendix II		149
Appendix III		151
Appendix IV		157
Appendix V		159
References		161

ABSTRACT

In this dissertation, the mechanical properties of RBC membrane and the deformability of erythrocytes under the optical traps with different applications were investigated. The applications include direct optical trap in radiation pressure of double laser beams, indirectly optical stretching by attached beads and single optical trap in fluid flow. The investigations were conducted by experimental approaches and by numerical simulations based on the established physical models.

In the experimental studies, a mechanical stretching experiment was designed for RBC membrane deformability testing. The mechanical behaviors of a spherically swollen erythrocyte stretched via attached microbeads by a single laser beam were studied in great details. From the measured experimental data or some experimental data extracted from previous work by others with different forms of optical trap applications, the mechanical responses of deformation (displacement/strain) and stretching force/radiation pressure were obtained and quantitatively analyzed under different loading conditions.

In order to interpret the experimental data with meaningful physical implications from them, several mechanical models were established to simulate the mechanical behaviors of deformed RBC under different optical loading conditions by numerical methods respectively. These models were based on different classic theories of continuum mechanics — membrane/shell theory of axisymmetric revolutions and general 3D hyperelastic theory by incorporating different constitutive laws. The profiles of simulated

Abstract

RBC shapes from different models were largely very close to the experimental observations under different loading conditions separately.

To extract the mechanical properties of RBC membrane from experimental and numerical data, an optimization approach for comparison was proposed to minimize the difference between experimental results and numerical results. Through this approach, several moduli to describe the material properties of RBC membrane from different models can be quantitatively predicted. The validity of the results was also evaluated by the literature surveys for the mechanical properties of RBC membrane, which were studied by different experimental techniques other than the optical traps. The comparability, similarity and approximation to previous work by others with different techniques prove that the work reported in this dissertation is reasonable, feasible and valid.

LIST OF PUBLICATIONS

- 1 Chuan Li, Y. P. Liu, K. K. Liu, J. H. Hsieh, A Parameter Study of the Material Properties in the Vesicle-Fluid Interaction, Computational Materials Science, Vol.30, 2004, 504-510.
- 2 Y. P. Liu, C. Li, A. C. K. Lai, Experimental Study on the Deformation of Erythrocytes under Optically Trapping and Stretching, Materials Science and Engineering A, Vol. 423, 2006, 128-133.
- 3 Y. P. Liu, C. Li, K. K. Liu, A. C. K. Lai, The Deformation of an Erythrocytes under the Radiation Pressure by Optical Stretch, ASME, Journal of Biomechanical Engineering, Vol.128 (6), 2006, 830-836
- 4 C. Li , Y. P. Liu, K. K. Liu, and A. C. K. Lai, 2007, "Correlations between the Experimental and Numerical Investigations on the Mechanical Properties of Erythrocyte by Laser Stretching", *IEEE Transactions on NanoBioscience* (accepted)

LIST OF FIGURES

Figure 2.1	Schematic representation of a spherical cell trapped by opposite, double laser beams	48
Figure 2.2	The geometry and coordinate system of an axisymmetric cell membrane. (a) the undeformed spherical cell with diameter a ; (b) and (c) the undeformed and deformed membrane in dimensionless coordinates (r^*, z^*) and (R^*, Z^*) respectively	49
Figure 2.3	The flowchart of numerical procedures	50
Figure 2.4	The profile of a spherical cell stretched by a pair of laser beams along the Z^* -axis	51
Figure 2.5	Comparison of the deformation for a spherical cell with $C = 1.0$ and 5.0	52
Figure 2.6	The transverse and longitudinal strains as function of the stretching forces for different membrane stiffness	53
Figure 2.7	The critical pressure loading for different membrane stiffness	54
Figure 2.8	The Taylor deformation parameter as a function of the stretching force adapted from the experimental results in (Guck et al, 2000; Guck et al, 2001)	55
Figure 2.9	The contour of errors as a function of C and bending stiffness B Corresponding shear stiffness G is shown in the top figure	56
Figure 3.1a	Schematic drawing of the optical tweezers (Module 1064/1500 from Cell Robotics Inc.)	74

List of Figures

Figure 3.1b	Scheme of a deformed RBC and the definition of deformation	74
Figure 3.2	Force calibrations by dragging a silica bead (2.34 μ m dia.) in Stoke's flow at the maximum escape velocity	75
Figure 3.3a	Edge detection by digital image processing using MATLAB	76
Figure 3.3b	Scheme of edge detection algorithm by MATLAB	76
Figure 3.4	Experimental data and images of deformed RBCs with attached silica microbeads (2.34 μ m in dia.) at different stretching forces	77
Figure 3.5	Numerical results for the deformed RBC with microbeads attached ($\delta^* = 0.185$)	78
Figure 3.6a	the longitudinal strain ϵ_l vs. the dimensionless force F^*	79
Figure 3.6b	the transverse strain ϵ_t vs. the dimensionless force F^*	79
Figure 3.7	Comparison of numerical and experimental results for deformed RBCs with the optimal tensile stiffness	80
Figure 4.1	The geometry and coordinate system of an axisymmetric cell membrane stretched by an attached bead. (a) the undeformed spherical cell with diameter a ; (b) and (c) the undeformed and deformed membrane in dimensionless coordinates (r^*, z^*) and (R^*, Z^*) respectively; the deformed membrane in dimensionless coordinates (R^*, Z^*) where $R^* = R/a$, $Z^* = Z/a$, $S^* = S/a$, and Z^* is the axis of symmetry. Note that $\delta^* = \delta/a$ is the dimensionless radius of attachment.	99

Figure 4.2	The flowchart of numerical calculation	100
Figure 4.3	The deformation of cell membrane with different stiffness under stretching by laser beams on silica beads at two ends along the Z^* -axis. The dimensionless stiffness C is a ratio of the in-plane shear to the out-of-plane bending. The average radius of attachment $\delta^* = 0.185$ is measured from the experimental data	103
Figure 4.4	The transverse strain as a function of stretching force for membrane with different stiffness	104
Figure 4.5	The transverse strain ($\varepsilon_t = R^*(\pi/2) - 1$) as a function of stretching force scaled by C	105
Figure 4.6	An illustration for the curve fittings of experimental data with simulated results through the minimization of errors	106
Figure 4.7	The contours of errors between the numerical and experimental results in the plane of G and C . Some errors are labeled by their values. The average of optimal G for $C = 10 \sim 100$ are also indicated by the solid lines	109
Figure 5.1	The geometry and finite element mesh of a vesicle in the Stokes' flow. The axisymmetry is in the y -direction and the thickness of membrane around the vesicle is 5nm	128
Figure 5.2	(a) Schematic representation of the laser trapping for a spherical vesicle. (b) If the trapping point is not in line with the center along with the axis of axisymmetry then the flow will push it to a new equilibrium position	129
Figure 5.3	Comparison between the numerical and analytical solutions of normal	130

	pressure and wall shear stress for fluid flow over a rigid vesicle at $V_{\infty} = 86 \mu\text{m/s}$	
Figure 5.4	Displacement field of vesicle for $V_{\infty} = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	131
Figure 5.5	the contour distribution of strain in Y direction, $V_{\infty}=86\mu\text{m/s}$, $Y_1=2000\text{Pa}$, $Y_2=20\text{Pa}$, $\nu_1=\nu_2=0.499$, $\beta_1=\beta_2=0.1$	132
Figure 5.6	Von Mises stress distribution of the vesicle for $V_{\infty} = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	133
Figure 5.7	Pressure distribution in the flow (water at room temperature) with $V_{\infty} = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	134
Figure 5.8a	Pressure distribution of the vesicle at $V_{\infty} = 86 \mu\text{m/s}$ for different E_1 ; The material properties are $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	135
Figure 5.8b	Wall shear distribution of the vesicle at $V_{\infty} = 86 \mu\text{m/s}$ for different E_1 . The material properties are $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	136

List of Figures

Figure 5.9	The Taylor deformation parameter (D_{12}), stretch ratio (η) and drag coefficient (C_d) vs. different combinations of Young's moduli of membrane and cytoskeleton. ($\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$)	137
Figure 5.10	Comparisons of the numerical solutions for deformable and rigid vesicle with the analytical solution. The material properties are $E_1 = 2000$ Pa, $E_2 = 20$ Pa, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$	138
Figure 5.11	The stretch ratio (η) as a function of β_1 and β_2 at $V_\infty = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000$ Pa, $E_2 = 20$ Pa and $\nu_1 = \nu_2 = 0.499$	139

LIST OF TABLES

Table 1.1 the measured values of RBC membrane elasticity by different techniques 28

Table 3.1 Characteristic properties of silica beads 72

Table 3.2 Experimental measurements of five RBCs and the estimated values of RBC membrane shear stiffness by a simple mechanical analysis 73

Table 4.1 Experimental measurements of five RBCs (from Chapter 3) and their optimized membrane shear stiffness by comparing with numerical results 98

Table 4.2 Predicted Values of Shear Stiffness and Bending Stiffness of RBC Membrane by mechanical models 98

Table 5.1 Comparison between the numerical and analytical solutions of fluid flow over a rigid vesicle for the finite domain shown in Figure 1 ($a=3.2\mu\text{m}$ is the radius of undeformed vesicle) 126

Table 5.2 Comparisons of the numerical solutions for deformable vesicle with the analytical solution for rigid vesicle. The material properties are $E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$ 127

Table 6.1 Summary of Predicted Material Properties from Different Experiment Results Membrane and by Different Mechanical Models with Optical Trap Applications 143

NOMENCLATURES

English Letter Symbols	Designation	Units
A	The Frontal Area of the Undeformed Vesicle	μm^2
a	Original Radius	μm
a_{10}	The First Material Constant in Two Parameter Mooney-Rivlin Model	N/m^2
a_{01}	The Second Material Constant in Two Parameter Mooney-Rivlin Model	N/m^2
B	Membrane Material Property	dyn/cm
	Membrane Bending Stiffness	N.m
C	Membrane Material Property	dyn/cm
	A Non-dimensionalized Parameter	
	Cauchy-Green Strain Tensor	
C_1	Coefficient of Material Properties of Mooney-Rivlin Material	dyn/cm^2
C_2	Coefficient of Material Properties of Mooney-Rivlin Material	dyn/cm^2
C_d	Drag Coefficient	
D	Diameter or Maximum Transverse Length	μm
D_{12}	Taylor Deformation Parameter	Dimensionless
E	Young's Modulus	dyn/cm^2
	The Lagrange Strain Tensor	
F	Stretching Force	pN
f	Deformation Gradient	

Nomenclatures

f_d	Drag Force	pN
G	Shear Stiffness	N/m
H	Membrane/Shell Stiffness against Change of Shape	N/m
h	Membrane Thickness	nm
I_i	Invariants of the Cauchy-Green Strain Tensor	
$\bar{I}_1, \bar{I}_2$	Invariants of Stretch Ratios	
$\bar{I}_i$	Reduced Strain Invariants	
K	Membrane Stiffness against Change of Area	N/m
L	The Maximum Longitudinal Length	
M	Membrane Bending Moment	N
P	External Pressure Loads or Resultant Stresses	Pa
Q	Transverse Shear Force	N/m
R	Dimension in the direction of "r" coordinate	μm
r	"r" direction in Cylindrical Coordinate System	
S	Arc Length	μm
	The Second Piola-Kirhhoff Stress Tensor	Pa
	Surface Area	μm^2
s	Arc Length	μm
T	Membrane/Surface Tension	N/m
	Isotropic Component of Membrane Principal Tensions	N/m
V	Velocity	$\mu\text{m/s}$

Nomenclatures

V_{∞}	Fluid Velocity at indefinite Distance	$\mu\text{m/s}$
W	Elastic Potential Energy	dyn/cm for 2D membrane/surface dyn/cm^2 for 3D objects
X	Position Vector of a Point in the Undeformed Shape	
x	Position Vector of a Point in the Deformed Shape	
Z	Dimension in the direction of "z" coordinate	
z	"z" direction in Cylindrical Coordinate System	

Greek Letter Symbols	Designation	Units
α		
β	Dimensionless Parameter of Material Properties	
γ	Deviatoric Component of Membrane Principal Tensions	N/m
σ	Optical Pressure/Stress	Pa
η	Stretch Ratio	
τ	Shear Stresses	Pa
λ	Stretch Ratio	Dimensionless
	Wave Length	nm
κ	Curvatures of Membrane Material Constant in Two Parameter Mooney-Rivlin Model	$1/\text{m}$
φ	Meridional Angle	
δ	Radius of Adhesion Area between RBC and Bead	μm

Nomenclatures

	Chroncher-delta	
θ	Circumferential Angle	
ε	Strain	Dimensionless
ρ	Density of Fluid	kg/m^3
π	A Constant	3.1415926
ν	Poisson's Ratio	
μ	Memebrane Shear Modulus	N/m
	Fluid Viscosity	Pa.S

Subscripts	Designation	
0	Original Dimensions / Original Geometry	
	Peak Values	
B	Bending Stiffness	
i	Indice of Principal Directions	$i = 1, 2, 3$
	Tensor Index	$i = 1, 2, 3$
	Material Index	$i = 1, 2$
j	Tensor Index	$j = 1, 2, 3$
l	Longitudinal	
r	Radial Direction	
s	Meridional Direction	
t	Transverse	

Nomenclatures

θ Circumferential Direction

Superscript	Designation
exp	Experimental Data
num	Numerical Data
R	Rest State of undeformed geometry
*	Non-dimensionalized Variables or Parameters

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND OF CELL MECHANICS

The cell is the basic unit of the biological system. Every human organism consists of either multiple or single cells. In order to fulfill their specific functions, cells have to perform many complicated processes involved in utilization of genetic information; synthesis, sorting, storage and transportation of biomolecules; conversion between different forms of energy; transduction of signals; maintenance of internal structures; and response to external environments.

Among these processes, some involve mechanical aspects. A straightforward way that cells sense mechanical perturbation is to transduce mechanical signals via the deformation of lipid membrane and cytoskeleton into physical or biochemical signals that will cause certain physiological reactions in cells or tissues in organism. It has been proposed that such deformation may result in membrane attachment and activation of cytoplasmic molecules, which triggers a cascade of biochemical events (*Gudi, et al, 1998*). Mechanosensitive ion channels have also been suggested by many studies, because their blockade by gadolinium can inhibit stretch-induced gene expression and morphological changes in endothelial cells (*Sokabe, et al, 1997*). For an instance, fluid shear stresses (*Davies, 1995*) not only up-regulated or down-regulated the expression of genes coding for growth factors, adhesion molecules, as well as protooncogenes, but also induce cytoskeleton remodeling, leading to elongation and alignment of endothelial cells in the direction of flow. Furthermore, it has been shown that tyrosine kinase

activity and intracellular calcium are important in fluid shear-induced cell shape change and cytoskeleton remodeling (Malek, *et al*, 1996).

Exactly, some biological responses in cells can be induced by these mechanical perturbations. Evidence shows that some normal functions and malfunctions of cells are dependent on or regulated by their mechanical environment. Therefore, a major interest in cell mechanics is the regulation of cellular functions by mechanical forces. The field of cell mechanics, as a part of the interdisciplinary endeavor, mainly concerns how cells move, deform, and interact, as well as how they sense, generate, and respond to mechanical forces.

In human body, many kinds of cells live in fluid environments, such as red blood cells, white blood cells and endothelial cells of vascular vessels. They all interact with the fluid flow. The red blood cell (RBC), with mean diameter of $\sim 8\mu\text{m}$, is known for its ability to withstand large deformation when it routinely passes through capillaries with inner diameter as small as $\sim 3\mu\text{m}$; and it can fully recover to its original shape when the constraints of load and geometry are released. The membrane's in-plane shear resistance and out-of-plane bending resistance of the erythrocyte must be very small; otherwise the cell could not undergo severe deformation easily in a capillary. On the other hand, the in-plane shear resistance must be also large enough to maintain the cell's shape and integrity during normal flow in the circulatory system. Therefore, the knowledge of RBC's *rheology* is very useful to understand the biodynamics of blood circulation system.

In addition, evidence shows that the mechanical behaviors of RBC are associated

with several blood diseases, which are dependent on or regulated by their mechanical environment. In the case of sickle cell disease, a defect in the haemoglobin structure causes the changes of red blood cell shape; and its deformability and biorheology are adversely affected (Platt, 1995). In the single-cell studies of normal and plasmodium-infected red blood cells (iRBCs), which are intended to develop sensitive diagnostics for malaria, it appears that malaria-induced organ failure is associated with capillary blockage and the increased rigidity of iRBCs. Micropipette (Shelby, *et al*, 2003) and rheoscope relying on measurements of fluid shear stress (Cranston, *et al*, 1984) have been utilized to establish differences in the deformability of normal and iRBCs. Therefore, the knowledge of RBC's mechanical properties is also very useful to understand the mechanisms that how diseases relevant to RBCs are dependent on or regulated by their external mechanical stimuli.

A dramatic revolution has been taking place in the research of cell mechanics during the past two decades due to the introduction of several new biophysical technologies. The scientific challenges in the study of the effective forces at the scales of piconewton (pN) or nanonewton (nN) on biomimetic structures is made possible by the advent of some advanced and sophisticated instruments, such as optical laser tweezers, confocal interference contrast microscopy and atomic force microscopy. With the huge increase in the computational efficiency, mechanical modeling and numerical simulation become more feasible in cellular mechanical behaviors under various external mechanical stimuli. Thus the current trend of cell mechanics research is to elucidate the relationship between the mechanical forces and cellular functions in living

organisms.

1.2 STRUCTURE OF ERYTHROCYTES

Since the rheology of erythrocyte plays an important effect on the biodynamics of blood circulation system and associates with some blood diseases, the deformability of erythrocytes has been a hot topic of biomechanics for its great scientific interest. To understand the structure of erythrocytes is the first necessary step for the investigation of their cellular mechanics.

The typical structure of a cell (eukaryotic) is composed of three major parts such as nuclear, membrane and cytoplasm consisting of cytosol and organelles (mitochondrion, golgi apparatus, lysosome, endoplasmic reticulum, etc.). Without a doubt, the overall mechanical behavior of a eukaryotic cell is influenced by its complex and intricate structure. However, for mature red blood cells, they have lost their nuclei of their youth and cannot divide themselves. In addition to the normal physiological environment, mature RBCs are also able to be perfectly alive at 20 ° C or 25 ° C in the absence of nutrients. The only requirement is that the osmotic pressure of their aqueous surroundings be kept at or near the equivalent of a sodium chloride concentration of 0.147 mol/dm⁻³, at which they retain their classical biconcave shape (*Len Fisher, 1993*).

RBCs vary considerably in size even within a single individual. For human red blood cells, the mean surface area is ~130 μm² and the mean volume is ~98 μm³. The range of sizes within a population is in Gaussian-distribution with standard deviations of ~15.8 μm² for area and ~16.1 μm³ for volume (*Fung, et al, 1981*). Cells from different species vary enormously in size. However, the sizes of RBCs from the same

class of species, such as mammalian resource, are relatively close to each other in statistics. Hawkey et al gave some statistical data for red blood cells from mammalian species (*Hawkey, et al, 1991*).

Due to the absence of nuclei, the structure of RBCs can be divided into two main spatial parts. The interior of RBCs is a concentrated solution of hemoglobin, the oxygen-carrying protein, and it behaves as a Newtonian fluid (*Cokelet, et al, 1968*). In a normal population of RBCs, there is a distribution of hemoglobin concentrations in the range of 29~39 g/dl. The viscosity of the cytosol depends on the hemoglobin concentration and temperature.

The outer shell encompassing the interior of a RBC is the cellular membrane. The structure and physic-chemical properties of mammalian red blood cell membrane are now quite well understood. From the physical point of view, a membrane is not just a lipid bilayer studded with proteins. A better definition is: a material with very small thickness in comparison with its radii of curvature, which separates two adjacent liquid-like domains and supports the stress created by the embedding medium. The membrane of cells is composed of two main components: a sheet of lipid bilayer embedding with some proteins and a subsurface network of protein filaments.

The phospholipid bilayer forms a stable barrier between two aqueous compartments. The major lipid components are unesterified cholesterol and phospholipids. The phospholipids composition of the membrane is mainly as follows: phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE) and phosphatidylserine (PS). In the lipid bilayer, the glycolipids are relatively minor membrane components, constituting only about 2% of the lipids of most plasma

membranes. However, cholesterol is also a major constituent, being present in about the same molar amounts as the phospholipids. The outer leaflet of the plasma membrane consists mainly of the choline-containing neutral phospholipids PC and SM; the negatively charged phospholipids PE and PS are the predominant phospholipids of the inner leaflet.

Unlike most eukaryotic cells in which the complex three-dimensional (3D) array of the inter-connected cytoskeleton (CSK) extends throughout the 3D spatial cytoplasm, the cytoskeleton of erythrocytes is just beneath the lipid bilayer. The cytoskeleton network, which mainly consists of spectrin, actin, ankyrin, protein 4.1 and band 3 (*Shen, 1989; Bennett, et al, 1993; Chien, et al, 1990*), provides the structural framework for cells, serving as a scaffold that determines cell shape and playing a major role in resisting to shape deformation. Because of this unique spatial structure, the cytoskeleton of RBCs can be assumed to be a flexible two-dimensional cross-linked protein network.

In a word, since erythrocyte has no significant intracellular structure, composed of viscous fluid (hemoglobin solution) inside, the resistance to stress is mainly attributed to the elastic properties of its membrane, a lipid bilayer reinforced on its inner face by a flexible two dimensional cross-linked brushy protein network (cytoskeleton).

1.3 RATIONALES AND PREMISE OF CELLULAR MECHANICS OF RBCS

Before outlining the theoretical mechanical models, a question must be first

elucidated. Are cellular behaviors governed by a continuous or discrete medium? The answer largely depends on the relevant biological issues and the scales involved. The mechanical models of cells can be grouped into two broad classifications: continuum and discrete.

The continuum model depicts the cell as stress-bearing multi-phase media (membrane and cytoplasm) that are continuous, homogeneous and evenly distributed in a finite space, without any explicit description of a precise micro-structural organization. The underlying assumption for treating a material as a continuum is that the smallest dimension to be considered is much larger than the space over which structures and properties vary significantly. Thus, treating a suspended cell as a continuum is valid when one is interested only in deformations on the whole-cell scale.

For continuum mechanical models, there are two hypotheses proposed for the load bearing capacity of the cell interior, which lead to two different research approaches. In the first hypothesis, the plasma membrane encloses an intercellular mixture with an overall fluid-like rheology. At steady state, the cells' resistance to deformation totally contributes to the plasma membrane, which is modeled as a thin elastic membrane. Under the second hypothesis, the cell interior and membrane express an overall solid-like rheology. The cell resists a static stress by a deformation from a zero stress state. Both of the two hypotheses can capture some essential features of cells and produce some quantitative and qualitative depictions of cell's elastic moduli and viscosity.

In the human blood circulation system, the mechanical behaviors of blood cells interacting with vascular flow are of extreme interest and importance. Among of them, erythrocytes and leukocytes typically exhibit fluid-like rheology. The erythrocyte has a relatively simpler intracellular structure. To a good approximation, it is simply a bag of fluid, a concentrated solution of hemoglobin surrounded by a thin microscopically homogeneous membrane. Thus, the sophisticated continuum models are most often employed to depict blood cells in mammals. The shapes and shape transitions of laboratory-prepared fluid-phase phospholipid vesicles (lacking any skeleton), which are used to simulate erythrocytes, have been successfully predicted by the area-difference-elasticity (ADE) model based on continuum mechanics (*Miao, et al, 1994*).

Experimental evidence supports both hypotheses for the rheology of the cell cytoplasm. Briefly, the data obtained from micropipette aspiration measurements of endothelial cells are consistent with both the fluid (*Sato, et al, 1987a*) and solid interior hypotheses (*Sato, et al, 1987b*). Furthermore, in vitro measurements on purified CSK filaments demonstrate both fluid-like and solid-like behaviors. The concentrated actin filaments show a nearly elastic response when a cell is exposed to small shear stresses; on the other hand, they respond to a large shear stress with a viscous flow (*Janmey, et al, 1991*).

The discrete models of cell mechanics are based on a priori idealized postulates of CSK organization. It takes the micro-structures of CSK into account. The prominent benefit of this approach is its potential to reveal the relationship between specific CSK structures and their mechanical functions in resisting deformation. The premise

underlying discrete models for the mechanics of cells is that the load bearing capacity of the cell originates from the response of an ensemble of individual intracellular structural components.

At present, the tractable discrete models for mechanics of CSK are spring networks (Hansen, *et al*, 1996; Hansen, *et al*, 1997) and tensegrity (Ingber, *et al*, 1981) models. They capture some essential mechanisms that govern CSK deformability and provide a powerful tool that can trace cell mechanics to the level of the CSK's resistance to deformation, although they all neglected some relatively minor factors of other microstructures in cytoplasm. More details about the discrete models can be found by reference to the relevant literature.

Due to its unique and simple structure, RBC membrane is generally described in the simplest way as a *two-dimensional* network of spectrin strands bound to the phospholipidic bilayer. With the assumption that the *interior* of red blood cells is a fluid being *purely viscous* and *no elasticity*, the plasma membrane is entirely responsible for the elastic response. The experiments of micropipette aspiration have demonstrated that the RBC membrane can be modeled as an effective continuum (Hochmuth, 2000). If only the mechanical behaviors of RBCs and its mechanical properties are concerned on whole-cell scale, the red blood cell is more amenable to the development of theoretical or computational models by assuming the composite of lipid bilayer, transmembrane protein and cytoskeleton network as continuum.

1.4 CONSTITUTIVE LAWS OF RBC MEMBRANE MECHANICS

Theoretical studies are essential to investigate the mechanical behaviors of cells. So far, there are several classic mechanical models that have been proposed for RBCs. Each of them can capture some features of RBC mechanical behaviors but not all. For continuum models, the most used ones are linear elasticity, hyperelasticity and viscoelasticity. In this section, they will be briefly introduced.

The simplest constitutive model to describe the mechanical behavior of RBC membrane is linear elasticity. It is widely used to establish the simplest mechanical models to interpret the data of various experiments. For instance, Hochmuth and Mohandas used a one-dimensional linear elastic model to approximately analyze and interpret their experimental data of cell elongation when a RBC was subject to shear flow in channel (*Hochmuth, et al, 1972*). Henon et al approximately described the biconcave RBC as two parallel discs and used a two-dimensional planar linear elastic model to approximately analyze and interpret their experimental data of a deformed RBC stretched by two point loads at the two poles (*Henon, et al, 1999*). Guck et al used a two-dimensional linear elastic membrane theory to analyze and interpret their experimental data of a deformed RBC strapped in an optical stretcher (*Guck, et al, 2001*). However, the modeling analyses based on linear elastics were restricted in the range of small deformation.

In fact, the phenomena that erythrocytes can traverse capillaries whose diameter is considerably less than their major diameter and that erythrocytes can undergo the multiple morphological transformations from normal biconcave shape to various other shapes such as echinocytes (crenated shapes), stomatocytes (cup-like shapes) and

spicules (*Ranjan, et al, 2002*), show that RBC can resist large deformation. Many artificial biomimetic counterparts of RBC, such as vesicles, sacs or microcapsules, have been made for observing their morphological transformations under various mechanical conditions in laboratories. For instance, a liposome is a small artificial vesicle only made of a single lipid bilayer; it both encloses water and is surrounded by water. Hotani observed some striking morphological transformations in his experiments (*Hotani, 1984*). The liposomes undergo a series of morphological transformations over a period of time as a result of osmotic pressure difference, as shown in the picture in Appendix I-1.

Apparently, the linear elasticity cannot describe such complicate mechanical behaviors of RBC or liposome. In order to capture some features of the mechanical mechanism standing behind the morphological transformations of RBC/liposome undergoing large deformation, Pamplona and Calladine proposed a very simple constitutive model of axisymmetric liposomes (*Pamplona, et al, 1993*); and Parker et al extended its application into the mechanics of RBC membrane (*Parker, et al, 1999*).

One of the main features of the mechanical behavior of lipid bilayer is that a lipid bilayer is like a two-dimensional liquid in the sense that the surface can change its shape rather easily, while preserving its area as constant. In the mechanical model proposed by Pamplona and Calladine, the response of bilayer element is supposed to be elastic; and the elasticity may be described by means of two distinct elastic constants K and H . The first one expresses the idea that in a state of “equal biaxial tension”, $T_1 = T_2$, the element is stiff, in the sense that there can be little enlargement of surface area. The second corresponds to the situation where $T_1 = -T_2$, i.e., the loading is a state of pure

shear with respect to axes at $\pm 45^\circ$ to the principal axes; there now may be significant pure shear distortion and strain with respect to the same axes. Since $K \gg H$, the elastic constitutive law of tensions may be simply written as by taking the limiting case $K/H \rightarrow \infty$.

$$T_1 - T_2 = H (\lambda_1 - \lambda_2) \quad (1.1)$$

where, K is the stiffness (N/m) against change of area; H is the stiffness (N/m) against change of shape; λ_i are the principal stretch ratios; here $i=1, 2$, representing the two principal directions, respectively.

Another important feature of the mechanical behavior of lipid bilayer is that it has a significant bending stiffness. Corresponding to the state of “equal biaxial tension” in the two layers, an equal biaxial or isotropic bending stress resultant is induced. The bending moments in the lipid bilayer can be assumed to be proportional to the change in the sum of the principal curvatures of the membrane surface. Therefore, the principal bending moments can be rewritten as

$$M_1 = M_2 = B \left[(\kappa_1 + \kappa_2) - (\kappa_1 + \kappa_2)^R \right] \quad (1.2)$$

where, B is the bending stiffness (N·m); κ_i are the principal curvatures; here $i=1,2$, representing the two principal directions, respectively; the superscript “R” denotes the rest state of undeformed geometry.

Using the constitutive law (1.1) and (1.2), a linear constitutive relation between the principal tensions and the principal stretch ratios was proposed by Pamplona and Calladine. With the constraint of area constant, i.e., $\lambda_1 \cdot \lambda_2 = 1$, they successfully simulated the morphological transformations of liposomes from original spherical shape to prolate spheroid, then to biconcave shape under various osmotic pressures.

Some classic theoretical analyses also argued that bending resistance of cell membrane is negligible compared to the shear resistance when RBC membrane is on large deformation. This approximation has been incorporated in many theoretical analyses and simulations (*Lin, et al, 1973; Secomb, et al, 1986*). Nevertheless, mechanical analysis of other membrane phenomena, such as the formation of tethers (*Bozic., et al, 1997*) and spicules (*Ranjan, et al, 2002*), emphasize the bending stiffness of membrane. At present, no strong evidence can show which approximation is more appropriate in interpreting the data such as from the laser tweezers experiments.

Unlike the perspective that Pamplona and Calladine directly proposed the constitutive law by carefully analyzing the chemical-physical structure of lipid bilayer, the constitutive relations can be also derived from the strain-energy function of RBC/liposome membrane. To capture the mechanical feature of large deformation, various strain-energy functions were proposed based on the uniaxial tension experiments. Generally, these strain-energy functions fall into hyperelastic category.

Hyperelasticity refers to materials that can experience finite elastic deformation that is completely recoverable. Many polymers fall into this category. The stresses for these materials are usually derived from strain energy density function. A material is said to be hyperelastic if there exists an elastic potential function W (or strain energy density function) that is a scalar function of one of the strain or deformation tensors, whose derivative with respect to a strain component determines the corresponding stress component. The theory employed for the family of hyperelastic elements is limited to isotropic materials. However, no limits are placed on the magnitude of the strain and the results are path independent.

In earlier studies, the RBC membrane was modeled as a rubber sheet with a constant thickness. Generally, rubber may be regarded as isotropic and incompressible. Such a typical material which has a rubber-like nonlinear elasticity is the so-called Mooney-Rivlin materials with a strain energy function as following form (*Green, et al, 1970*).

$$W = C_1(\bar{I}_1 - 3) + C_2(\bar{I}_2 - 3) \quad (1.3)$$

where, $\bar{I}_1$ and $\bar{I}_2$ are the invariants of stretch ratios.

The constitutive relations derived from Eq.1.3 or their simplified forms were widely used to describe the mechanical behaviors of liquid-filled cellular entities. For instance, Barthes et al analyzed the dynamics of a small deformable capsule freely suspended in a viscous fluid undergoing a pure shear flow (*Barthes-Biesel, et al, 1981*); they also investigated the mechanics of two-dimensional membrane on shear flow-induced capsule deformation (*Barthes-Biesel, et al, 2002*) and the mechanical behaviors of capsules moving in cylindrical channels (*Christophe, et al, 1997*). In addition, Liu et al investigated the compressive behaviors of single microcapsules deformed between two parallel plates (*Liu, et al, 1996*). The studies in these literatures surely captured some features of the mechanics of two-dimensional (2D) membrane of microcapsules which was modeled as hyperelastic material.

To model the mechanical behaviors of RBCs on large deformation, Skalak proposed the following strain energy function for 2D membrane of red blood cell (*Skalak, et al, 1973*).

$$W = \frac{B}{4} \left(\frac{1}{2} I_1^2 + I_1 - I_2 \right) + \frac{C}{8} I_2 \quad (1.4)$$

where, B and C are the two membrane material properties, dyn/cm.

The constitutive relations derived from Eq.1.4 or their modified forms were applied to simulate the large deformation of RBC in various experiments, such as red blood cell in sphering and sieving experimentations (Skalak, 1973), the morphological transformations of microcapsules/liposomes from original spherical shape to prolate spheroid, then to biconcave shape under various osmotic pressures (Zarda, et al, 1977), and RBC motion in capillary (Secomb, et al, 1986). These applications showed that a constitutive law derived from the strain-energy function can describe the mechanical behaviors of RBC membrane. The constitutive laws derived from the hyperelastic (Evans, et al, 1980) or hyperelastic effective models were also used to simulate the cell behaviors through finite element analyses (C. T. Lim, et al, 2004).

For mechanical analyses with the strain energy functions in the forms of Eq.1.3 and Eq.1.4, there are two parameters associated with the material properties of RBC membrane, which need to be determined through experimental tests. In the homogenous and incompressible rubber RBC membrane model defined by the Eq.1.3, the two membrane material properties C_1 and C_2 can be estimated by adjusting their values to give the Young's modulus of membrane measured in uniaxial tension for example by Suter et al (Sutera, et al, 1970). That is, $C_1 \cong 2.57 \times 10^6$ dyn/cm² and $C_2 \cong 0.257 \times 10^6$ dyn/cm² for a given Young's modulus of membrane $E \cong 10^7$ dyn/cm² and initial membrane thickness $h_0 \cong 10$ nm. For another hyperelastic RBC membrane model defined by the Eq.1.4, the two membrane material properties B and C can be estimated by comparing with available experimental data for the uniaxial tension test by Hochmuth et al (Hochmuth, et al, 1972), that is, $B \cong 0.005$ dyn/cm and $C \cong 5.0$ dyn/cm for

a given assumption that Young's modulus of membrane $E \cong 2 \times 10^4$ dyn/cm² and membrane thickness $h \cong 10$ nm.

The constitutive relations between the principle membrane tension forces and the principle extension ratios derived from the strain energy functions, such as those proposed by Evans and Skalak, show that the material parameters B and C are associated with the specific membrane moduli: the shear modulus/stiffness and the area dilation modulus/stiffness respectively. If incorporated with the constitutive relations of bending moments with such forms as Eq.1.2, there are three material parameters that need to be determined for the mechanical analyses of RBC. However, no such studies are available at present.

1.5 REVIEWS OF PREVIOUS EXPERIMENTAL WORKS ON RBCs

In addition to the theoretically model on the overall deformation and mechanical behaviors of cells under certain loads, it is essential to know some key parameters that characterize the material properties of a whole cell or its components, depending on which kind of model, continuum or discrete, is adopted. Usually, experimental study is the unique way to obtain this information by measuring and interpreting certain kind of relationship of deformation (strain or displacement)-forces (or stresses). In the experimental study of cell mechanics, following techniques have been used to different kinds of cells: micropipette aspiration (MA) (*Evans, et al, 1989*), optical trap (such as: optical tweezers(OT) and optical stretcher (OS)), cell poker (CP) (*Zahalak, et al, 1990*), atomic force microscope (AFM) (*Radmacher, et al, 1996*), magnetic twisting cytometry

(MTC) (Wang, *et al*, 1993) and Magnetic bead microrheometry (MBM) (Bausch, *et al*, 1998). Since the theme of this thesis is to conduct experimental study on RBCs by optical traps, previous works done with this technique will be briefly reviewed; and the results from other techniques will also be outlined.

1.5.1 The Principle of Optical Trapping Techniques

In the past decade, a well-known novel technique is optical laser trap that is being applied in the study of cell biology due to its capability to manipulate objects ranging in size from atoms to cells in several research and technological areas such as biology, medicine, physics and engineering.

The principle of laser traps is that whenever a ray of light projects on a dielectric, homogenous particle with a refractive index different from that of its surrounding medium, a part of the incoming light is reflected; a part is transmitted and scattered, and even some may be possibly absorbed. Thus, a change occurs between the momentums of the incoming light and the out-going light. Because of the conservation of total momentum, the change of momentum flux of photons will produce forces exerted on the surface of a trapped particle. The force per unit surface area is known as “radiation pressure”. There are several kinds of instruments that are designed to use the forces of laser radiation pressure to trap small particles. The forces that such an instrument with a single laser beam is capable of measuring are of the order of one to 100 pN. This technique has been used for over 30 years to manipulate and study the properties of micron-size dielectric particles. The more detailed illustrations can reference to Appendix I-2. However, it is only in the last decade that this precise force

measurement instrument has been applied to the study of biological systems. Detailed philosophy and principles of optical traps and the instructions for building up the instruments of optical traps can be found in relevant references (*Ashkin, 1998; Denk, et al, 1990; Chu, 1991; Ashkin, et al, 1986*).

Using laser traps, spherical microbeads or cells can be manipulated through the optical forces. The most often used instruments on the basis of optical traps in cell mechanical experiments are the optical tweezers and the optical stretcher.

1.5.2 Experimental Works on RBC by Optical Trapping Techniques

Generally, the optical tweezers can only produce a single optical trap by a single laser beam. The mechanical behaviors of biconcave erythrocytes directly trapped by a single laser beam have been reported by *Grover et al (Grover, et al, 2000)* and *Dharmadhikari et al (Dharmadhikari, et al, 2004)*. All of them found the phenomena of rotation, reorientation and morphological changes in their experiments. However, they did not give further investigations on the material properties and their effects on the cell's deformation. The difficulties of mechanical investigation on RBCs directly trapped in optical laser lie in two aspects. Firstly, the radiation pressure on the biconcave shape of RBCs is hard to determine; thus the deformation induced by this undetermined radiation pressure is not feasible to the quantitative analysis. Secondly, the highly focused laser beam will induce local temperature increase. As a consequence, the trapped RBCs may be possibly damaged due to temperature increase (*Liu, et al, 1995*).

Due to the uncertainty of the effects of optical pressure on the mechanical

behavior of RBCs directly trapped in an optical laser, a more typical application of optical tweezers is that the laser beam illuminates on the microbead bound to the surface of a cell, but not directly on the cell itself. The microbead is used as a handle to stretch the cell. By measuring the data of deformation and the forces exerted on the trapped microbead by optical laser, a mechanical response of displacement (or strain)-force can be quantitatively characterized.

A specially improved optical tweezers installed with two perpendicular galvanometric mirrors, by which a single laser beam can be split into two, was used by Henon et al (*Henon, et al, 1999*). The two laser beams trap the two microbeads radially bound to a RBC. The RBC can be stretched since laser traps apply identical but opposite forces to the two beads. With a pure 2D planar linear elasticity, Henon et al interpreted their experimental data and predicted the shear modulus of RBC membrane with the magnitude of $\sim 2.5 \pm 0.4 \mu\text{N/m}$ according to the displacement-force response.

The optical tweezers with a single optical trap can also be used for RBC stretching experiment. Such an experimental design was first introduced by Dao et al (*M. Dao, et al, 2003 and C. T. Lim, et al, 2004*). The main feature of this experiment design is that one of the two beads bound to a RBC is required to be stuck to the bottom glass coverslip; another is free for movement and trapped by optical laser. Dao et al and Mills (*Mills, et al, 2004*) simulated their experimental results of biconcave RBC stretched by the optical tweezers with finite element modeling and conducted a parametric analysis to the mechanical properties of RBC membrane.

A more novel instrument using optical traps is the optical stretcher. In contrast, the main feature of an optical stretcher is that it is based on a double-beam trap, in which

two slightly divergent, horizontal, co-axis and identical Gaussian laser beams but in opposite propagation directions trap an object in the middle. If a soft biological dielectrics trapped in the optical stretcher, it will deform under the optical stresses on its surface. Generally, this optical technique can provide larger forces than its predecessor, the optical tweezers. The forces are on the order of hundreds of piconewtons. These forces can produce measurable deformations in a wider range of cells. With this technique, an entire cell can be deformed without the addition of beads to the surface.

However, to analyze the mechanical behaviors of cells trapped in the optical stretcher, it is necessary to investigate the optical stress distribution on the surface of a trapped object. The simplest optical theory for this purpose is the ray optics (RO), which was successfully used to predict the gradient force and propulsive force of a spherical microbead trapped by a single laser beam (*Bakker Schut, et al, 1991; Nemoto, et al, 1998*). It was Guck et al who first analyzed the optical stress distribution on a spherical cell trapped in optical stretcher by the approach of RO when the circumference of the trapped spherical object is much greater than the wavelength of laser beam, i.e., $2\pi R_0/\lambda \gg 1$ (*Guck, et al, 2001*). They found that the axisymmetrical radiation stress profiles can be closely proximately expressed by a simple function $\sigma = \sigma_0 \cdot \cos^2 \varphi$ ($0 \leq \varphi \leq \pi/2$) when the ratio of waist size of Gaussian laser beams to the radius of RBC is slightly larger than 1.0. Wherein, σ_0 is the peak stress (Pa) on the surface of the trapped red cell along the centered axis of laser beam and its magnitude depends on the power of laser beam and the relative sizes of the waist of laser beam and the radius of red cell. With the approximate optical stress profile function, Guck et al established a mechanical model by pure linear elastic membrane

theory and predict the Young's modulus (1.3 kPa) of RBC membrane by fitting the experimental data (Guck, *et al*, 2000).

However, the elastic modulus estimated in the above study is a three-dimensional constant that is unlike the cortical tension that represents solely the two-dimensional membrane tension. Furthermore, large deformation of RBC membrane was obviously observed in Guck's experiments. For small deformation, the models based on Hooke's law can give a good prediction consistent with the experimental observations. However, for large deformation, the discrepancy becomes distinct compared with experimental results. This phenomenon that the non-linear mechanical behaviors of the membrane of RBC will be prominent at large deformation has been also demonstrated in other experiments. Furthermore, the bending resistance of membrane was not incorporated in Guck's analysis.

1.5.3 Experimental Testing on RBC Deformation in Fluid Flow

Among all mechanical stimuli, flow-induced stress exists in the circulation system of most organisms and serves as a natural link between physiological fluid and cells/tissues. Thus, the elucidation of cell deformation triggered by physiological flow provides valuable information for engineering cellular behavior and clinical diagnoses (Dong *et al*, 2000; Lei *et al*, 1999).

The earlier experimental study and mechanical analyses on the deformation of RBC caused by flow-induced stresses were conducted by Hochmuth and Mohandas (Hochmuth, *et al*, 1972). In their experiment, a RBC was attached on the coverslip and was immersed in flow channel. The elongation of the RBC and the flow-induced

stresses on the cell surface were quantitatively measured and analyzed. Through the mechanical analyses based on the Hooke's law elastic model, they predicted the Young's modulus of RBC membrane with a value of $E \cong 2.5 \times 10^4 \text{ dyn/cm}^2$ for a given membrane thickness $h=10\text{nm}$. Although Hochmuth and Mohandas' work gave a fairly good estimation, the mechanical model seems to be too simple.

A more recent experimental study on RBC mechanics by optical traps is that a biomimetic vesicle trapped by a single optical laser trap is subject to Stoke's flow and its deformation under viscous drag of flow is observed and quantitatively evaluated under constant temperature or various temperatures (*Foo, et al, 2003*). In the work reported by Foo et al, the deformed shapes of vesicles under different velocities of viscous flow were captured by an in-line CCD camera; the velocity and pressure in the flow field around the optically trapped vesicle were numerically simulated and the corresponding viscous drag force of vesicle in flow for each case were calculated. They argued that the flow- induced stress leads to a significant vesicle deformation and the observed deformation is mainly caused by the flow-induced stresses acting on the surface of a trapped vesicle.

In essence, *Foo's* work merely discussed the effects of deformed shapes of vesicles on the flow field (velocity and pressure) and on the drag forces. How the flow-induced stresses exert effects on the morphology of a vesicle itself was not involved in their work. Furthermore, it deserves to further investigate whether the shape changes of vesicles in the Stoke's flow were only contributed to flow-induced stresses in their experiments.

Guck's work showed that the optical radiation stresses on a trapped soft object

might also lead to its deformation. That means the deformation of an optically trapped vesicle in fluid flow should be contributed to a combination of the coupling effects from multiphysical fields, i.e., optical field and fluid field. Apparently, it is very difficult to analyze the mechanical behaviors of an optically trapped cell or vesicle in fluid flow since it is in fact a coupling problem of triple-physical fields. For an interest of this problem, some attention will be paid to the question how and in what extent the flow-induced stresses influence the morphology of an optical trapped cell or vesicle in fluid flow and vice versa.

Optical traps of particles in fluid flow are a kind of non-intrusive technique since the laser beam does not exert any effect on flow field when it is used to control cell in fluid flow. Therefore, it seems to be feasible that an experiment and its related mechanical modeling similar to what Hochmuth and Mohandas had done can be more effectively conducted by using this novel tool. If a cell/vesicle can be held in flow by focusing the laser beam in very small area on the surface of RBC or focusing on a very small bead which is attached on the surface of RBC and whose radius is much less than that of RBC. Then, it can be expected that the cell will be deformed and elongated much like the experimental results from Hochmuth and Mohandas' work.

1.5.4 Summary

As foregoing introduction, different techniques have been used to conduct experimental study on RBC membrane mechanical properties; and various physical models have been used to simulate RBC mechanical behaviors. As far as the RBCs are concerned, erythrocytes and their artificial biomimetic counterparts, such as

microcapsules, liposomes and vesicles, have been studied by various experimental techniques. For an instance, micropipette aspiration produces much information on RBC's mechanics. A summary of the main material property parameters reported in the literature by different techniques is listed in Table 1.1. However, different experimental techniques yield very different data of the parameters characterizing the material properties of cells even for the same cell type (*Rachel, 2000*). This point is also exhibited in Table 1.1.

1.6 Objectives and Scopes

As is known well, the significance of mechanical properties of cells and their mechanical stimuli are evident in a number of physiological pathologies. It is very intriguing for many scholars to understand the mechanism of the mechanical behaviors of cells. Although much information on mechanical properties of RBC has been obtained from previous work mainly from micropipette aspiration experiments, the discrepancy among from techniques' results still exists. A reason is that the measured values may depend on the length scale and the time scale of the mechanical probe and perturbation involved in the techniques (*Thoumine, 1997*), which may cause mechanical response of cells in different elastic regimes for different measurement techniques. Another reason is that the mechanical models used for data analysis are likely to be overly simplified.

The advent of more novel biophysical techniques (such as optical laser traps) provides more feasible and precise technical skills, which make it possible to manipulate micron-scaled cells in a totally different form from micropipette aspiration.

The experimental data from this novel technique may greatly enrich the information on the mechanical properties of RBCs. Therefore, the research work in this thesis focuses on following four aspects.

- First, a numerical analysis on the mechanical behaviors of a RBC under radiation pressure will be conducted. Compared the numerical results with the experiment results of Guck's work, the material properties of RBCs membrane will be predicted.
- Second, an experimental study on RBC deformation by using optical tweezers will be conducted. It is generally believed that RBCs of mammalian animals have the maximum similarities in their physical and chemical properties. Some evidences had been shown by previous research works (*Hawkey, et al, 1991; Fung, 1990; Len Fisher, 1993*). Therefore, for convenience, rat RBC will be used for the experimental study in this these. The experimental data of the deformation-force response are collected and analyzed.
- Third, mechanical models based on different constitutive laws will be established to simulate the deformation of RBC stretched by optical tweezers. The mechanical behaviors will be analyzed. Prediction or estimate on the mechanical properties of RBC membrane can be given after comparison of the numerical results and the experimental results.
- Finally, a finite element analysis based on a 3D hyperelastic solid model will be conducted for the coupling problem of the fluid-cell/biomimetic vesicle interaction in fluid flow. With analyzing the mechanical behaviors of cell deformation in the simple laminar fluid flow (Stoke's flow), the material property,

Young's modulus of Cell Membrane, can also be estimated through a totally different way.

In a word, the effort of this thesis will be contributed to mechanical modeling, experimental study and prediction of the mechanical properties on erythrocyte membrane. The organization of this thesis is briefly outlined below.

1.7 Layout of this Thesis

This report is organized into six chapters. In Chapter 1, a summary about the background of cell mechanics was presented; the rationales and premise underlying Cellular Mechanics are also introduced; previous experimental and theoretical work on erythrocyte mechanics in literature was briefly reviewed; main results from previous work were summarized. The scope and objective of this research were outlined.

In Chapter 2, to better describe the mechanical behaviors of RBCs under radiation pressure, a non-linear constitutive law derived from the strain-energy function of RBC membrane will be used to describe the mechanical behaviors of a RBC trapped in an optical stretcher. Based on Guck's experimental data (*Guck et al, 2000 and 2001*), the material properties of RBCs membrane will be predicted by minimizing the error between experimental data and simulated data with an optimization method.

In chapter 3, an experimental study of RBC stretching deformation is conducted and introduced. The experimental setup and process will be designed by mainly following the method in the literature (*Dao, et al, 2003*). A spherically swollen RBC from white rat will be deformed by stretching the two attached beads on the surface of the RBC. The displacement-force response will be recorded and measured. The

collected data will be used for mechanical modeling analyses. To examine the applicability of the constitutive law proposed by Pamplona (*Pamplona et al, 1993*) and to interpret the experimental data, a simple mechanical model (no bending resistance) will be established.

In chapter 4, since the Pamplona's constitutive relation between the principal tensions and the principal stretch ratios is essentially linear, a mechanical model based on a non-linear constitutive law derived from strain-energy function will be established to simulate the deformation of RBC and to interpret the experimental data. Prediction or estimate on the material properties of RBC membrane will be given. Comparison of the results from two mechanical models based on different constitutive laws will be also conducted.

In chapter 5, a finite element modeling (FEM) will be conducted for a structure-fluid coupling problem of the interaction between a biomimetic vesicle and fluid flow in this study. The purpose of the FEM on this problem is to investigate the effects of the flow-induced stresses on the deformation of a vesicle, which is manipulated and held by the optical tweezers in fluid flow. Since the obvious advantage of this non-intrusive manipulation by laser beam, the deformation of the vesicle will be completely caused by flow-induced stresses when it is immersed in the Stoke's flow. The material properties of vesicle membrane can be estimated by a parametric study on the relationships between the membrane stiffness and the quantitative deformation of the vesicle under the certain flow velocities. The simulation results will be compared with those in the work of Hochmuth (*Hochmuth, et al, 1972*)

In chapter 6, a summary of this study and the contributions will be presented and a

recommendation is suggested.

Table 1.1 the measured values of RBC membrane elasticity by different techniques

Techniques	References	Measured elastic modulus of membrane	Models of the elasticity	Comments
Micropipette aspiration	(Waugh, et al, 1995) (Hochmuth, 1987)	Shear Stiffness: $\mu \approx (6.0 \sim 9.0) \times 10^{-6}$ N/m Area modulus: $K_A \approx 480 \times 10^{-3}$ N/m Viscosity: $\eta \approx 3.6 \times 10^{-7}$ N·s/m	$\frac{\Delta P \cdot R_p}{\mu} = 2.45 \frac{\Delta L_p}{R_p},$ $\frac{L_p}{R_p} > 1$	device dependence
Optical tweezers	(Henon, et al, 1999)	Shear Stiffness: $\mu \approx (2.5 \pm 0.5) \times 10^{-6}$ N/m	2D planar linear elasticity	Applicable for small deformation
Optical stretcher	(Guck, et al, 2001) (Guck, et al, 2000)	$E \cdot h \approx 3.9 \times 10^{-5}$ N/m Young's modulus: $E \approx 1.3$ kPa for the given membrane thickness $h = 30$ nm	The linear elastic membrane theory	Applicable for small deformation
AC electric field	(Engelhardt, 1988)	Shear Stiffness: $\mu \approx 6.0 \times 10^{-6}$ N/m Viscosity: $\eta \approx 3.4 \times 10^{-7}$ Ns/m	The linear elastic membrane theory	Applicable for small deformation
Atomic force microscopy	(Radmacher, et al, 1996)	Young's modulus: $E \approx 1.0 \sim 50.0$ kPa Shear Stiffness: $\mu \approx (0.33 \sim 16.7) \times 10^{-5}$ N/m for the given membrane thickness $h = 10$ nm	Hooke's law of elasticity, the theory of Hertz and the mechanics of Sneddon	device dependence
uniaxial tension tests	(Sutera, et al, 1970)	Material parameters: $C_1 \approx 2.57 \times 10^5$ N/m ² $C_2 \approx 0.257 \times 10^5$ N/m ² for a given Young's modulus $E \approx 10^6$ N/m ² and membrane thickness $h_0 \approx 10$ nm	the rubber-like hyperealstic RBC membrane model	Standard test inapplicable for RBC directly
Elongation in shear flow channel	(Hochmuth, et al, 1972)	Material parameters: $B \approx 0.005 \times 10^{-3}$ N/m $C \approx 5.0 \times 10^{-3}$ N/m for a given Young's modulus $E \approx 2.5 \times 10^3$ N/m ² and membrane thickness $h \approx 10$ nm	Linear elasticity	Applicable for small deformation

CHAPTER 2 THE DEFORMATION OF AN ERYTHROCYTE UNDER THE RADIATION PRESSURE BY OPTICAL STRETCH^{*}

2.1 INTRODUCTION

Recently, the optical stretcher has emerged as a novel tool for characterizing mechanical behaviors through applying sub-nano-scale force to deform cell membranes. A scheme of a spherical RBC trapped by two horizontal and co-axis laser beams but in opposite propagation directions is illustrated in Fig.2.1. Since the cell is under stretching by a pair of laser beams applied in opposite directions, its surface is loaded by so called “radiation pressure” which is normally generated from the electromagnetic fields. It was *Guck et al.* who first experimentally investigated cell deformation induced by this radiation pressure, and they found the pressure distribution is approximately in the form of Cosine Square (*Guck et al, 2000; Guck et al, 2001*). Some of their experimental results are shown as Figures (a), (b) and (c) in Appendix II. However, so far only few studies have reported on the cell membrane deformation in response to such pressure. The study in this chapter aims to investigate numerically the mechanical behaviors of erythrocyte membrane under such optical-mechanical loading.

The specific problem reported in this chapter is a spherical cell membrane stretched by two opposite laser beams. A mechanical model is developed to study how the cell membrane deforms and transforms under the radiation pressure. The mechanical behavior of a spherically swollen erythrocyte stretched by a pair of laser

^{*} Y. P. Liu, C. Li, K. K. Liu, A. C. K. Lai, The Deformation of an Erythrocytes under the Radiation Pressure by Optical Stretch, *Journal of Biomechanical Engineering*, Vol.128 (6), 2006, 830

beams applied in opposite directions has been studied in great details. In addition, the simulated results will be compared with the experimental data (*Guck et al, 2000; Guck et al, 2001*) through a numerical optimization to facilitate the determination of the membrane mechanical properties. The comparison provides the optimal values of membrane properties through minimizing the errors between experimental and numerical data. Computational results were assessed by an experimental work from literature to determine the optimal values of membrane properties.

2.2 MECHANICAL MODELING

2.2.1 Geometry of Cell Membrane

The geometry of a cell is shown in Figure 2.2(a) where a spherical membrane with radius a is axisymmetric in the z -direction. Several dimensionless cylindrical coordinates: $r^* = r/a$, $z^* = z/a$ and $s^* = s/a$, have been introduced for numerical simulation and they satisfy the following relation

$$r^* = \sin s^* \quad (2.1)$$

$$z^* = 1 - \cos s^* \quad (2.2)$$

where s is the arc length and φ is the meridional angle, as depicted in Figure 2.2(a) & (b). Note that the dimensionless arc length s^* is identical with the meridional angle φ , because of $s = a\varphi$.

Figure 2.2(c) shows the deformed cell where a new set of dimensionless coordinates, $R^* = R/a$, $Z^* = Z/a$ and $S^* = S/a$, are used to describe the geometry. Unlike the geometry of the undeformed cell, the arc length S^* is no longer equal to the meridional angle φ because the radius of the deformed membrane varies from point to

point after deformation. Therefore R^* and Z^* are functions of S^* and φ and they both satisfy the following relations

$$\frac{dR^*}{dS^*} = \cos \varphi \quad (2.3)$$

$$\frac{dZ^*}{dS^*} = \sin \varphi \quad (2.4)$$

Curvature is another key geometric parameter which can be expressed in the two principal directions, i.e., the circumferential (θ) and meridional (s or φ) directions. If the dimensionless curvatures are defined as $\kappa_s^* = a\kappa_s$ and $\kappa_\theta^* = a\kappa_\theta$, then it is obvious $\kappa_s^* = \kappa_\theta^* = 1$ for the undeformed cell. When the cell membrane is deformed, dimensionless curvatures are found to be

$$\kappa_s^* = a\kappa_s = \frac{d\varphi}{dS^*} \quad (2.5)$$

$$\kappa_\theta^* = a\kappa_\theta = \frac{\sin \varphi}{R^*} \quad (2.6)$$

2.2.2 Kinematic Relation for Deformation

Relations between the undeformed (r^* , z^*) and deformed (R^* , Z^*) states of a cell can be determined by defining two principal stretches (strains) along the circumferential (θ) and meridional (s or φ) directions as (Pozrikidis, 2003 and Pamplona, et al, 1993)

$$\lambda_s = \frac{dS^*}{ds^*} \quad (2.7)$$

$$\lambda_\theta = \frac{R^*}{r^*} = \frac{R^*}{\sin s^*} \quad (2.8)$$

Physically, equation (2.7) represents the change of arc length along the meridional direction and equation (2.8) denotes the radius change along the circumferential direction.

2.2.3 Constitutive Law

Pamplona and Calladine (*Pamplona, et al, 1993 and 1996*) considered the phospholipid bi-layer as a shell structure at the continuum level and assume no relative sliding between layers during deformation. Their constitutive model was adapted from the Love-Kirchhoff hypothesis in the classic theory for thin-shells. Based on such assumptions, several important mechanical mechanisms governing the cell membrane deformation, including the resultant tensile forces (force/length, T_s , T_θ), the bending moments (force, M_s , M_θ) and the transverse shear (force/length, Q_s , Q_θ) across the layer thickness for any infinitesimal element of a membrane (cf. Figure 2.2(c)), have been well taken into account in the model. For an axisymmetric liposome, the transverse shear is independent of θ . Moreover, the principal direction coincides with the circumferential (θ) and meridian (s or φ) directions.

In this chapter, the basic concepts and definitions of membrane forces, moments, stretch ratios and geometric variables will comply with the work of Pamplona and Calladine. However, the constitutive law of RBC membrane derived from the work of Evans and Skalak (ES) (*Evans, et al, 1980*) was used to describe the mechanical behaviors of a RBC trapped by the optical stretcher. Because of the large modulus of dilatation relative to elastic shear, the RBC membrane is highly resistant to changes in area but shears readily (*Secomb, et al, 1986*). Therefore, the red-cell membrane, although highly flexible, can be assumed to maintain a constant area under deformation. This assumption imposes a constraint on the principal extension ratios in the meridional

and circumferential directions by requiring that the second invariant of the Green strain tensor vanish. This constraint gives rise to the concept of the isotropic membrane tension component T^* which is the two-dimensional analog to pressure in three-dimensional materials and the deviatoric component γ^* .

The two principal tensions (T_s and T_θ , $\text{N}\cdot\text{m}^{-1}$) can be decomposed into dilatational and deviatoric parts as

$$T_s = T + \gamma \quad (2.9)$$

$$T_\theta = T - \gamma \quad (2.10)$$

where $T = \frac{(T_s + T_\theta)}{2}$ and $\gamma = \frac{(T_s - T_\theta)}{2}$ are the dilatational and deviatoric tensions respectively. The constitutive relation between the deformation and resultant tensions for an isotropic membrane has been studied by Evens and Skalak and cited in their work of Hansen et al (*Hansen, et al, 1996*), in which a proposed strain energy function leads to the following relation

$$\gamma = G\left(\frac{1}{\lambda_\theta^2} - \frac{1}{\lambda_s^2}\right) \quad (2.11)$$

where G is the shear stiffness ($\text{N}\cdot\text{m}^{-1}$) (with factor $\frac{1}{2}$ being absorbed); λ_s and λ_θ are the principal stretch ratios along the meridional and circumferential directions, respectively. For incompressible membrane, i.e., cell surface area remains constant during deformation; the two principle stretches satisfy the following relation

$$\lambda_\theta \lambda_s = 1 \quad (2.12)$$

The bending moments are assumed to be proportional to the curvature change of membrane through the following expressions (*Pamplona, et al, 1993 and 1996*)

$$M_s = M_\theta = B[(\kappa_s + \kappa_\theta) - (\kappa_s + \kappa_\theta)_0] \quad (2.13)$$

where M_s and M_θ are principal bending moments (N); B is the bending modulus of membrane (N·m); κ_s and κ_θ are the principal curvatures (m^{-1}); the subscript 0 denotes the undeformed geometry.

2.2.4 Equilibrium and Governing Equations

Equilibrium equations for the cell membrane can be found from the classical theory for axisymmetric shells (*Flügge, 1973; Ugural, 1998*) as follows:

$$\frac{1}{R} \frac{d(RQ_s)}{dS} + \kappa_\theta T_\theta + \kappa_s T_s = P_r \quad (2.14)$$

$$\frac{1}{R} \frac{d(RT_s)}{dS} - \kappa_\theta T_\theta \cot \varphi - \kappa_s Q_s = -P_t \quad (2.15)$$

$$\frac{d(RM_s)}{dS} - M_\theta \cos \varphi + RQ_s = 0 \quad (2.16)$$

where P_r and P_t are external pressure/stress loads ($\text{N}\cdot\text{m}^{-2}$) on the membrane surface in the radial and meridional directions, respectively. For cells in an aquatic environment, P_r is the osmotic pressure across the cell membrane and P_t is the shear stress normally generated by fluid flow. Equations (2.14) – (2.16) represent the equilibrium of forces and moments in the radial and meridional directions, as reported by (*Pamplona, et al, 1993*). Contrary to the classic shell theory (*Timoshenko, et al, 1959*) where governing equations are derived based on φ , the current differentiations are performed along with the arc length S in order to avoid the singularity at $\varphi = 0$.

Equations (2.3) - (2.5) and (2.14) – (2.16) are the governing equations for our simulations. To simplify the numerical study, several dimensionless parameters, $T_s^* = T_s a^2 / B$, $T_\theta^* = T_\theta a^2 / B$, $P_r^* = P_r a^3 / B$, $P_t^* = P_t a^2 / B$, $Q_s^* = Q_s a^2 / B$,

Chapter 2

$M_s^* = M_s a / B$ and $M_\theta^* = M_\theta a / B$, are introduced and therefore the constitutive relations (2.9) and (2.10) can be recast as

$$T_s^* = T^* + C\left(\frac{1}{\lambda^2} - \lambda^2\right) \quad (2.17)$$

$$T_\theta^* = T^* - C\left(\frac{1}{\lambda^2} - \lambda^2\right) \quad (2.18)$$

where $T^* = T a^2 / B$, $C = G a^2 / B$ and

$$\lambda = \lambda_\theta = \lambda_s^{-1} = R^* / \sin s^* = ds^* / dS^* \quad (2.19)$$

Equation (2.19) is derived based on equations (2.7) and (2.8) in combination with the membrane incompressibility, as stated in equation (2.12). The new parameter C represents the ratio of in-plane shear to out-of-plane bending resistance in the cell membrane deformation. It is also a proportional constant for the membrane area change to the deviatoric tension component $\gamma = \frac{(T_s - T_\theta)}{2}$. With all the aforementioned dimensionless variables and equations (2.17)-(2.19), the governing equations can be non-dimensionalized as

$$\frac{dR^*}{ds^*} = \frac{\cos \varphi \sin s^*}{R^*} \quad (2.20)$$

$$\frac{dZ^*}{ds^*} = \frac{\sin \varphi \sin s^*}{R^*} \quad (2.21)$$

$$\frac{d\varphi}{ds^*} = \frac{\kappa_s^* \sin s^*}{R^*} \quad (2.22)$$

$$\frac{d\kappa_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[-Q_s^* - \frac{\kappa_s^* \sin \varphi}{R^*} + \frac{\sin \varphi \cos \varphi}{R^{*2}} \right] \quad (2.23)$$

$$\frac{dQ_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[P_r^* - \left(T^* + C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \right) \kappa_s^* - \left(T^* - C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \right) \frac{\sin \varphi}{R^*} - \frac{Q_s^* \cos \varphi}{R^*} \right] \quad (2.24)$$

$$\frac{dT^*}{ds^*} = \frac{\sin s^*}{R^*} \left[\kappa_s^* Q_s^* - P_t^* - 2C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \frac{\cos \varphi}{R^*} \right] + C \cdot \left(\frac{2}{\lambda^3} + 2\lambda \right) \cdot \left(\frac{\cos \varphi}{R^*} - \frac{R^* \cos s^*}{\sin^2 s^*} \right) \quad (2.25)$$

where the last term in the right hand side of equation (2.25) is obtained by differentiating the function $\left(\frac{1}{\lambda^2} - \lambda^2 \right)$ with respect to s^* . Note that the derivatives are changed from the current configuration $\left(\frac{d}{dS^*} \right)$ back to the original $\left(\frac{d}{ds^*} \right)$ by the chain rule described in equation (2.19). Equations (2.20)-(2.25) form a set of non-linear, first order ordinary differential equations (ODEs) and can be solved only numerically. In these equations, the unknowns are R^* , Z^* , φ , κ_s^* , Q_s^* and T^* . In order to obtain the solution, proper boundary conditions (BCs) have to be imposed and they are discussed in the next section.

2.2.5 Boundary Conditions

Geometry of an axisymmetric cell membrane in Figure 2.2 directly leads to the following boundary conditions at the pole $s^* = 0$,

$$R^*(0) = 0 \quad (2.26)$$

$$Z^*(0) = 0 \quad (2.27)$$

$$\varphi(0) = 0 \quad (2.28)$$

However, boundary conditions for forces at the pole ($s^* = 0$ or $\varphi = 0$) are rather obscure. We notice that the shear force Q_s^* has to vanish at the pole to maintain the

axisymmetry. Following the same reasoning, the axisymmetry can be further extended to other functions: variables such as Z^* , λ , κ_s^* , κ_θ^* , T_s^* , T_θ^* and T^* are even functions of s^* whereas R^* , φ and Q_s^* are odd functions (Pamplona, et al, 1993). One implication of a continuous, odd function is that its value vanishes at zero. Therefore, it can be found that

$$Q_s^*(0) = 0 \quad (2.29)$$

The tension at the pole can be found from the original equation (2.14). Based on the dimensionless terms, it can be deduced by recognizing that $Q_s^*(0) = 0$ and both κ_s^* and κ_θ^* approach the same value $\kappa_s^*(0)$ as $s^* \rightarrow 0$. Substituting T_s^* and T_θ^* from (2.17) and (2.18) into (2.14), the deviatoric component of tensions is cancelled out, leading to the following expression

$$T^*(0) = \frac{1}{\kappa_s^*(0)} \left[\frac{1}{2} P_r^*(0) - \frac{dQ_s^*(0)}{ds^*} \right] \quad (2.30)$$

where $P_r^*(0)$ is the pressure loading at $s^* = 0$. Note that neither $\kappa_s^*(0)$ nor $\frac{dQ_s^*(0)}{ds^*}$ is known at the beginning of numerical simulation and the former is related to the curvature

$$\frac{d\varphi(0)}{ds^*} = \kappa_s^*(0) \quad (2.31)$$

For the stretching by a pair of laser beams in the opposite direction, one further simplification can be introduced due to the two-fold symmetry, i.e., the axisymmetry plus symmetry at $\varphi = \frac{\pi}{2}$. This simplification results in another two boundary conditions as

$$\varphi\left(\frac{\pi}{2}\right) = \frac{\pi}{2} \quad (2.32)$$

$$Q_s^*(\pi/2) = 0 \quad (2.33)$$

In other words, only a quarter of the cell membrane is needed for the numerical analysis.

2.2.6 Radiation Pressure from the Optical Field

The laser trapping is created by the force gradient, known as the “radiation pressure” generated by the electromagnetic fields. From a microscopic point of view, the force is generated from the momentum change of photons which first hit the cell membrane then reflect back to the medium or transmit into the cell. The optical-mechanical interaction between the light and cell membrane can be modeled by the theory of ray optics (*Hecht, 2001*). However, the theoretical approach can only be applicable to the object which is in size much larger than the wavelength and also takes no account of the diffraction (*Hulst, 1957*). Since the size of typical Erythrocytes is about 10 times, or more, than the laser wavelength, this approach is suitable for the study. Detailed calculations for the stress distribution by the theory of ray optics can be found in the literature (*Bakker Schut, et al, 1991; Nemoto, et al, 1998*) under the assumption of a Gaussian laser beam.

The empirical relationships given by Guck et al (*Guck et al, 2000; Guck et al, 2001*), through curve fitting with their experimental measurements, show that the tangential stress $P_t = 0$ and the normal pressure is in the form of

$$P_r = \sigma_0 \cos^2 \varphi \quad (2.34)$$

where $-\frac{\pi}{2} \leq \varphi \leq \frac{\pi}{2}$ and σ_0 is the peak value of radiation pressure at $\varphi = 0$. This peak

value can be non-dimensionalized as $\sigma_0^* = \sigma_0 a^3 / B$ by the dimensionless

parameter $P_r^* = P_r a^3 / B$. It is noticed that the pressure loading in equation (2.34) shall change if cell deforms. Because the biconcave at $\pi/2$ (middle of oblate cell) was not observed in Guck's experiments and the front ends of the deformed RBC with oblate shape are approximately semi-spherical surface, the normal pressure distributions on the front ends are still approximate to Eq.2.34. However, numerical results as discussed in subsequent sections show the biconcave deformation at $\pi/2$ may take place when σ_0 for each C value is beyond a threshold. Under this situation, the optical pressure distributions in the front ends of the trapped RBC will greatly deviate from the form of Eq.2.34. Then, numerically a coupling problem between optical force field and cell deformation has to be solved iteratively and the convergence cannot be guaranteed. Therefore, the coupling problem is ignored in current study.

2.3 NUMERICAL METHOD

The governing equations (2.20) – (2.25) with the BCs (2.26) – (2.33) are able to well define a non-linear, two-point boundary value problem, which can be solved by numerical integration methods such as standard Runge-Kutta method (*Sandford, et al, 1972*). Based on the method, the values of the six variables R^* , Z^* , φ , κ_s^* , Q_s^* and T^* can be obtained by integrating the ODEs (2.20)-(2.25) from $\varphi = 0$ to $\frac{\pi}{2}$. However, the initial values of $P_r^*(0)$, $\kappa_s^*(0)$ and $\frac{dQ_s^*(0)}{ds^*}$ have to be guessed or given for the integrations. The accurate solutions can be obtained through iterative adjustment of these initial values by Newton-Raphson method to meet the BCs, described by the equations (2.32) – (2.33). To expedite the simulations, the following starting conditions at the pole are normally recommended

$$\frac{dR^*(0)}{ds^*} = 1 \quad (2.35)$$

$$\frac{dZ^*(0)}{ds^*} = 0 \quad (2.36)$$

$$\frac{d\varphi(0)}{ds^*} = \kappa_s^*(0) \quad (2.37)$$

$$\frac{d\kappa_s^*(0)}{ds^*} = 0 \quad (2.38)$$

$$\frac{dQ_s^*(0)}{ds^*} = Q'_0 \quad (2.39)$$

$$\frac{dT^*(0)}{ds^*} = 0 \quad (2.40)$$

These conditions are derivable. It is noticed that ratio $R^* / \sin s^* \rightarrow 1$ as $s^* \rightarrow 0$. Then from equation (2.19), it is found that $\lambda = \lambda_\theta = \lambda_s^{-1} = ds^* / dS^* \rightarrow 1$ as s^* approaches to

0. This leads to $\frac{dR^*(0)}{ds^*} = 1$, $\frac{dZ^*(0)}{ds^*} = 0$, $\frac{d\varphi(0)}{ds^*} = \kappa_s^*(0)$, $\frac{d\kappa_s^*(0)}{ds^*} = 0$ and

$\frac{dT^*(0)}{ds^*} = 0$, respectively, based on the equations (2.20)-(2.23) and (2.25). As

mentioned before, three unknown parameters, the curvature $\kappa_s^*(0)$, pressure $P_r^*(0)$ and

gradient of shear tension $\frac{dQ_s^*(0)}{ds^*}$, all need to be specified along with the material

property C before the numerical integration.

Detailed calculation procedures are illustrated concisely by a flowchart in Figure

2.3. Noted that among the three unknowns, only the curvature $\kappa_s^*(0)$ is specified, but

the initial values of $P_r^*(0)$ and $\frac{dQ_s^*(0)}{ds^*}$ are guessed. The reason for such specifications

will be given later in the Section 2.4.1. The iterative adjustment of initial guess is done by linear search based upon the Newton-Raphson method to solve the boundary

conditions: $\varphi\left(\frac{\pi}{2}; P_r^*(0), \frac{dQ_s^*(0)}{ds^*}\right) = \frac{\pi}{2}$ and $Q_s^*\left(\frac{\pi}{2}; P_r^*(0), \frac{dQ_s^*(0)}{ds^*}\right) = 0$, if they are

treated as two simultaneous equations with variables $P_r^*(0)$ and $\frac{dQ_s^*(0)}{ds^*}$.

2.4 RESULTS AND DISCUSSION

2.4.1 Profile of Deformed Cell

Firstly investigation is conducted on the deformation of an initially spherical cell with various values of property C under different pressure loading $P_r^* = \sigma_0^* \cos^2 \varphi$.

The dimensionless parameter C is the ratio of the product of in-plane shear multiplying radius square to the out-of-plane bending stiffness. Higher values of C represent less deformability of membrane. Figure 2.4 clearly demonstrates this phenomenon: as the

increase of C , a larger stretching stress (σ_0^*) has to apply to achieve the same degree of deformation that is occurred in a more deformable membrane. All these solutions were obtained following the previously proposed numerical scheme by gradually increasing

curvature $\kappa_s^*(0)$ from 1.0 to determine the corresponding $P_r^*(0)$ and $\frac{dQ_s^*(0)}{ds^*}$. After

computations, all deformation profiles were shifted to center at $\varphi = \pi/2$ for a plausible presentation.

One important issue found in the Figure 2.4 is that for different stiffness, almost identical deformation can be obtained from the same curvature at $\varphi = 0$ (cf. Figure 2.5, the comparison of $C = 1.0$ and 5.0). This implies that a minor difference in $\kappa_s^*(0)$ can

lead to a significant change in $P_r^*(0)$. Therefore to avoid numerical instability and divergence, we choose to specify $\kappa_s^*(0)$ and try our initial guess on $P_r^*(0)$ when devising the algorithm.

2.4.2 Critical Pressure, Transverse and Longitudinal Strains

To further validate the simulated results, two parameters, the transverse and longitudinal strains (Parker, et al, 1999; Guck et al, 2000; Guck et al, 2001), have been introduced as

$$\varepsilon_l = Z^*(0) - 1 \quad (2.41)$$

$$\varepsilon_t = R^*(\pi/2) - 1 \quad (2.42)$$

These two represent quantitatively the radius change of a cell at the pole ($\varphi = 0$) and equator ($\varphi = \pi/2$). Since the stretching is applied mainly along the polar direction, a negative value of ε_t and a positive value of ε_l should be anticipated after deformation.

Figure 2.6 shows both the parameters as a function of the peak stretching stress σ_0^* for different values of the mechanical property C . In the figure, there exists a critical value of σ_0^* for cells to maintain their deformation as an *ellipse*. Beyond the critical value (the shadowed area), a *bi-concave* shape emerges when the curvature at the equator ($\kappa_s^*(\pi/2)$) becomes negative and the current analyses are no longer valid. Physically, this is due to the blocking of laser beams whereby the two humps prevent them from reaching $\varphi = \pm \pi/2$. Thus a finite angle instead of $\frac{\pi}{2}$ should be imposed on φ for the range of pressure loading. A complicate numerical algorithm is needed to resolve this situation with iterations to determine the finite angle, which is never known before the deformation can be found.

With the current numerical scheme, the critical σ_0^* can be found for $C \leq 20$. This scheme becomes sensitive to the initial guess of $P_r^*(0)$ and $\frac{dQ_s^*(0)}{ds^*}$ for $C > 20$, because the stiffness of the nonlinear ODEs increases. Therefore, results for $C > 20$ were stopped somewhere when enough iterations have been performed by the computer program. This is similar to the numerical algorithm presented in (Parker, et al, 1999) where a similarity factor $C^{1/3}$ leads to the curves of longitudinal strain vs. stretching force $\times C^{1/3}$ for $C \geq 20$, clustering into a small region with values up to a certain limit.

To further investigate the simulated results, a trend line of the critical pressure corresponding to various values of C is provided in Figure 2.6. It shows that critical σ_0^* occurs around $\varepsilon_l \approx 0.5$ and $\varepsilon_t \approx -0.3$ for $C \leq 10$ but the trend line of ε versus σ_0^* becomes slope-down more significantly as the membrane becomes stiffer in shear, particularly for $C > 20$. The limiting case of $C = 0$ is interesting for that the critical σ_0^* is found and shown as the inflection point of both strain curves (cf. the vertical dotted line in Figure 2.6).

Figure 2.7 utilizes the data from Figure 2.6 to show the critical σ_0^* as a function of C . The critical value increases monotonically with the C value. A trend line is also provided for readers' reference.

2.4.3 Optimized Values of Membrane Properties

This section will discuss how the optimal values of membrane properties C can be obtained by comparing the experimental data to numerical results. It is worthwhile to introduce first the experimental work by Guck et al (Guck et al, 2000; Guck et al, 2001) in which an osmotically swollen erythrocyte (red blood cell) was trapped by a

pair of non-focused laser beams from two optical fibers placed in opposite directions. The wavelength of the laser is 785 nm with maximum power up to 800 mw. The radius of the undeformed cell is $3.13 \pm 0.05 \mu\text{m}$, and the distance between the cell and either the fiber tip is $60 \mu\text{m}$. The peak value of the loading pressure by laser beams is from 0.19 to 1.47 Nm^{-2} , calculated based on the theory of ray optics. The transverse and longitudinal strains can be measured from the images of the deformed cell, shown as Figure (c) in the Appendix II.

In order to give a comprehensive presentation, we introduce the Taylor deformation parameter D_{12} (Chang, *et al*, 1993; De Hass, *et al*, 1997) as a function of ε_l and ε_t in combination:

$$D_{12} = \frac{Z^*(0) - R^*(\pi/2)}{Z^*(0) + R^*(\pi/2)} \quad (2.43)$$

The experimental results from (Guck *et al*, 2000; Guck *et al*, 2001) then were re-plotted in terms of D_{12} against the peak stretching stress σ_0 (Nm^{-2}) as shown in Figure 2.8. It is notable that the data primarily consists of three segments. The slope of the second region is steeper than the other two and this may be originated from the material non-linearity of the cell membrane.

We also reinforce Figure 2.6 through fitting the data of D_{12} with the various values of C , whereby the results provide an explicit comparison between theoretical predictions and experimental measurements, shown as Figure 2.8. Such fitting can be

simply done by scaling the dimensional pressure as $\frac{\sigma_0 a^3}{B} (= \sigma_0^*)$, if a bending stiffness

B can be possibly determined. However, when both C and B are unknown, the fitting is by no means trivial and needs to be optimized. Such optimization can be achieved by defining an objective function as

$$\min \sqrt{\sum_{\text{experimental data point}} \left(\frac{\sigma_0^{\text{exp}} a^3}{B} - \sigma_0^{\text{num}} \right)^2} \quad (2.44)$$

where the superscripts “num” and “exp” stand for the numerical and experimental results respectively. Since σ_0^{num} is a function of C , equation (2.44) is the error in the form of 2-norm which represent a two dimensional optimization in the space of C and B . Figure 2.9 shows the contour of errors which are calculated based on equation (2.44) with some local minimum values labeled. Overall, the errors (dark zone) are greatly reduced at lower value of C and higher value of B . In other words, membranes with lower shear stiffness but higher bending stiffness resemble the experimental results best. However, further calculations on the values of shear stiffness G show that a relatively constant value of G can be obtained in the range from $C = 10$ to 300 (cf. the top figure and the triangular region). The average value of shear stiffness between $C = 10$ and 300 is about $4.611 \times 10^{-6} \text{ Nm}^{-1}$ which is close to those found in earlier works by Lenormand et al (Lenormand, et al, 2001) as $3.4 \pm 1.5 \sim 4.7 \pm 1.3 \times 10^{-6} \text{ Nm}^{-1}$ (in isotonic buffer); Waugh et al (Waugh, et al, 1979) as $6.6 \times 10^{-6} \text{ Nm}^{-1}$; or in a broader range by Lee et al (Lee, et al, 2001) as $1.0 \sim 10.0 \times 10^{-6} \text{ Nm}^{-1}$; Hochmuth (Hochmuth, et al, 1987) and Lelievre (Lelievre, et al, 1995) as $4.0 \sim 10.0 \times 10^{-6} \text{ Nm}^{-1}$. Therefore, we may conclude that between $C = 10$ and 300 exists an optimal property for the Guck’s experimental results. This further implies that in reality an erythrocyte membrane may have its shear stiffness much higher than bending. More importantly, quantitative determination of the cell membrane properties can achieved by the comparison of numerical to experimental data through numerical optimization, i.e., the minimization of the function (2.44) subjected to the constraint, i.e., a constant value of G .

On the other hand, the bending stiffness of RBC membrane can be estimated according to the expression $B = Ga^2/C$ in the range of $C = 10 \sim 300$ with the optimized

and averaged value of membrane shear stiffness, that is, $4.517 \times 10^{-18} \sim 1.506 \times 10^{-19} \text{ N}\cdot\text{m}$. This estimation of bending stiffness of RBC membrane is very close to the value (1.8×10^{-19}) listed in the literature (*Len Fisher, 1993*).

2.5 CONCLUSIONS

In this chapter, the mechanical behaviors of a spherically swollen erythrocyte stretched by a pair of laser beams in opposite directions were carefully studied. The work first addresses the optical-mechanical effect (*Guck et al, 2000; Guck et al, 2001*) on the cell membrane deformation. The critical peak pressure which triggers the transform of membrane deformation from an ellipse to biconcave shape was studied. The results indicate that the critical pressure can be found at a constant magnitude of transverse and longitudinal strains for membranes with low shear stiffness. However, the level decreases significantly if the shear stiffness of the membrane is increased. The simulated results also demonstrate that the critical pressure increases monotonically with the shear stiffness or C values.

In order to validate the numerical results, the experimental data reported by Guck were used for a comparison. Such comparison was achieved by minimizing the errors between the simulated values and measured data of the Taylor deformation parameter for deformed cell membrane. Through such optimization process, minimum error can be simply found for membranes with lower C , i.e., bending effects dominate the deformation. However, further calculations show that a constant value of shear stiffness can be obtained for the range from $C = 10$ to 300. This range corresponds to average membrane shear stiffness around $4.611 \times 10^{-6} \text{ Nm}^{-1}$, which is comparable with those values reported by various research groups in literature. It also implies that in reality

Chapter 2

erythrocyte membrane may have its shear stiffness much higher than bending stiffness;
and the estimated value of bending stiffness is $4.517 \times 10^{-18} \sim 1.506 \times 10^{-19} \text{ N}\cdot\text{m}$.

Figure 2.1 Schematic representation of a spherical cell trapped by opposite, double laser beams.

Figure 2.2 The geometry and coordinate system of an axisymmetric cell membrane. (a) the undeformed spherical cell with diameter a ; (b) and (c) the undeformed and deformed membrane in dimensionless coordinates (r^*, z^*) and (R^*, Z^*) respectively.

Figure 2.3 The flowchart of numerical procedures

Figure 2.4 The profile of a spherical cell stretched by a pair of laser beams along the Z^* -axis.

Figure 2.5 Comparison of the deformation for a spherical cell with $C = 1.0$ and 5.0 .

Figure 2.6 The transverse and longitudinal strains as function of the peak stretching stress for different membrane stiffness.

Figure 2.7 The critical pressure loading for different membrane stiffness.

Figure 2.8 The Taylor deformation parameter as a function of the peak stretching stress adopted from the experimental results in references (*Guck et al, 2000*; *Guck et al, 2001*); Labels 1, 2 and 3 represent three segments with different slopes.

Figure 2.9 The contour of errors as a function of C and bending stiffness B . Corresponding shear stiffness G is shown in the top figure.

CHAPTER 3 AN EXPERIMENTAL STUDY ON THE DEFORMATION OF ERYTHROCYTES UNDER OPTICALLY TRAPPING AND STRETCHING*

3.1 INTRODUCTION

In this chapter, an experiment is designed to quantitatively investigate the mechanical behaviors of RBCs by using a precise force measurement instrument, optical tweezers, which can trap and deform erythrocytes. The mechanical behavior of erythrocytes is studied experimentally.

An advantage of laser trapping is that it is a non-destructive method of measuring overall material behavior under external loadings because the force field can be directly applied on the cell to manipulate it in a fluid environment. To achieve a high accuracy, the laser should be highly focused to attain a high force field. The smallest focus that can be made is determined by the wavelength of laser. Typical wavelengths for trapping purpose are 1064, 980 and 830 nm. Shorter wavelength could penetrate the membranes of cell and nucleus to damage the DNA and kill the cell.

For a selected wavelength, the cell can be targeted more easily and accurately by the laser beam if small microbeads are attached to its surface as a marker (*Hénon, et al, 1999; Sleep, et al, 1999; Svoboda, et al, 1994*). This approach reduces the direct incidence of the laser beam on the cell and thus avoid cell being deformed by the radiation pressure. Since microbeads are rigid, they can be used as handles to stretch/drag the cells in a fluid.

* Y. P. Liu, C. Li, A. C. K. Lai, 2006, An Experimental Study on the Deformation of Erythrocytes under Optically Trapping and Stretching, Materials Science and Engineering A, Vol.423, 128.

3.2 EXPERIMENT

3.2.1 Cell and Microbead Preparation

Fresh blood was drawn from white rats and stored in acid citrate dextrose (Sigma C3821) at 4 °C in refrigerator. An aliquot of blood sample was first diluted in phosphate-buffered saline (PBS, Sigma P4417) then rinsed and fractionated by centrifugation three times. Similarly, silica microbeads purchased from Bangs Laboratories (Fishers, IN, US) were rinsed in deionized water by centrifugation for several times. The purpose of washing RBCs and microbeads repeatedly is to guarantee the spontaneous adhesion between RBCs and microbeads in later processes. Some important physical properties of silica microbeads are listed in Table 3.1 for readers' reference.

The washed RBCs and microbeads were diluted again by PBS to the concentration of $\sim 1 \times 10^5/\mu\text{l}$ and $\sim 2 \times 10^5/\mu\text{l}$ respectively. After diluting, 20 μl of each suspension was mixed together in a little vial. By using equal amounts of solutions, it is most likely that there will be two beads adhered to one erythrocyte. The mixed suspension was incubated at 4°C for 1 hour, allowing spontaneous and nonspecific adhesion of microbeads to RBC membrane (*Hénon, et al, 1999; Sleep, et al, 1999; Svoboda, et al, 1994*). After confirming the adhesion between RBCs and beads by observing a small portion under an optical microscope, the suspension of mixture was further diluted to $\sim 1 \times 10^3$ cells/ μl in a hypotonic buffer (10mM potassium phosphate, pH7.4, 75mM NaCl, i.e., 155mOsm/kg), with a small amount ($\sim 1\text{mg/ml}$) of Bovine serum albumin (BSA, Sigma A4503) added to prevent RBCs from sticking to the glass plate (*Hénon, et al, 1999*). This suspension should be kept for about 10~20 minutes before the experiment of laser trapping can be started, for allowing RBCs to swell into spherical or nearly spherical shape.

More details about the procedures of the preparation of erythrocytes, microbeads and their adhesion can be found in the Appendix III.

3.2.2 Optical tweezers

A schematic drawing of the optical tweezers is shown in Fig.3.1a. The whole system was purchased from Cell Robotics Inc., NM, US. Source of laser is Nd: YAG and pumped by a 1.5W diode. Laser is then reflected through dichroic mirrors and focused by an inverted microscope (Nikon optical microsystem) before it reaches the objects. The wavelength of 1064nm is chosen to minimize the absorption by water and hemoglobin to avoid the possible damage of a trapped RBC due to heating (*Liu, et al, 1995*). Since the laser beam was never directly focused on the cell but on the attached bead in the current study, the actual heating is only marginal.

The chamber for observation is assembled of glass slides and coverslips. Prior to the experiment, all slides and coverslips were treated with BSA (100mg/ml). In addition, the bottom glass slides were silanized with Dichlorodimethylsilane solution ($C_2H_6Cl_2Si$, Sigma 85126) (*Svoboda, et al, 1992*). Dichlorodimethylsilane solution is commonly used in protein analysis for silanizing the glass to prevent sticking of proteins (*Svoboda, et al, 1992*). All observations and measurements were made at room temperature in the laboratory ($\sim 25^\circ C$). The expected deformed shape of RBC stretched by optical tweezers is shown as Fig.3.1b.

3.2.3 Force Calibrations

One main purpose of the experimental study is to find the relation between external loading and the deformation of cells. However, the force can be calibrated with input power of laser but force not measured directly. Using silica beads only, the laser beam can trap a bead and let the fluid flow over it, similar to the wind tunnel test. At a

certain power level, there is a maximum fluid velocity under which the trapping force can withhold the bead. This is an equilibrium state where the trapping force is balanced by the viscous drag force (*Lenormand, et al, 2001*). Therefore, following the Stokes' flow, the trapping force is

$$F = 6\pi R\mu V \quad (3.1)$$

where R is the radius of microbeads, μ the liquid viscosity (1.01×10^{-3} Pa·s at 25 °C for water) and V the maximum velocity directly measured by the system from its motor speed.

Fig.3.2 shows the calibration of trapping force vs. input power of laser where a linear fitting is also given. The linear relation was explained earlier by *Lenormand et al (Lenormand, et al, 2001)*. The relative accuracy in the calibration is estimated to be ~10% mainly due to the uncertainty in size distribution of microbeads shown in Table 3.1.

3.2.4 Image Processing

In order to measure the deformation of erythrocytes, digital image processing for each picture taken by CCD camera is done by the toolbox of MATLAB. Since the average size of microbeads is known and all pictures are in the format of bitmap, the task is to find the edges of erythrocytes and microbeads in each picture. Fig.3.3a indicates an example result of the edge detection by MATLAB. The schematic diagram of image edge detection algorithm is shown in Fig.3.3b. Based upon the size of microbeads ($2.34 \mu\text{m}$ in dia.), a conversion between the pixels and the real physical length can be established in a statistical way by measuring pixels between any two opposite points on the perimeter of a bead. This mapping is fairly accurate as long as

the measures are made at many different locations. For example, the conversion is found to be $0.023237 \mu\text{m/pixel}$ for the cell No.1 in Table 3.2.

3.2.5 Experimental Results

Experimental results of five example cells are shown in Fig.3.4 along with selected images. Corresponding values of all measurements are listed in Table 3.2. The geometry of the cells is measured by image processing as mentioned previously. The stretching force is calculated by the correlation of the trapping force and input power from Fig.3.2. To obtain more accurate measurements, experiments were specifically carried out each time at different force level within a small range instead of running through the whole breadth from 2 to 15 pN. Note that the radius of attachment δ by beads is also measured and tabulated. Strictly speaking, it was estimated by reading the pixels of “contact edge” many times and taking the averaged value. The standard deviation of contact edge’s diameter is ranged in 10%~40% of its averaged value in each picture. This small dimension has to be included later in the mechanical analysis. To further quantify the experimental results, a transverse (ε_t) and longitudinal strains (ε_l) are introduced as following

$$\varepsilon_t = \frac{\Delta D}{D_0} = \frac{D_0 - D}{D_0} \quad (3.2)$$

$$\varepsilon_l = \frac{\Delta L}{D_0} = \frac{L - D_0}{D_0} \quad (3.3)$$

where D_0 is the original diameter ($= 2R$) of RBCs before deformation; D is the maximum length perpendicular to the stretching direction and L the maximum length parallel to the stretching. To avoid confusion, Eq.(3.2) and (3.3) are specifically defined to make both strains positive. It is found that the transverse strain (ε_t) in this study was

easier to accurately measure than the longitudinal one because the boundaries between cell and beads cannot be precisely determined by the edge detection as shown in Fig.3.3a. Fig.3.4 demonstrates a correlation between the stretching force and the positive transverse strain for five selected cells. In other words, the more stretching, the more oblate cell could be.

3.3 A SIMPLE MECHANICAL MODEL ANALYSIS

3.3.1 Assumption and Simplification for Mechanical Analysis

Generally, RBC membrane is described in the simplest way as a *two-dimensional* network of spectrin strands bound to the phospholipidic bilayer. Since the inner fluid of the red blood cells is purely viscous and has no elasticity, the plasma membrane is entirely responsible for the elastic response.

Some Experimental results, such as micropipette aspiration, did show that the membrane of RBCs exhibits some viscoelastic effects (*Hochmuth, 2000; Chang, 1993; Sato, 2000*). But, the assumption of *non-viscoelasticity* can be made upon certain conditions. It will be assumed that if a given deformation is applied to a RBC membrane sufficiently rapidly, that a definite stress will occur which is independent of the strain rate in some range of loading time. Behaviors such that membrane experiences a finite elastic deformation that is completely recoverable on unloading rapidly is usually called the elastic response. This assumption implies, in terms of a crude model, that a series elastic element exists. Fung (*Fung, 1972*) has shown that this is a reasonable assumption for some biological tissues and Rand (*Rand, 1964*) suggests it is also required to interpret his experiments on red blood cells.

On the other hand, the phospholipidic bilayer is a kind of fluid sheet that is incompressible; it is often assumed that the area compressibility modulus K (bulk modulus) of cell membrane is controlled by the phospholipidic bilayer. On the contrary,

shear modulus μ is determined by the elastic properties of the cytoskeleton network. The assumption of *incompressibility* is based upon such experimental results. Experiments of micropipette aspiration showed that K is on the order of $(300\sim 500)\times 10^{-3}$ N/m (Evans, et al, 1976; Waugh, et al, 1979; Hochmuth, et al, 1987) which is far larger than the shear modulus ($K \gg \mu$). As a consequence, an approximation that RBC membrane keeps constant area is made. In addition, because *constant volume* of a whole cell is a very strong constraint, it can be expected that its dominant effects on cell's equilibrium configuration may overwhelm the effects of the material property parameters, shear modulus and bending modulus. The assumption of constant membrane area is more convenient to be used for exploring the effects of elastic moduli on the mechanical behaviors of cell membrane. (Parker, et al, 1999)

Therefore, the composite of lipid bilayer, transmembrane protein and cytoskeleton network is simplified and assumed to be effective *continuum* material, which is *homogeneous, non-viscoelastic, incompressible and permeable* (Liu, 1996; Hanns, 1997; Evans, 1979; Dao, et al, 2003; Parker, et al, 1999). With the above simplification and assumption for RBC membrane's structure and its elastic characteristics, a simple mechanical analysis can be conducted based on the works from Pamplona and Calladine, who developed a simple and linear constitutive law for RBC membrane to describe the large deformation of RBCs.

3.3.2 Geometry and Kinematic Relation

Considering a spherical cell membrane with radius a is axisymmetrically deformed along the stretching direction (z -direction, cf. the small panel inside Fig.3.5). Introducing two non-dimensional coordinates: $r^* = r/a$, $z^* = z/a$, $s^* = s/a$ and

Chapter 3

$R^* = R/a$, $Z^* = Z/a$, $S^* = S/a$ for the membrane before and after deformation respectively, then the followings have to be valid (Pamplona, et al, 1993).

$$r^* = \sin s^* \quad (3.4)$$

$$z^* = 1 - \cos s^* \quad (3.5)$$

$$\frac{dR^*}{dS^*} = \cos \varphi \quad (3.6)$$

$$\frac{dZ^*}{dS^*} = \sin \varphi \quad (3.7)$$

where φ is the meridional angle, s and S are arc lengths before and after deformation.

Note that s^* is exactly the meridional angle φ because $s = a\varphi$.

Also, we can have two dimensionless, principal curvatures of membrane in the meridional (s or φ) and circumferential (θ) as (Pamplona, et al, 1993)

$$\kappa_s^* = a\kappa_s = \frac{d\varphi}{dS^*} \quad (3.8)$$

$$\kappa_\theta^* = a\kappa_\theta = \frac{\sin \varphi}{R^*} \quad (3.9)$$

Note that $\kappa_s = \kappa_\theta = \frac{1}{a}$ in the undeformed cell. κ_θ^* can be directly calculated once R^*

and φ are determined.

Correspondingly, two principal stretches (strains) along the circumferential (θ) and meridional (s or φ) directions are introduced as (Pamplona, et al, 1993)

$$\lambda_s = \frac{dS^*}{ds^*} \quad (3.10)$$

$$\lambda_\theta = \frac{R^*}{r^*} = \frac{R^*}{\sin s^*} \quad (3.11)$$

Physically, the first term represents the change of arc length along the meridional direction and the second term the radius change along the circumferential one.

3.3.3 Constitutive Relations

Here we adopted the earlier studies by Pamplona and Calladine (*Pamplona, et al, 1993*) on bi-lipid layer to assume there exist tensions (resultant tensile forces, T_s and T_θ in Fig.3.5, $\text{N}\cdot\text{m}^{-1}$) across the membrane thickness in any infinitesimal element. Note that the principal direction is coincident with the circumferential and meridian directions for axisymmetric cases. The principal membrane tensions can be expressed in terms of the approximate mean value (T) of T_s and T_θ and their corresponding principal stretch ratios as proposed by Pamplona and Calladine (*Pamplona, et al, 1993*)

$$T_s = T + H \frac{1}{\lambda} \quad (3.12)$$

$$T_\theta = T + H\lambda \quad (3.13)$$

where H is the modulus of stiffness ($\text{N}\cdot\text{m}^{-1}$) and

$$\lambda = \lambda_\theta = \lambda_s^{-1} = R^* / \sin s^* = ds^* / dS^* \quad (3.14)$$

Note that this relation is valid provided that membrane is incompressible (constant area), i.e. $\lambda_\theta \lambda_s = 1$.

3.3.4 Governing Equations

Equilibrium equations for the cell membrane can be found from the classical theory for shells (*Flügge, 1973; Ugural, 1999*). For axisymmetry, those equations reduce to two for the tension T^* and curvature κ_s^* along the arc length s^* . Combining the geometric requirements Eq. (3.6) – (3.8), we can have the whole set of governing equations in a dimensionless form as

$$\frac{dR^*}{ds^*} = \frac{\cos \varphi \sin s^*}{R^*} \quad (3.15)$$

$$\frac{dZ^*}{ds^*} = \frac{\sin \varphi \sin s^*}{R^*} \quad (3.16)$$

$$\frac{d\varphi}{ds^*} = \frac{\kappa_s^* \sin s^*}{R^*} \quad (3.17)$$

$$\frac{dT^*}{ds^*} = \frac{\cos \varphi - \cos s^*}{R^*} \quad (3.18)$$

with

$$\kappa_s^* = \frac{-(T^* + \lambda) \cdot \sin \varphi / R^*}{T^* + 1/\lambda} \quad (3.19)$$

The last two, (3.18) and (3.19), are equilibrium equations for tension and curvature with replacements of κ_θ^* , λ_s and λ_θ from (3.8) – (3.11). Note that for computational purposes the derivative is changed from the current configuration ($\frac{d}{dS}$) back to the original one ($\frac{d}{ds}$) by the chain rule in (3.14). The dimensionless tensions are defined as

$$T_s^* = T_s / H, \quad T_\theta^* = T_\theta / H \quad \text{and} \quad T^* = T / H.$$

3.3.5 Boundary Conditions and Numerical Calculations

The boundary conditions were imposed by considering the beads attachment at

$$s^* = s_0^* = \sin^{-1} \delta^*,$$

$$R^*(s_0^*) = \delta^* \quad (3.20)$$

$$Z^*(s_0^*) = 0 \quad (3.21)$$

$$\varphi(s_0^*) = \varphi_0 \quad (3.22)$$

$$T^*(s_0^*) = \frac{F^*}{2\pi\delta^* \sin \varphi_0} - \frac{\sin(s_0^*)}{R^*(s_0^*)} \quad (3.23)$$

and two-fold symmetry at $s^* = \frac{\pi}{2}$,

$$\varphi(\pi/2) = \pi/2 \quad (3.24)$$

$$T^*\left(\frac{\pi}{2}\right) = \frac{F^*}{2\pi R^*(\pi/2)} - \frac{1}{R^*(\pi/2)} \quad (3.25)$$

where $F^* = F/aH$ is the dimensionless stretching force and $\delta^* = \delta/a$. Eq.(3.23) and (3.25) were obtained by the overall force balance between F and T_s^* around the cell assuming that F are highly concentrated in the adhesion area.

Eq.(3.15) – (3.18) together with conditions (3.20) – (3.25) form a non-linear, boundary value problem which can be solved by Runge-Kutta method. But to start the procedure, the initial values of φ and κ_s^* at s_0^* have to be guessed. With successive adjustment of both initial values by simplex method to meet the conditions (3.24) – (3.25) at $\frac{\pi}{2}$, accurate solutions can be obtained. To facilitate the whole simulations,

following starting conditions at s_0^* are recommended also.

$$\frac{dR^*}{ds^*} = \cos \varphi_0 \quad (3.26)$$

$$\frac{dZ^*}{ds^*} = \sin \varphi_0 \quad (3.27)$$

$$\frac{d\varphi}{ds^*} = \kappa_s^*(s_0^*) \quad (3.28)$$

$$\frac{dT^*}{ds^*} = \frac{\cos \varphi_0}{\delta^*} - \frac{\sqrt{1 - \delta^{*2}}}{\delta^*} \quad (3.29)$$

3.3.6 Numerical Solutions

Since the adhesion between RBC and microbeads is spontaneous and non-specific, the sizes δ^* of the adhesion areas are different from each case. Although the adhesion areas of the selected cases for analyses are intended to be as close to each other as possible, the effect of δ^* on RBC deformation still needs to be investigated for selecting an appropriate deformation parameter (strain) to probe the mechanical properties of RBC membrane. As shown in Fig.3.6, the variation of δ^* has a distinct effect on the longitudinal strain ε_l , but almost has no effect on the transverse strain ε_t . That means the size of adhesion area between erythrocyte and bead has a prominent effect on the longitudinal strain.

A plausible explanation is that the smaller the contact areas are, the higher the membrane resultant force per unit circumferential length is near the poles. This higher local resultant membrane force can induce local large deformation near smaller contact regions. However, the plasma membrane of a stretched RBC was subject to a relative small deformation near the equatorial region ($\varphi = \pi/2$). In addition, the deviation of δ must exist since the adhesion between microbeads and individual RBCs is spontaneous and nonspecific. Due to reason that ε_t is not sensitive to the deviation of δ^* in experiments, the transverse strain is most appropriately used to probe the mechanical properties of RBC membrane by comparing the numerical results with experimental data.

For numerical simplification, using the average value $\delta^* = 0.185$ from Table 3.2 and the previously proposed numerical scheme, the profile of deformed cell by different stretching force can be obtained as shown in Fig.3.5. The higher stretching

force, the more elongation of cell in the Z^* direction as expected even though the membrane could become concave locally near the adhesion area if stretching is high enough. In fact, the local concave deformation of membrane was not observed in experiments, either. However, this possibly takes place if the stretching force is high enough in experiments. At this moment, the deformation is approaching to the critical point at which membrane is extremely stretched and becomes almost torn. With the maximum force limit of the optical tweezers, this phenomenon cannot be observed.

3.4 COMPARISON OF NUMERICAL AND EXPERIMENTAL RESULTS

Comparisons of the experimental and numerical results are made based upon the transverse strain ε_t defined earlier in Eq.(3.2) to find the stiffness H of membrane. It is first noticed that H is used as a scale to non-dimensionalize the stretching force ($F^* = F/aH$). Therefore for each ε_t measured in the experiment, F^* can be determined correspondingly from the numerical solutions in Fig.3.7. Then using the measured data of a and F from the 3rd and 4th column in Table 3.2, the stiffness H can be calculated. In other words, the experimental and numerical data are matched by the same ε_t but different values of H . Calculation results are shown in the second to last column in Table 3.2 for every measurement. Conversely, a single value of H for each cell can be found. But this single value cannot match all numerical values of ε_t with experiment completely. Thus, an optimization of

$$\text{Error} = \min \left(\sqrt{\sum_{i=1}^3 (\varepsilon_{t,\text{exp}}^i - \varepsilon_{t,\text{num}}^i)^2} \right) \quad (3.30)$$

has to be solved for every cell to determine the optimized value of H . The constraint of H is restricted to the maximum and minimum of three H values for each cell in

Table 3.2. The optimized values of H , listed in the last column of Table 3.2, results a good correlation between the experiments and computations as shown in Fig.3.7. Its range, $4.56\sim 5.18\ \mu\text{N}\cdot\text{m}^{-1}$, is close to the studies in (Hénon, *et al*, 1999) ($2.5\pm 0.5\ \mu\text{N}\cdot\text{m}^{-1}$), and (Hochmuth, *et al*, 1987; Evans, 1973; Lelievre, *et al*, 1995; Engelhardt, *et al*, 1988) ($4.0\sim 10.0\ \mu\text{N}\cdot\text{m}^{-1}$) for human RBCs measured by other techniques such as micropipette aspiration or high frequency electric field.

The numerical results from this set of governing equations show that an almost linear $\varepsilon_t - F^*$ relation, close to the experimental results in Fig.3.4, can be obtained under negligible bending stiffness. Since bending stiffness is neglected, this relation only reflects the effect of tensile stiffness on the cell deformation in this study. By above optimization method, the magnitude of tensile stiffness can be predicted by comparisons of the experimental and numerical results.

3.5 CONCLUSIONS

In this chapter, an experimental work of single beam laser stretched erythrocytes was conducted. The erythrocytes were specially treated by buffer solutions to become spherical shape and attached by prepared silica microbeads. In order to find the relation between the stretching force and the cell deformation, two measurements were taken separately. First, the stretching force is calibrated with the laser power following the Stokes' law for a creeping flow over a laser-trapped silica bead. Secondly, the deformation of cells is measured through the image processing of digitally recorded photos. Results are presented in terms of the transverse strain that is a measure of the change of radius in a spherical cell along its equator. In addition, a simple mechanical model was introduced to simulate the deformation of cell membrane by stretching. Therefore, some features can be found from the work in this Chapter.

-
- (1) Experiments showed that optical tweezers is a powerful tool in cell biology to conduct a micro-mechanical stretching experiment on cells for its capacity of applying calibrated forces in the pN range.
 - (2) Experimental data plots show that the transverse strain ε_t versus the stretching force of different RBC has a good similarity and consistency. Numerical results show that the relationship of F - ε_t is sensitive to the deviation of δ but F - ε_l is not. Therefore, the transverse strain ε_t is most appropriate to quantitatively describe and evaluate the mechanical behaviors and the deformability of RBC numerically and experimentally.
 - (3) Comparisons of the experimental and numerical data were made by matching a defined transverse strain to determine the shear stiffness of membrane. An optimized stiffness of each example cell in the experiment was also calculated by minimizing the errors between the experimental and numerical data. The optimized shear stiffness ranging $4.56\sim 5.18 \mu\text{N}\cdot\text{m}^{-1}$ is very close to those from other studies by different experimental techniques ($2.5\pm 0.5 \mu\text{N}\cdot\text{m}^{-1}$ by Hnon, *et al*, 1999; and $4.0\sim 10.0 \mu\text{N}\cdot\text{m}^{-1}$ by Hochmuth, *et al*, 1987; Evans, 1973; Lelievre, *et al*, 1995; Engelhardt, *et al*, 1988).

Mean Diameter (μm)	Standard Deviation (μm)	Density (g/cm^3)	Solid content (wt.%)	Number of beads (ml^{-1})	Number of beads (g^{-1})	Surface functional groups	Buffer solution
2.34	0.23	1.96	30.6	2.737×10^{10}	7.605×10^{10}	Si-OH	Deionized water

Table 3.1 Characteristic properties of silica beads

Cell No.	Diameters (pixels)	Original radius a (μm)	Trapping force F (pN)	Length of L (pixels)	Length of D (pixels)	average 2δ (pixels)	$\delta^* = \delta a$	ϵ_t	ϵ_l	$H \times 10^{-6}$ ($\text{N} \cdot \text{m}^{-1}$)	Optimal $H \times 10^{-6}$ ($\text{N} \cdot \text{m}^{-1}$)
1	127.606	1.482	2.415	148.082	120.003	25.26	0.198	0.0596	0.1605	4.1721	5.1821
			3.623	157.295	117.641			0.0786	0.2327	4.6817	
			4.830	169.896	117.380			0.0801	0.3314	6.0700	
2	138.007	1.602	2.415	156.944	130.750	24.07	0.174	0.0526	0.1372	4.4048	4.5648
			3.623	173.534	128.250			0.0707	0.2574	4.8195	
			4.830	188.858	124.500			0.0979	0.3685	4.5115	
3	123.548	1.435	4.830	178.594	110.013	23.71	0.194	0.1096	0.4456	4.4484	4.7484
			6.038	183.249	107.380			0.1309	0.4832	4.5559	
			7.245	188.286	106.250			0.1400	0.5240	5.0637	
4	125.841	1.465	8.453	207.580	105.001	23.03	0.184	0.1656	0.6495	4.7821	5.0021
			9.660	225.143	102.626			0.1845	0.7891	4.8183	
			10.868	240.670	102.251			0.1875	0.9125	5.3194	
5	142.743	1.657	9.660	181.598	121.000	24.81	0.174	0.1523	0.2722	5.3063	5.0008
			12.075	197.298	114.000			0.2014	0.3822	4.7878	
			14.490	218.007	110.750			0.2241	0.5273	5.0543	

Table 3.2 Experimental measurements of five RBCs and the estimated values of RBC membrane shear stiffness by a simple mechanical analysis

Fig.3.1a Schematic drawing of the optical tweezers (Module 1064/1500 from Cell Robotics Inc.)

Fig. 3.1b Schematic of a deformed RBC and the definition of deformation

Fig.3.2 Force calibrations by dragging a silica bead ($2.34\mu\text{m}$ dia.) in Stoke's flow at the maximum escape velocity

Fig.3.3a Edge detection by digital image processing using MATLAB

Fig.3.3b Scheme of edge detection algorithm by MATLAB

Fig.3.4 Experimental data (transverse strain ϵ_t) and images of deformed RBCs with attached silica microbeads ($2.34\mu\text{m}$ in dia.) at different stretch forces

Fig.3.5 Numerical results for the deformed RBC with microbeads attached ($\delta^* = 0.185$)

Figure 3.6a the longitudinal strain ϵ_l vs. the dimensionless force F^*

Figure 3.6b the transverse strain ϵ_t vs. the dimensionless force F^*

Fig.3.7 Comparison of numerical and experimental results for deformed RBCs with the optimal tensile stiffness

CHAPTER 4 NUMERICAL ANALYSES ON THE MECHANICAL PROPERTIES OF ERYTHROCYTE BY LASER STRETCHING*

4.1 INTRODUCTION

From biological viewpoint, mammal erythrocytes have a simpler structure than many other cells since they only consist of membrane (phospholipid bi-layer and a 2D cytoskeleton network beneath) and cytoplasm (mainly hemoglobin with atomic irons), i.e., without nucleus or mitochondria, as mentioned in Section 1.2 in Chapter 1. The membrane enclosing whole cell is stiffer than the cytoplasm and thus maintains the integrity of cellular structure. Therefore, the deformation behaviors of RBCs are normally governed by their membranes under various external loadings. For this reason, the mechanical modeling for static erythrocytes is conventionally built upon the membrane instead of the whole cell.

In Chapter 3, a simple mechanical analysis was conducted to interpret the experimental data. Essentially, the constitutive relations between membrane tensions and stretch ratios, proposed by Pamplona et al, are linear; and no membrane bending resistance was taken into account in the simple mechanical analysis. To better interpret the experimental data and analyze the mechanical properties of erythrocyte's plasma membrane, more delicate and complex mechanical models need to be established to describe the mechanical behaviors of erythrocytes stretched optically.

* C. Li, Y. P. Liu, K.K.Liu, A. C. K. Lai, 2007, "Correlations between the Experimental and Numerical Investigations on the Mechanical Properties of Erythrocyte by Laser Stretching", *IEEE Transactions on NanoBioscience* (accepted)

In addition to the assumptions given in Section 3.3.1 in Chapter 3, the relationships of stretch ratios and tensions are assumed to be nonlinear based on the large deformation of stretched erythrocyte observed in our experiments and the similar observations by other researchers or in other experiments by micropipette aspiration, etc. Since the elastic response observed in our experiments is almost independent of strain rate in the transient loading time, the mechanical behaviors of RBC membrane will be modeled by a hyperelastic material model.

The physical problem to be simulated is that a spherically swollen RBC bound with two beads is approximately axisymmetrically stretched to deform by optical tweezers, as described in Chapter 3. Parker and Winlove (*Parker, et al, 1999*) numerically calculated the profiles of a deformed vesicle when two identical but opposite forces are exerted its two poles. An assumption in their calculations is that the forces are acting on very small contact areas on which $P = F/\pi\delta^2$, where the dimensionless radius δ of the contact area is assumed to be less than 0.01. In addition, they analyzed the effect of a dimensionless material property parameters C on the deformation of vesicles, but no quantitative prediction for shear modulus or bending modulus were made. In our cases, the forces are acting on finite areas at the poles of a RBC. The experimentally measured data of δ is far larger than 0.01. The simulations of our cases in this chapter are based on the experimentally measured data, which will be fed into our mechanical models for calculations. The purposes of simulations are to analyze the mechanical behaviors of RBCs stretched optically and try to predict the isotropic elastic moduli of RBC membrane.

For the convenience of illustrating and formulizing the boundary conditions in our mechanical models, a complementary geometric relation $s_0 = \sin^{-1} \delta$ is given by referring to Fig.4.1.

4.2 MECHANICAL MODELING

4.2.1 Geometry of Cell Membrane

The geometry of a cell attached by a silica bead is shown in Fig.4.1 (a) where a spherical membrane with radius a is axisymmetric in the z -direction. Dimensionless cylindrical coordinates: $r^* = r/a$, $z^* = z/a$ and $s^* = s/a$, have been introduced for numerical simulation and they satisfy the following relation,

$$r^* = \sin s^* \quad (4.1)$$

$$z^* = 1 - \cos s^* \quad (4.2)$$

where s is the arc length and φ is the meridional angle, as depicted in Fig.4.1(a) & (b).

Note that the dimensionless arc length s^* is identical with the meridional angle φ because of $s = a\varphi$.

Fig.4.1(c) shows the deformed cell where a new set of dimensionless coordinates: $R^* = R/a$, $Z^* = Z/a$ and $S^* = S/a$, are used to describe the geometry. Unlike the undeformed cell, the arc length S^* is no longer equal to the meridional angle φ because the radius of the membrane varies from point to point after deformation. Therefore R^* and Z^* are functions of S^* and φ and they both satisfy the following relations,

$$\frac{dR^*}{dS^*} = \cos \varphi \quad (4.3)$$

$$\frac{dZ^*}{dS^*} = \sin \varphi \quad (4.4)$$

Curvature is another key geometric parameter which can be expressed in the two principal directions, i.e., the circumferential (θ) and meridional (s or φ) directions. If the dimensionless curvatures are defined as $\kappa_s^* = a\kappa_s$ and $\kappa_\theta^* = a\kappa_\theta$, then obviously

$\kappa_s^* = \kappa_\theta^* = 1$ for the undeformed cell. When the cell membrane is deformed, dimensionless curvatures are found to be,

$$\kappa_s^* = a\kappa_s = \frac{d\varphi}{dS^*} \quad (4.5)$$

$$\kappa_\theta^* = a\kappa_\theta = \frac{\sin \varphi}{R^*} \quad (4.6)$$

4.2.2 Kinematic Relation for Deformation

Relations between the undeformed (r^*, z^*) and deformed (R^*, Z^*) states of a cell can be determined by defining two principal stretches (strains) along the circumferential (θ) and meridional (s or φ) directions as (Pozrikidis, 2003; Pamplona, et al, 1993; Pamplona, et al, 1996)

$$\lambda_s = \frac{dS^*}{ds^*} \quad (4.7)$$

$$\lambda_\theta = \frac{R^*}{r^*} = \frac{R^*}{\sin s^*} \quad (4.8)$$

Physically, Eq.(4.7) represents the change of arc length along the meridional direction and Eq.(4.8) denotes the radius change along the circumferential direction. For the original spherical cell, $\lambda_s = \lambda_\theta = 1$.

4.2.3 Equilibrium Equations

Equilibrium equations for the cell membrane can be found from the classical theory for axisymmetric shells (Flugge, 1973; Ugural, 1998) as follows:

$$\frac{1}{R} \frac{d(RQ_s)}{dS} + \kappa_\theta T_\theta + \kappa_s T_s = P_r \quad (4.9)$$

$$\frac{1}{R} \frac{d(RT_s)}{dS} - \kappa_\theta T_\theta \cot \varphi - \kappa_s Q_s = -P_i \quad (4.10)$$

$$\frac{d(RM_s)}{dS} - M_\theta \cos \varphi + RQ_s = 0 \quad (4.11)$$

where P_r and P_t are external pressure/stresses ($\text{N}\cdot\text{m}^{-2}$) on the membrane surface in the radial and meridional directions, respectively. For cells in an aqueous environment, P_r is the normal pressure across the cell membrane and P_t the shear stresses normally generated by fluid flow. For the case under study in this paper, we have both terms vanish. Eq.(4.9) – (4.11) represent the equilibrium of forces and moments in the radial and meridional directions of a membrane or shell element in axisymmetric revolution. Contrary to the classic shell theory (*Timoshenko, et al, 1959*) where governing equations are derived based on φ , the current differentiations are performed along the arc length S instead in order to avoid the singularity at $\varphi = 0$.

4.2.4 Governing Equations

Constitutive law

Because of the large modulus of dilatation relative to elastic shear, the RBC membrane is highly resistant to changes in area but shears readily (*Secomb, et al, 1986*). Therefore, the red-cell membrane, although highly flexible, can be assumed to maintain a constant area under deformation. This assumption imposes a constraint on the principal extension ratios in the meridional and circumferential directions by requiring that the second invariant of the Green strain tensor vanish. This constraint gives rise to the concept of the isotropic membrane tension component T which is the two-dimensional analog to pressure in three-dimensional materials and the deviatoric component γ . (*Pozrikidis, 2003*)

The constitutive law can be derived from a strain energy function. Such a function is in terms of principal stretches: λ_θ and λ_s . The basic line of thought is that the

membrane tension can be decomposed into two parts, the dilatational and deviatoric components. The former is responsible for the area change and the latter for the shear deformation. Corresponding strain measures are $(\lambda_\theta \lambda_s - 1)$ to the area change and $(\frac{1}{2}(\frac{\lambda_\theta}{\lambda_s} + \frac{\lambda_s}{\lambda_\theta}) - 1)$ to the shear deformation. As a result, each tension is the partial derivative of the strain energy function with respect to each corresponding strain measure. Thus the following expressions for the two principal membrane tensions (T_s and T_θ) can be obtained as:

$$T_s = T + \gamma \quad (4.12)$$

$$T_\theta = T - \gamma \quad (4.13)$$

where $T = \frac{(T_s + T_\theta)}{2}$ and $\gamma = \frac{(T_s - T_\theta)}{2}$ are the dilatational and deviatoric components of tensions, respectively. Explicit expressions of the constitutive relation for an isotropic membrane has been studied by Evans and Skalak, cited by Hansen et al in their work (*Hansen, et al, 1996*), in which a proposed strain energy function leads to the following relation

$$\gamma = G\left(\frac{1}{\lambda_\theta^2} - \frac{1}{\lambda_s^2}\right) \quad (4.14)$$

where G is the shear stiffness ($\text{N}\cdot\text{m}^{-1}$) (with factor $\frac{1}{2}$ being absorbed in). With the constant area constraint of membrane, the two principle stretches satisfy the following relation

$$\lambda_\theta \lambda_s = 1 \quad (4.15)$$

This constraint implies an assumption that the membrane is permeable so that the volume of cell can be changed and osmotic pressure will be automatically balanced.

Under this constraint, the dilatational component T is independent of the strain measure $(\lambda_\theta \lambda_s - 1)$ and therefore it becomes an independent variable in this analysis.

In addition to the membrane tensions, the bending moments are assumed to be proportional to the curvature change of membrane through the following expressions (Pamplona, et al, 1993; Pamplona, et al, 1996)

$$M_s = M_\theta = B[(\kappa_s + \kappa_\theta) - (\kappa_s + \kappa_\theta)_0] \quad (4.16)$$

where M_s and M_θ are the principal membrane resultant bending moments (N); B is the bending stiffness of membrane (N·m); κ_s and κ_θ are the principal curvatures (m⁻¹); and the subscript “0” denotes the curvature in the original geometry.

To simplify the numerical task, several dimensionless parameters, $T_s^* = T_s a^2 / B$, $T_\theta^* = T_\theta a^2 / B$, $Q_s^* = Q_s a^2 / B$, $M_s^* = M_s a / B$ and $M_\theta^* = M_\theta a / B$, are introduced and therefore the constitutive relations (4.12) and (4.13) can be recast as

$$T_s^* = T^* + C\left(\frac{1}{\lambda^2} - \lambda^2\right) \quad (4.17)$$

$$T_\theta^* = T^* - C\left(\frac{1}{\lambda^2} - \lambda^2\right) \quad (4.18)$$

where $T^* = T a^2 / B$, $C = G a^2 / B$ and

$$\lambda = \lambda_\theta = \lambda_s^{-1} = R^* / \sin s^* = ds^* / dS^* \quad (4.19)$$

Eq.(4.19) is derived based on Eq.(4.7) and (4.8) in combination with the membrane incompressibility, as stated in Eq.(4.15). The new parameter C represents the ratio of in-plane shear to out-of-plane bending resistance in the cell membrane deformation (with factor $\frac{1}{2}$ being absorbed). It is also a proportional constant for the membrane area

change to the deviatoric component of tensions $\gamma = \frac{(T_s - T_\theta)}{2}$.

Governing Equations

Non-dimensionalizing the equilibrium equations (4.9) – (4.11) with all foregoing mentioned dimensionless variables and substituting Eq. (4.17) – (4.19) into them, the following dimensionless governing equations can be obtained

$$\frac{dR^*}{ds^*} = \frac{\cos \varphi \sin s^*}{R^*} \quad (4.20)$$

$$\frac{dZ^*}{ds^*} = \frac{\sin \varphi \sin s^*}{R^*} \quad (4.21)$$

$$\frac{d\varphi}{ds^*} = \frac{\kappa_s^* \sin s^*}{R^*} \quad (4.22)$$

$$\frac{d\kappa_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[-Q_s^* - \frac{\kappa_s^* \sin \varphi}{R^*} + \frac{\sin \varphi \cos \varphi}{R^{*2}} \right] \quad (4.23)$$

$$\frac{dQ_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[- \left(T^* + C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \right) \kappa_s^* - \left(T^* - C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \right) \frac{\sin \varphi}{R^*} - \frac{Q_s^* \cos \varphi}{R^*} \right] \quad (4.24)$$

$$\frac{dT^*}{ds^*} = \frac{\sin s^*}{R^*} \left[\kappa_s^* Q_s^* - 2C \left(\frac{1}{\lambda^2} - \lambda^2 \right) \frac{\cos \varphi}{R^*} \right] + C \cdot \left(\frac{2}{\lambda^3} + 2\lambda \right) \cdot \left(\frac{\cos \varphi}{R^*} - \frac{R^* \cos s^*}{\sin^2 s^*} \right) \quad (4.25)$$

where the last term in the right hand side of Eq. (4.25) is obtained by differentiating the

function $\left(\frac{1}{\lambda^2} - \lambda^2 \right)$ with respect to s^* . Note that the derivatives are changed from the

current configuration $\left(\frac{d}{dS^*} \right)$ back to the original $\left(\frac{d}{ds^*} \right)$ by the chain rule described in

Eq.(4.19). Eq.(4.21)-(4.25) form a set of non-linear, first order ordinary differential equations (ODEs) and can only be solved numerically. In these equations, the unknowns are R^* , Z^* , φ , κ_s^* , Q_s^* and T^* . In order to obtain the solution, proper

boundary conditions (BCs) have to be imposed and they are discussed in the next section.

4.2.5 Boundary Conditions (BCs)

The area of attachment by silica beads is relatively smaller when compared to the overall size of cell. It is thus assumed that the membrane tension distribution (T_s^*) along the perimeter of the attached area is uniform and its resultant can be obtained by force balance analysis at the perimeter of the attached area (cf. Fig.4.1(c)). The stretching force can be non-dimensionalized by $F^* = Fa/B$. This is a very important simplification in that the adhesive contact problem can be avoided within the attached area and the boundary conditions for tensions can be determined purely by force balance.

Following boundary conditions are imposed by considering the location at attachment $s^* = s_0^* = \sin^{-1} \delta^*$,

$$R^*(s_0^*) = \delta^* \quad (4.26)$$

$$Z^*(s_0^*) = 0 \quad (4.27)$$

$$\varphi(s_0^*) = \varphi_0 \quad (4.28)$$

$$\kappa_s^*(s_0^*) = \kappa_{s0}^* \quad (4.29)$$

$$Q_s^*(s_0^*) = Q_{s0}^* \quad (4.30)$$

$$T^*(s_0^*) = \frac{F^*}{2\pi\delta^* \sin \varphi_0} - \frac{Q_s^* \cos \varphi_0}{\sin \varphi_0} \quad (4.31)$$

$$\text{and at } s^* = \frac{\pi}{2},$$

$$\varphi(\pi/2) = \pi/2 \quad (4.32)$$

$$Q_s^*(\pi/2) = 0 \quad (4.33)$$

$$T_s^*(\frac{\pi}{2}) = \frac{F^*}{2\pi R^*(\pi/2)} - C(\frac{1}{R^{*2}(\pi/2)} - R^{*2}(\pi/2)) \quad (4.34)$$

where $\delta^* = \delta/a$ and $s_0^* = s_0/a$; s_0 is the half arc length within the contact area. Eq. (4.26) and (4.27) are directly from the geometry shown in Fig.4.1(c). In (4.28) – (4.30), the initial angle φ_0 , curvature κ_{s0}^* and shear tension Q_{s0}^* are unknown and therefore initial guessed values are needed before the numerical analysis. Eq. (4.31) is obtained by the balance among forces (T_s^* , Q_s^* and F^*) along the stretch direction (Z^*) at s_0^* . Since the cell is stretched by two opposite aligned forces, it preserves the axisymmetry and as well the symmetry with respect to $\varphi = \frac{\pi}{2}$. Such a *two-fold symmetry* results a zero shear and a horizontal (along Z^* direction) tension T^* at $\varphi = \frac{\pi}{2}$. Eq. (4.32) is then established by the balance between $T_s^*(\pi/2)$ all over the membrane and stretching force F^* . Note that there are two important facts that lead to the boundary conditions (4.26) – (4.34): one is $\lambda = R^*/\sin s^* = ds^*/dS^* = 1$ at $s^* = s_0^*$ and another $\lambda = R^*$ at $s^* = \pi/2$. The first yields a zero deviatoric component of tensions at s_0^* .

4.3 NUMERICAL METHOD

Another set of governing equations Based on Pamplona and Calladine's Constitutive Law are formulized in Appendix IV. Essentially, the same numerical method to be elaborated below can be applied to solve these two sets of governing equations. In this chapter, detailed numerical explanation, numerical results, analyses, discussion and experimental data fitting are only given to the governing equations

derived from the Evans & Skalak's Constitutive Law in the subsequent sections. However, the final results derived from the governing equations based on the Pamplona and Calladine's Constitutive Law will be also given for comparison.

Eq.(4.20) – (4.25) together with conditions (4.26) – (4.31) form a non-linear, two-point boundary value problem which can be solved by numerical integration such as Runge-Kutta method (*Sandford, et al, 1972*). But to start the procedure, the initial values of φ , κ_s^* and Q_s^* at s_0^* have to be guessed. These initial guesses then will be successively adjusted by an optimization method until the conditions (4.32) – (4.34) at $\frac{\pi}{2}$ are accurately met. Figure 4.2 shows the numerical algorithm for solving this problem. As shown in the flow chart, the stretching force F^* and membrane property C were first specified. The initial guesses on the values of φ_0 , κ_{s0}^* and Q_{s0}^* then were given to solve Eq.(4.20) – (4.25). The function “ode15s” in MATLAB® is chosen to be the solver for its ability to deal with stiff ODEs. Once solutions were found (convergent), Eq.(4.32) – (4.34) are checked and the search for new φ_0 , κ_{s0}^* and Q_{s0}^* are set off if those conditions are not satisfied. The search for new initial values is carried out by the function “fminsearch” in MATLAB®, which uses an unconstrained nonlinear optimization for finding the minimum of a multiple-variable function. Technically, it is implemented by combining (4.32) – (4.34) into a single objective function as

$$\left[\varphi\left(\frac{\pi}{2}\right) - \frac{\pi}{2} \right]^2 + \left[Q_s^*\left(\frac{\pi}{2}\right) \right]^2 + \left[T^*\left(\frac{\pi}{2}\right) - \left(\frac{F^*}{2\pi R^*(\pi/2)} - C \left(\frac{1}{R^{*2}(\pi/2)} - R^{*2}(\pi/2) \right) \right) \right]^2 \quad (4.35)$$

This function is considered as an implicit function of φ_0 , κ_{s0}^* and Q_{s0}^* . If the minimum found is smaller than 10^{-4} then the program will stop. For otherwise, the new

φ_0 , κ_{s0}^* and Q_{s0}^* will be used to solve Eqs.(4.20) – (4.25) again. The iteration continues until both governing equations and conditions (4.35) converge.

To expedite the whole simulations, following starting conditions at s_0^* are also recommended.

$$\frac{dR^*}{ds^*} = \cos \varphi_0 \quad (4.36)$$

$$\frac{dZ^*}{ds^*} = \sin \varphi_0 \quad (4.37)$$

$$\frac{d\varphi}{ds^*} = \kappa_s^*(s_0^*) \quad (4.38)$$

$$\frac{d\kappa_s^*}{ds^*} = -Q_{s0}^* + \frac{\sin \varphi_0 \cos \varphi_0}{\delta^{*2}} - \frac{\kappa_{s0}^* \cos \varphi_0}{\delta^*} \quad (4.38)$$

$$\frac{dQ_s^*}{ds^*} = -\left(\frac{F^*}{2\pi\delta^* \sin \varphi_0} - \frac{Q_{s0}^* \cos \varphi_0}{\sin \varphi_0} \right) \left(\kappa_{s0}^* + \frac{\sin \varphi_0}{\delta^*} \right) - \frac{Q_{s0}^* \cos \varphi_0}{\delta^*} \quad (4.40)$$

$$\frac{dT^*}{ds^*} = \kappa_{s0}^* \cdot Q_{s0}^* + 4C \left(\frac{\cos \varphi_0}{\delta^*} - \frac{\sqrt{1 - \delta^{*2}}}{\delta^*} \right) \quad (4.41)$$

These auxiliary conditions are obtained simply by substituting $s^* = s_0^* = \sin^{-1} \delta^*$ and BCs. (4.26) – (4.31) into the governing equations (4.20) – (4.25).

4.4 NUMERICAL RESULTS

4.4.1 Profile of Cell Deformation

We first study the deformation of cell membrane with different stiffness (C values) by stretching. Note that since the radius of attachment δ^* is much smaller than the cell, its variation on the cell deformation is very minor. Using the average value $\delta^* = 0.185$ from Table 4.1, Fig.4.3 demonstrates the profile of deformation under

different stretching force according to different C values of the membrane. For membrane with zero tensile stiffness ($C = 0$), the original circular membrane is stretched to an elliptic shape. As the tensile stiffness increases (or bending stiffness decreases), the cell deforms into a sausage shape ($C = 0.3$). When C approaches 0.5, the deformed cell is like a spindle if stretching force is high enough. It is important to remind readers that $C = 0.5$ represents a value of the product of shear stiffness and the square of original radius of cell, which is equal to the bending stiffness in the membrane; so the deformation for $C > 0.5$ is controlled by the membrane tensions rather than bending moment. A significant influence due to this change is the appearance of negative curvature near the two ends of stretching in the deformation profiles for $C \geq 1$. Physically, large C implies the membrane is rather resistant to the tension than bending. This effect remains for a very stiff membrane ($C = 100$) and thus maintains similar deformation profiles as in the case of $C = 1$.

4.4.2 Transverse Strains

To further characterize the simulated results, the transverse and longitudinal strains (Guck, *et al*, 2001; Parker, *et al*, 1999) introduced earlier are found to be

$$\varepsilon_l = 1 - Z^*(0) \quad (4.42)$$

$$\varepsilon_t = R^*(\pi/2) - 1 \quad (4.43)$$

corresponding to the numerical analysis. These two strains quantitatively represent the radius change of a cell at the pole ($\varphi = 0$) and equator ($\varphi = \pi/2$). As explained earlier in the experimental part, only the transverse strain ε_t will be used to characterize the deformation from our numerical results. Fig.4.4 demonstrates the ε_t as a function of the stretching force F^* for different values of C . Overall speaking, the $\varepsilon_t - F^*$

relation changes from non-linear when $C < 1$, to linear when $C \geq 100$. In between, ε_t increases monotonically with the stretching force but the slope of curves reduces as the membrane stiffened in tension. To further examine the results, we scale the stretching force F^* by C and re-plotting Fig.4.4 in Fig.4.5. This is a different way to non-dimensionalize our variables following Parker and Winlove (*Parker, et al, 1999*), which allows us to find the $\varepsilon_t - F^*$ relation at zero bending stiffness ($C \rightarrow \infty$). Results indicate that ε_t varies linearly with F^*/C when $C > 10$, and non-linearly for $C < 1$. Furthermore, the curves of $C = 1$ and $C = 10$ seem to envelope all cases for $C > 1$. For $C \geq 100$, it almost coincides with the case of zero bending. In fact, the case of zero bending stiffness is solved separately and then imposed in the figure for a comparison. Generally speaking, Fig.4.5 demonstrates the scaling effect of C on the change of transverse strain more remarkably than Fig.4.4; so we will use it in the following section for a comparison to experimental results.

4.5 COMPARISON BETWEEN NUMERICAL AND EXPERIMENTAL RESULTS

The numerical and experimental results, namely, Fig.3.4 and Fig.4.5, are now put alongside each other for a comparison. Though both figures present the change of transverse strain due to stretching, they are different in the abscissas, i.e. the dimensional and dimensionless force - F and F^*/C . Since $F^*/C = F/(aG)$, a direct comparison can be made possible simply by the search of proper bending stiffness G to fulfill either of the following circumstances:

$$\min \sqrt{\sum_{\text{experimental data point}} (\varepsilon_t^{\text{exp}} - \varepsilon_t^{\text{num}})^2} \text{ at the same } F/(aG) \quad (4.44)$$

or

$$\min \sqrt{\sum_{\text{experimental data point}} \left(\frac{F^{\text{exp}}}{aG} - \left(\frac{F^*}{C} \right)^{\text{num}} \right)^2} \text{ at the same } \varepsilon_t \quad (4.45)$$

These two conditions are illustrated in Fig.4.6 and in general they are curve fittings of the experimental data by numerical results at various G and C values. Mathematically, Eq.(4.44) and (4.45) are *equivalent* at the limit of *zero* errors. However, since the transverse strain ε_t is already small, in order to catch the numerical significance during calculations, we use only (4.45) as the objective to search for the optimized shear/bending stiffness. Results are shown in Fig.4.7 where contours of errors in the plane of G and C for each of the five cells are presented respectively with some values labeled. The average of optimized G for $C = 10 \sim 100$ are also indicated by the solid lines.

An important finding is that minimum errors can be obtained at *almost constant* G when $C > 10$ for *all* five cells. We list numerical values of the average optimal G in Table 4.1 for a comparison. The shear stiffness ($2G$), ranging from 2.35218 to $4.28934 \times 10^{-6} \text{ Nm}^{-1}$, is close to those found in other works such as: $3.4 \pm 1.5 \sim 4.7 \pm 1.3 \times 10^{-6} \text{ Nm}^{-1}$ (in isotonic buffer) by Lenormand et al (Lenormand, et al, 2001); $6.6 \times 10^{-6} \text{ Nm}^{-1}$ by Waugh (Waugh, et al, 1979); $1.0 \sim 10.0 \times 10^{-6} \text{ Nm}^{-1}$ by Lee et al (Lee, et al, 2001); $4 \sim 10 \times 10^{-6} \text{ Nm}^{-1}$ by Hochmuth (Hochmuth, et al, 1987) and Lelievre (Lelievre, et al, 1995). Therefore, we may conclude that an optimal material property exists for the experimental results when $C > 10$. This further implies that in reality an erythrocyte membrane may have its shear stiffness much higher than bending. Moreover, the proposed optimization in Eq.(4.44) or (4.45) provides a viable way for the quantitative determination of the cell membrane properties by the comparison of numerical to experimental data.

On the other hand, the bending stiffness of RBC membrane can be estimated according to the expression $B = Ga^2/C$ with the optimized and averaged value of membrane shear stiffness, that is, $5.889 \times 10^{-19} \sim 2.422 \times 10^{-20}$ N·m. This estimation of bending stiffness of RBC membrane is smaller than the estimated one in Chapter 2, but their mean value is closer to the value (1.8×10^{-19}) listed in the literature (*Len Fisher, 1993*).

With the same method and procedure of experimental & numerical data optimization and data fitting, the values of membrane shear stiffness and bending stiffness can be also predicted and estimated based on the numerical results from the Governing Equations derived from Pamplona and Calladine's Constitutive Law in Appendix IV. The final results are listed in the Table 4.2 for comparison.

4.6 CONCLUSIONS

To better interpret the experimental data in Chapter 3 and to predict the mechanical properties of RBC membrane, two physical models for cell membrane including the effect of membrane bending resistance are introduced by following a linear constitutive law from Pamplona and Calladine's original work and by following a non-linear constitutive law proposed by Evans and Skalak. Proper boundary conditions are carefully imposed to comply with the real experimental conditions, i.e., silica beads attached cell stretched by laser beam. Numerical simulations are then carried to solve the non-dimensionalized governing equations. Using the dimensionless material properties (ratio of shear to bending stiffness), a detailed parametric analysis is conducted to study the relation between this material properties and cell deformation. We cast the simulated results in terms of the transverse strain and compare it to the experimental data. The comparison is made by the method of optimization, i.e., the

search of proper shear stiffness in order to minimize the errors between the two results.

Therefore, following features can be revealed from the work in this Chapter.

- (1) Numerical results indicate that at high tensile stiffness, cell can deform into a spindle shape with negative curvature close to the ends of stretch.
- (2) Comparison of experimental data plots and numerical curves shows that the dimensionless mechanical property parameter C shall be more than 10. That means the shear resistance of membrane plays a dominant effect on the mechanical behaviors of RBCs stretched optically. The effect of membrane bending resistance can be neglected with increases of C .
- (3) Quantitative comparison of the experimental data and numerical results by optimization, the mechanical properties of RBC membrane can be estimated. It shows that an optimal material property exists for the experimental results and smaller errors can be obtained if the erythrocyte membrane has its shear stiffness much higher than bending. Numerically, the average optimal shear stiffness is around $2.35218 \times 10^{-6} \sim 4.28934 \times 10^{-6} \text{ Nm}^{-1}$, which is comparable to those found in the literature. Table 4.2 shows the moduli of membrane predicted by two different models.
- (4) The predictions of mechanical properties of membrane are dependent on the mechanical models using different constitutive law of membrane tensions. Compared with the values of membrane shear modulus ($2.5 \pm 0.5 \mu\text{N} \cdot \text{m}^{-1}$) obtained by Henon et al, whose experimental work was also conducted by optical tweezers and whose experimental data were interpreted by the pure linear elasticity, the constitutive law of Evans and Skalak is better to describe the mechanical behaviors of RBC membrane.

Table 4.1 Experimental measurements of five RBCs (from Chapter 3) and their optimized membrane shear stiffness by comparing with numerical results

Cell No.	Diameters (pixels)	Original radius a (μm)	Trapping force F (pN)	Length of L (pixels)	Length of D (pixels)	average 2δ (pixels)	$\delta' = \delta/a$	ϵ_1	ϵ_2	Average Optimal $G \times 10^{-6}$ ($\text{N} \cdot \text{m}^{-1}$)	$2G \times 10^{-6}$ ($\text{N} \cdot \text{m}^{-1}$)
1	127.606	1.482	2.415	148.082	120.003	25.26	0.198	0.0596	0.1605	1.33547	2.67094
			3.623	157.295	117.641			0.0786	0.2327		
			4.830	169.896	117.380			0.0801	0.3314		
2	138.007	1.602	2.415	156.944	130.750	24.07	0.174	0.0526	0.1372	1.17124	2.34248
			3.623	173.534	128.250			0.0707	0.2574		
			4.830	188.858	124.500			0.0979	0.3685		
3	123.548	1.435	4.830	178.594	110.013	23.71	0.194	0.1096	0.4456	1.17609	2.35218
			6.038	183.249	107.380			0.1309	0.4832		
			7.245	188.286	106.250			0.1400	0.5240		
4	125.841	1.465	8.453	207.580	105.001	23.03	0.184	0.1656	0.6495	1.21987	2.43974
			9.660	225.143	102.626			0.1845	0.7891		
			10.868	240.670	102.251			0.1875	0.9125		
5	142.743	1.657	9.660	181.598	121.000	24.81	0.174	0.1523	0.2722	2.14467	4.28934
			12.075	197.298	114.000			0.2014	0.3822		
			14.490	218.007	110.750			0.2241	0.5273		

Table 4.2 Predicted Values of Shear Stiffness and Bending Stiffness of RBC Membrane by mechanical models

	Pamplona & Calladine's constitutive law	Evans & Skalak's constitutive law
Shear stiffness	$4.2719 \times 10^{-6} \sim 5.2537 \times 10^{-6} \text{ N/m}$	$2.3425 \times 10^{-6} \sim 4.2893 \times 10^{-6} \text{ N/m}$
Bending stiffness	$1.442 \times 10^{-18} \sim 9.573 \times 10^{-20} \text{ N} \cdot \text{m}$	$5.889 \times 10^{-19} \sim 2.422 \times 10^{-20} \text{ N} \cdot \text{m}$

Figure 4.1 The geometry and coordinate system of an axisymmetric cell membrane stretched by an attached bead. (a) the undeformed spherical cell with diameter a ; (b) and (c) the undeformed and deformed membrane in dimensionless coordinates (r^*, z^*) and (R^*, Z^*) respectively; the deformed membrane in dimensionless coordinates (R^*, Z^*) where $R^* = R/a$, $Z^* = Z/a$, $S^* = S/a$, and Z^* is the axis of symmetry. Note that $\delta^* = \delta/a$ is the dimensionless radius of attachment.

Figure 4.2 The flowchart of numerical calculation

Figure 4.3 The deformation of cell membrane with different stiffness under stretching by laser beams on silica beads at two ends along the Z^* -axis. The dimensionless stiffness C is a ratio of the in-plane shear to the out-of-plane bending. The average radius of attachment $\delta^* = 0.185$ is measured from the experimental data

Figure 4.4 The transverse strain as a function of stretching force for membrane with different stiffness.

Figure 4.5 The transverse strain ($\varepsilon_t = R^*(\pi/2) - 1$) as a function of stretching force scaled by C

Figure 4.6 An illustration for the curve fittings of experimental data with simulated results through the minimization of errors.

Chapter 4

Figure 4.7 The contours of errors between the numerical and experimental results in the plane of G and C . Some errors are labeled by their values. The average of optimal G for $C = 10 \sim 100$ are also indicated by the solid lines.

CHAPTER 5 FINITE ELEMENT ANALYSIS OF A FLOW- DEFORMED BIOMIMETIC VESICLE MANIPULATED BY OPTICAL TRAP: A PARAMETER STUDY*

5.1 INTRODUCTION

In biological systems, most cells exist in an aqueous environment because the fluid exists ubiquitously in organisms and serves as a natural link between cells/tissues. The interaction between cells and fluid is primary to all biological processes. On the viewpoint of Biomechanics, the acquisition of the fundamental mechanical properties underlying biological processes is very important for engineering biological behaviors.

The earliest experimental study and mechanical analyses on the deformation of RBC caused by flow-induced stresses were conducted by Hochmuth and Mohandas (*Hochmuth, et al, 1972*). In their experiment, a RBC was stuck to the coverslip in the forms of line attachment or point attachment; then it was subjected to flow in a channel and was elongated by the flow-induced stresses on its surface. The experiment setup and some experimental pictures are shown in Appendix V. Hochmuth and Mohandas gave a fairly good estimation of the elasticity modulus of RBC membrane based on a linear elastic model by incorporating their experimental data.

Since the optical tweezers has emerged as a novel tool for manipulating cells and macromolecules for biophysical characterizations (*Ashkin et al, 1987; Greulich, 1997; Konig, 2000*), it seems to be feasible to use this novel tool to conduct more effective experimental study on cells as a non-destructive method. With a highly focused laser beam, an optical trap in a fluid is created to attract nearby particles by the differential

* Chuan Li, Y. P. Liu, K. K. Liu, J. H. Hsieh, A Parameter Study of the Material Properties in the Vesicle-Fluid Interaction, Computational materials Science, Vol.30, 2004, 504-510

momentum of photons (force). Consequently, the laser beam can be used to confine and move particles within the focal region. On the other hand, in biomechanical study, a vesicle or microcapsule with phospholipid membrane has been widely used as a model cell for investigating the deformation responses of a biological cell under various mechanical stimuli.

The latest experimental study on RBC/vesicle mechanics with optical traps was conducted by Foo et al (*Foo et al, 2003; 2004*) who observed the deformation of a biomimic vesicle trapped by a single optical trap in Stoke's flow. As mentioned in Section 1.4.2 chapter 1, although the real-life problem of tri-physical field coupling is really formidable, it still gives us an implication that a study on this phenomenon can be conducted by numerical simulation on a simplified physical prototype if the precision and the size of highly focused laser beam are good and small enough.

In this chapter, a mechanical analysis for a simplified coupling problem of fluid-vesicle interaction can be conducted if the effect of the undeterminable radiation pressure can be excluded. Using the laser trapping technique, a cell/vesicle can be held static in Stoke's flow by focusing the laser beam in very small area on the surface of RBC or by focusing it on a very small bead attached on the surface of RBC, as shown in Figure 5.2 (b). Then, it can be expected that the cell/vesicle will be elongated due to flow-induced stresses. The Finite Element Modeling will be applied to simulate the mechanical responses of a vesicle manipulated by laser trapping in a simple Newtonian fluid. However, the emphasis is first on the material modeling of vesicle and subsequently on its responses to the external fluid quantified by proposed parameters of mechanical properties.

Traditionally, the mechanical properties based on the continuum approach have been widely utilized to describe the deformation behaviors of various materials. However, it is less effective as the same method applied to the biological system due to

the intricacy of its own. A common thought to overcome such hurdles is to study a simplified system with numbered parameters. For which we will address in the following context.

5.2 STATEMENT OF THE PROBLEM AND ASSUMPTIONS

A continuum solid vesicle to model a cell based on the theory of hyperelasticity is established to simulate the mechanical behaviors of a deformable cell in the Stokes fluid flow. A vesicle with diameter $6.4\ \mu\text{m}$ ($6400\ \text{nm}$) shown in Figure 5.1 consists of two materials representing the plasma membrane and cytoplasm in the cell respectively. Note that this problem is axisymmetric in the y -direction and so does the free flow. For simplification, both the membrane and cytoplasm are assumed to be homogeneous and incompressible (*Liu et al, 1996; De Hanns et al, 1997; Evans et al, 1979*). The assumption of incompressibility is based upon the following physical reasons. First is the chemical structure of bi-layer lipid membrane only allows a limited deformation in the direction perpendicular to the membrane when the vesicle is interacting with external fluid pressure. Inside the cell, various protein molecules form a highly concentrated hemoglobin solution. Collectively, this fluid-like interior is mechanically assumed to behave as a very soft gel-like solid. Hence, the total volume of cytoplasm is more or less conservative during a finite deformation. It is also worth mentioning that the volume fraction of bi-layer membrane to the whole cell is very small and therefore many investigators postulate that the membrane maintains a constant volume during deformation (*Evans et al, 1979*). Another important assumption arises from the selectable permeability of the membrane to ions. In spite of the membrane's permeability to water, the inability of salts to cross the membrane, prevents significant

water loss because of the requirement for colloidal osmotic equilibrium. This osmotic mechanism generally contributes the incompressibility of a cell in aqueous environment.

Experimental results (*Hochmuth, 2000; Chang et al, 1993; Sato et al, 2000*) show that both the membrane and the inner content of a cell exhibit some viscoelastic effects when a cell is sucked into a micropipette or a vesicle moves in the shear flow. However, the viscoelastic effect can be neglected for steady state, sufficiently small deformation or relatively short stress relaxation time. The last condition has been found in the earlier experimental studies of the deformed vesicles in optical trap where the steady state can be reached within quite short time (*Foo et al, 2003; Foo et al, 2004*). Hence, a quasi-static equilibrium method can be used for the current simulations. That is, the cell is assumed to reach the static equilibrium in an infinitesimal short time after the fluid pressure exerted on it and vice versa for the flow field due to the cell deformation. In other words, both fluid and solid moves simultaneously and equally at the interface, which thus allows interactive computations between fluid and solid domains.

With the assumptions of constant volume and elastic deformation for both the membrane and cytoplasm, the constitutive law can be derived based on the hyperelasticity theory. Here we have to point out that although the two major assumptions are based upon “sufficiently small deformation”, it does not mean a “linear elastic” material behavior. In reality, most cells behave like nonlinear elastic materials (*Barthes-Biesel et al, 1985*). Therefore, a nonlinear constitutive law is necessary to describe the mechanical behaviors of cells and its corresponding mathematical formula will be introduced in the next section.

5.3 THE HYPRETELASTIC SOLID MODEL OF CELLS

Hyperelasticity refers to materials that are able to recover completely from a finite deformation to their undeformed status. Many polymers fall into this category. The constitutive law (stresses-strain relation) for these materials can be derived from a strain energy density function. Among various forms of the function, the Mooney-Rivlin law has been most prevalingly applied in the molding of cell deformation (Skalak, 1973; Lardner *et al*, 1978; Barthes-Biesel *et al*, 2002).

Denoting the strain energy density function as W , the constitutive relation can be determined by the following

$$S_{ij} = \frac{\partial W}{\partial E_{ij}} = 2 \frac{\partial W}{\partial C_{ij}} \quad (5.1)$$

where S_{ij} is the second Piola-Kirchhoff stress tensor, E_{ij} and C_{ij} are, respectively, the Lagrange and Cauchy-Green strain tensors. The second equality is due to the relation between the Lagrange and Cauchy-Green strain tensor $E_{ij} = \frac{1}{2} (C_{ij} - \delta_{ij})$ where δ_{ij} is the Kronecker-delta. Furthermore, the kinematic (strain-displacement) relation for Cauchy-Green strain is

$$C_{ij} = f_{ik} f_{kj} \quad (5.2)$$

where $f_{ij} = \partial x_i / \partial X_j$ is the deformation gradient, and x_i , X_j , are components of the position vectors, in direction i and j , of a point in the deformed and undeformed shape respectively.

For a given W , equations (5.1) and (5.2) together determine a completed set of constitutive law which directly links the stresses to displacements for solving the equilibrium equations. In the case of Mooney-Rivlin, the special functional form used in our study is

$$W = a_{10}(\bar{I}_1 - 3) + a_{01}(\bar{I}_2 - 3) + \frac{\kappa}{2}(\bar{I}_3^2 - 1)^2 \quad (5.3)$$

where $\bar{I}_i$ are the reduced strain invariants defined as

$$\bar{I}_1 = I_1 I_3^{-1/3}, \quad \bar{I}_2 = I_2 I_3^{-2/3}, \quad \bar{I}_3 = I_3^{1/2} \quad (5.4)$$

with I_i , the invariants of the Cauchy-Green strain tensor are given as $I_1 = C_{ii}$,

$I_2 = \frac{1}{2}(I_1^2 - C_{ij}C_{ij})$ and $I_3 = \det(C_{ij})$. The parameters a_{10} , a_{01} and κ are material

constants and the relation among them is

$$\kappa = \frac{2(a_{10} + a_{01})}{(1 - 2\nu)} = \frac{E}{3(1 - 2\nu)} \quad (5.5)$$

where ν is the Poisson's ratio. Since the third invariant represents the volume change,

κ is the bulk modulus and accordingly $6(a_{10} + a_{01})$ is equal to the Young's modulus.

For incompressible materials, the third term in Eq. 5.3 would vanish because I_3 equals to one.

The second Piolla-Kirchoff stress defined in equation (5.1), is algebraically related to the Cauchy stress by the deformation gradient as

$$S_{ij} = (f_{ik})^{-1} \sigma_{kl} (f_{lj})^{-T} \quad (5.6)$$

where the superscript -1 and T represent the inverse and transpose operations on a tensor respectively. Note that in equation (5.6), the material density is considered to be a constant throughout the deformation.

5.4 NUMERICAL ANALYSIS

5.4.1 Numerical Setup for Finite Element Analysis

The interaction between the flow and the vesicle deformation is simulated by the commercial finite element software ANSYS 6.1. Using its macro language APDL, we

can control the process of simulations for fluid and solid in alternative sequence with specifying iteration limit and numerical convergence. Element types used in this analysis are FLUID141 for fluid and HYPER56 for solid, respectively. Both are four-node quadrilateral elements which are suitable for axisymmetric problems. Assuming the fluid is water at room temperature, our analysis therefore can focus on the properties of solid (vesicle).

In the sequential analysis of fluid-solid interaction, it must be noted that the geometry of interface between two domains changes at the end of every iteration due to the deformation of solid. This change inevitably leads to a requirement of re-meshing in the fluid or the solid domain. Therefore, it is very critical to update the position of interface in the beginning of very new iteration. The iteration between two domains will perform until the numerical convergence is satisfied. For the numerical efficiency, re-meshing is limited to the area surrounding the fluid-solid interface and its size is determined empirically.

Up to this point, no address has been made yet on how to fix a vesicle, which is subjected to a fluid flow. The vesicle has to be trapped in some ways to against the fluid flow for experimental measurements. One of the common techniques for performing such a task is the optical laser tweezers, as shown in Figure 5.2 (a), which use laser to trap particles in a liquid. Through the mechanism of focusing in different wavelengths, the particle can be trapped by the momentum of photons. The area of focus on a particle is typically very small and determined by several factors such as the wavelength of laser, refractive indices of particle and fluid, and intensity of the laser beam (power). For example, the area is about 87 nm in radius for laser wavelength 709 nm by the LaserTweezers[®] from Cell Robotics International Inc. This size is used in all our analysis. Furthermore, Figure 5.2(b) schematically elucidates how the vesicle spontaneously aligns itself with the axisymmetric axis, if an arbitrary trapping point is

applied. Such a phenomenon has been observed in the earlier experimental studies of the laser-trapped vesicles in liquid medium (*Foo et al, 2003; Foo et al, 2004*). Therefore, in steady state a small trapping area has to be on the line of y-axis to fix the vesicle. If the fixed point is located on the stagnation point, the largest vesicle deformation will occur.

5.4.2 Parameters for Analysis

In order to investigate the effects of various material properties of the vesicle systematically, we introduce a few parameters to reduce the overall number of simulations. Since the fluid is assumed to be water, the only variable for the fluid flow is its velocity which we take the value between 0~100 $\mu\text{m/s}$, falling in the controlled capability of the optical laser tweezers (*Foo et al, 2003; Foo et al, 2004*). For the solids, there are totally eight material properties of the two solid parts (Poisson's ratio, Young's modulus, a_{10} and a_{01} from the Mooney-Rivlin model for each). Throughout this study, the values of Poisson's ratio are taken as 0.499 for both membrane and cytoplasm to avoid the numerical singularity. Apparently, as far as the solid cellular model used in this study is concerned, the cellular deformation is naturally more sensitive to the properties of the cytoplasm. The reasons lie in two aspects. One is that the premise of volume constant is a very strong constraint whose effect on cellular mechanical behaviors (deformation) may overwhelm that of the material properties of cell themselves. On the other hand, the cytoplasm occupies most space of a cell, so the change of its stiffness will also greatly dominate the mechanical behaviors of cell/vesicle. However, for blood cells and their biomimic counterparts (vesicles or liposome), the conventional belief is that membrane keeps the cell as a whole by taking most external loads. So, here we treat the membrane stiffer than cytoplasm. The values

of Young's modulus range from 2×10^3 to 3×10^5 Pa for the membrane and 2×10^1 to 2×10^2 Pa for the cytoplasm. These values cover a wide range of data obtained by either experimental measurements or theoretical models in various literatures (*Ragsdale et al, 1997; Rotsch et al, 1999; Hofmann et al, 1997; Gibson et al, 1982*). Actually, it would pose difficulties for the numerical convergence if the cytoplasm has a very low Young's modulus (c.a. 10 Pa). On the other hand, if the lower bound of cytoplasm stiffness can be estimated by numerical tests, then the difficulties in numerical computation can be avoided in the analysis of cell deformation with cytoplasm much softer than the membrane. Therefore, the lower bound is served as an indicator for the limit of numerical algorithm and constitutive law as well.

Regarding the values of the two coefficients a_{10} and a_{01} from the Mooney-Rivlin constitutive law, there are no demonstrative experiments in literature to provide substantial data for cells. Thus, a new parameter is introduced as

$$\beta_i = \left(\frac{a_{01}}{a_{10}} \right)_i \quad (5.7)$$

where the subscript i denotes the membrane as the material 1 and cytoplasm as material 2. Along with equation (5.5), these two coefficients can be expressed in terms of the Young's modulus and β as:

$$a_{10} = \frac{E}{6(1 + \beta)}, \quad a_{01} = \frac{E\beta}{6(1 + \beta)} \quad (5.8)$$

The value of β is typically less than 1 because the first invariant I_1 usually dominates over I_2 for many materials. As an example, the value of β in the literature (*Liu et al, 1996*) of the biomimetic microcapsule membrane was taken as 0.1. Considering both the effectiveness and extensiveness for our investigation, the range of β is chosen from ~ 0 to 0.5.

There are two ways to characterize the extent of deformation. One is the Taylor deformation parameter D_{12} , used by Chang and Olbricht (*Chang et al, 1993*) and De Hass et al. (*De Hass et al, 1997*), defined as:

$$D_{12} = \frac{L - D}{L + D} \quad (5.9)$$

where L and D are the major (y) and minor (x) axes of an ellipsoidal deformed cell whose original shape is a sphere. However, since most deformed shape cannot be deemed as an ellipsoid, this parameter can only be used for a qualitative measure of the deformation ratio between the two directions. Another parameter, the stretch ratio, defined in the following is more suitable for the current problem

$$\eta = \frac{\delta L}{L_0} \quad (5.10)$$

where L_0 is the characteristic length of the undeformed shape (the diameter of the undeformed vesicle) and δL is the maximum displacement along the major (y) axis of the vesicle. Generally, a common convention for using equations (5.9) and (5.10) is that the major axis is not only perpendicular to the minor axis but also coincident with the free flow direction.

5.5 NUMERICAL RESULTS

5.5.1 Benchmark Check for the Size of Computational Domain

As shown in Figure 5.1, the width and height of fluid domain are taken to be 25 and 50 times the diameter of vesicle. The optimal size of computational domain (fluid) should be “large” enough to avoid any effects due to this finite boundary but “small” enough to cope with the time of computation. To verify this, we carry out the analysis on the case of flow over a rigid sphere at first. Results are then compared with the analytical solution as shown in Table 5.1. Note that the three different flow velocities

were arbitrarily chosen below $100\mu\text{m/s}$ for the simulations and the results for drag coefficients (c.f. Eq. (5.12)) are all in a good agreement with the analytical solutions. Further checking on the distribution of normal pressure and wall shear stress along the circumferential direction is shown in Figure 5.3. The comparison of numerical to analytical calculations for velocity $V_\infty = 86\mu\text{m/s}$ shows only minor deviations. Thus in principle the preset computational domain yields reasonably accurate solutions and can be applied for our further analysis. The calculations of the drag coefficient for numerical solutions were done in the following. We first calculate the drag force as

$$f_d = \int_S (P \cos \phi - \tau \sin \phi) dS \quad (5.11)$$

where P is the pressure, τ is the wall shear stress and S is the surface area of vesicle. For axisymmetry, a differential area is the arc length between finite element nodes of the deformed shape times 2π . The integration is then computed by summation of all segments between nodes with the angle ϕ , pressure and wall shear stress at the corresponding nodes. For a non-dimensional presentation, the drag coefficient is introduced as

$$C_d = \frac{f_d}{\left(\frac{\rho V_\infty^2 A}{2}\right)} \quad (5.12)$$

where A is the frontal area of the undeformed vesicle.

5.5.2 Numerical Results for the Vesicle

There are dozens of solutions from the finite element analysis due to various combinations of Young's modulus (E_1, E_2) and Mooney-Rivlin constants (β_1, β_2) under different flow conditions. For illustrations, only the cases of low stiffness, i.e., $E_1 = 2000\text{Pa}$, $E_2 = 20\text{Pa}$, $\beta_1 = \beta_2 = 0.1$ and $\nu_1 = \nu_2 = 0.499$ at various flow velocities,

are presented, as listed in Table 5.2. Figure 5.4 and Figure 5.5 show the deformed shape is like a droplet due to the small area fixed by laser trapping. It can be found from the figure that most part of the vesicle is translated downstream by the flow instead of the deformation. This can be further verified from the distribution of stress (Von Mises) shown in Figure 5.6 where a zoom in around the trapping area is presented. Since the Young's modulus of membrane in this case is 100 times stiffer than the cytoplasm (2000 Pa/20 Pa), the membrane inevitably takes most of the pressure loads from the external flow. Figure 5.6 also reveals important information that the deformation mechanism in membrane changes from bending to stretching along the circumferential direction away from the trapping area. A close analogy to this phenomenon can be interpreted by the bending of a cantilever beam, as shown in Figure 5.6. Nevertheless, a relatively large bending force might influence the local biological process through the lipid bi-layer membrane because the structure of such membrane is usually weaker in bending than in stretching (Skalak[#], 1973; Waugh *et al*, 1995).

5.5.3 Numerical Results from the Fluid Field

The pressure distribution in the flow field is shown in the Figure 5.7 where the zone of negative pressure is larger than the case of rigid sphere. The anti-symmetrical pattern of pressure with respect to x-axis is broken due to the deformation of the vesicle. Figure 5.8a and 5.8b shows the numerical values of pressure and wall shear distribution for different Young's modulus of membrane (E_1) in details. We notice that the zero pressure point is shifted slightly downstream ($<90^\circ$) if the stiffness of membrane is reduced. This can be understood in that the vesicle with less stiff membrane is easier to streamline its deformed shape. However, by looking into the region very near the stagnation point, we found a peak increase in pressure as the Young's modulus E_1

reduced. For the cases of $E_1 = 2000 \text{ Pa}$, the pressure gradient flips between negative and positive over a very small region ($<10^\circ$) because of the very sharp peak of pressure at 0° . This phenomenon is even pronounced in the distribution of wall shear stress where a transition from smooth curves to a sharp peak can be seen when E_1 reduced from 10^5 Pa to 2000 Pa . This small region actually coincides with the bending-moment dominating area as shown in Figure 5.6 from a zoom-in check. It implies the possibility of a locally unstable flow due to the deformed vesicle near the stagnation point by laser trapping.

5.6 DISCUSSION

5.6.1 Deformation and Drag Force

To evaluate overall numerical results, three parameters introduced earlier in equations (5.9), (5.10) and (5.12) were used to quantify the system behavior. These parameters are, the Taylor deformation parameter (D_{12}), stretch ratio (η) and the drag coefficient (C_d). We first study the influence of different material properties of membrane and cytoplasm. Figure 5.9 shows the results of deformation and drag coefficient for different combinations of Young's moduli. Note that the lowest attainable stiffness of the membrane and cytoplasm to yield numerical solutions are 2000 Pa and 20 Pa respectively. The right-hand side of the figure is the cases of fixed E_2 (cytoplasm, 20 Pa) while increasing E_1 (membrane) and vice versa, the left-hand side is the cases by increasing E_2 . For both sides, there is a consistent trend for the deformation (D_{12} , η), i.e.; the vesicle deforms less as the membrane or cytoplasm becomes stiffer. The drag coefficient (C_d) nevertheless changes the other way around because a stiffer vesicle would cause a larger drag force in the flow field, but more

scattering are found from the results. The relative large degree of scatter is possibly caused by the numerical technique. In iteration processing of numerical calculation, the fluid field and the solid material field will be re-meshed to adapt for the deformed interface of fluid and solid. Even for the case with identical initial input parameters, the mesh grids are somewhat different for two times of repeats. This will cause the minor fluctuations of numerical results (wall shear distribution, pressure distribution, and drag force).

Since the deformation is directly affected by the flow velocity, an investigation on the drag coefficient of the deformed vesicle for different Reynolds numbers is necessary. As shown in Figure 5.10, the comparison between the numerical and analytical solutions clearly demonstrates that the deformation of vesicle is significant locally but negligible in the overall flow field. Table 5.2 shows the deviation of drag coefficient from the analytical solution of a rigid vesicle is around +3.2% lower than +3.5% in Table 5.1. This directly results from the effect of streamlining a deformable vesicle by the flow.

From the results of dozens of numerical simulation cases of different values of E_1 and E_2 in the selected calculation domain, it can be found that when the Young's modulus (E_1) of membrane is in the range of 2000~3000 Pa and the Young's modulus (E_2) of cytoplasm approaches to the lower limit (20 Pa) of the selected domain, the deformed shape of a locally optically trapped cell/vesicle is much like a droplet, as shown in Figure 5.4 and Figure 5.5. This numerical graph is somewhat similar to the pictures taken in Hochmuth and Mohandas' experimental work (*Hochmuth, et al, 1972*), which is shown as (c) in Appendix V. In addition, the value of Young's modulus (E_1) of membrane is very close to the estimated one by Hochmuth and Mohandas with a simple elastic analysis.

5.6.2 Influence of Mooney-Rivlin Coefficients

With the parameter β_i defined in equation (5.8) and Young's modulus, we can study the effects of Mooney-Rivlin coefficients a_{10} and a_{01} without their numerical values. Their influence on the deformation of a vesicle is illustrated in Figure 5.11 with flow $V_\infty = 86 \mu\text{m/s}$. Using the stretch ratio (η) as an overall deformation index, we find β_2 has a dominant influence on the deformation of the vesicle. For a fixed value β_2 , the change of deformation is nearly a constant throughout the range of β_1 . This is due to the overwhelming volume fraction of cytoplasm inside a cell. However, we should note that Figure 5.11 is for cases of very low stiffness of membrane ($E_1 = 2000 \text{ Pa}$). As the membrane becomes stiffer, the β_2 will lose its dominance on the part of non-linear deformation.

5.7 CONCLUSION

The interaction of a biomimetic vesicle and fluid flow in a laser trap is studied numerically by the finite element method. Using the Mooney-Rivlin law for the solid vesicle, a flow-induced nonlinear deformation in the steady state can be simulated. The numerical study reveals several important features in this system:

1. Most part of the vesicle in a flow field is translated downstream instead of deforming. In other words, the deformation is highly localized near the region of laser trapping.
2. Due to the highly localized deformation, a peak increase of pressure and thus negative pressure gradient can be found near the laser-trapped region if the stiffness of membrane is low enough. It implies the possibility of a locally unstable flow due to the deformation.

-
3. Stress analysis reveals that the membrane takes almost all the pressure loads from the external flow if it is relatively stiffer than cytoplasm. The deformation mechanism in membrane can change from bending to stretching along the circumferential direction away from the trapping area. Typically, a relatively large bending force might influence the local biological process through the lipid bi-layer membrane because the structure of such membrane is usually weaker in bending than in stretching.
 4. The drag coefficient can be used to evaluate the overall numerical results. A comparison between the numerical and analytical solutions for the different values of Reynolds number confirms that the deformation of vesicle is significant only in the local region.
 5. With the parameter β_i defined in equation (5.8), the effects of Mooney-Rivlin coefficients can be studied systematically. The β_2 of cytoplasm has a dominated influence on the part of non-linear deformation of vesicle if the stiffness of membrane is low.
 6. The estimated value of Young's modulus (E_1) of membrane is reasonably in the order of 2000~3000 Pa, which is fairly in accordance with Hochmuth and Mohandas' results.

Table 5.1 Comparison between the numerical and analytical solutions of fluid flow over a deformable vesicle for the finite domain shown in Figure 5.1. ($a=3.2\mu\text{m}$ is the radius of undeformed vesicle)

Flow velocity ($\mu\text{m/s}$)	Reynolds number $\text{Re} = \frac{2\rho V_\infty a}{\mu}$	Drag coefficient $C_d = 6\pi\mu a V_\infty \bigg/ \frac{\rho V_\infty^2}{2} A$	Numerical results	Deviation (%)
35	2.240E-04	1.0714E+05	1.1089E+05	+3.50
63	4.032E-04	5.9524E+04	6.1606E+04	+3.50
86	5.504E-04	4.3605E+04	4.5130E+04	+3.50

Table 5.2 Comparisons of the numerical solutions for a deformable vesicle with the analytical solution for a deformable vesicle. The material properties are $E_1 = 2000\text{Pa}$, $E_2 = 20\text{Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$

Re	Drag coefficient (C_d)		Deviation (%)
	Analytical solution rigid vesicle ($24/\text{Re}$)	Numerical solution of deformable vesicle	
1.280E-04	1.8750E+05	1.9359E+05	3.25%
1.792E-04	1.3393E+05	1.3828E+05	3.25%
2.240E-04	1.0714E+05	1.1054E+05	3.17%
2.560E-04	9.3750E+04	9.6726E+04	3.17%
2.880E-04	8.3333E+04	8.6010E+04	3.21%
3.200E-04	7.5000E+04	7.7392E+04	3.19%
3.584E-04	6.6964E+04	6.9104E+04	3.19%
4.032E-04	5.9524E+04	6.1417E+04	3.18%
4.480E-04	5.3571E+04	5.5272E+04	3.17%
4.992E-04	4.8077E+04	4.9605E+04	3.18%
5.504E-04	4.3605E+04	4.5009E+04	3.22%

Figure 5.1. The geometry and finite element mesh of a vesicle in the Stokes' flow. The axisymmetry is in the y-direction and the thickness of membrane around the vesicle is 5 nm.

Figure 5.2 (a) Schematic representation of the laser trapping for a spherical vesicle. (b) If the trapping point is not in line with the center along with the axis of axisymmetry then the flow will push it to a new equilibrium position.

Figure 5.3 Comparison between the numerical and analytical solutions of normal pressure and wall shear stress for fluid flow over a rigid vesicle at $V_{\infty} = 86 \mu\text{m/s}$.

Figure 5.4 Displacement field of vesicle for $V_\infty = 86 \mu\text{m/s}$. The material properties are

$E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Fig.5.5 the contour distribution of strain in Y direction, $V_{\infty}=86\mu\text{m/s}$, $Y_1=2000\text{Pa}$, $Y_2=20\text{Pa}$, $v_1=v_2=0.499$, $\beta_1=\beta_2=0.1$

Figure 5.6 Von Mises stress distribution of the vesicle for $V_{\infty} = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000 \text{ Pa}$, $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Figure 5.7 Pressure distribution in the flow (water at room temperature) with $V_\infty = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000\text{Pa}$, $E_2 = 20\text{Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Figure 5.8a Pressure distribution of the vesicle at $V_\infty = 86 \mu\text{m/s}$ for different E_1 . The material properties are $E_2 = 20 \text{ Pa}$, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Figure 5.8b Wall shear distribution of the vesicle at $V_\infty = 86 \mu\text{m/s}$ for different E_1 . The material properties are $E_2 = 20$ Pa, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Figure 5.9 The Taylor deformation parameter (D_{12}), stretch ratio (η) and drag coefficient (C_d) vs. different combinations of Young's moduli of membrane and cytoplasm. ($\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$).

Figure 5.10 Comparisons of the numerical solutions for deformable and rigid vesicle with the analytical solution. The material properties are $E_1 = 2000$ Pa, $E_2 = 20$ Pa, $\nu_1 = \nu_2 = 0.499$ and $\beta_1 = \beta_2 = 0.1$.

Figure 5.11 The stretch ratio (η) as a function of β_1 and β_2 at $V_\infty = 86 \mu\text{m/s}$. The material properties are $E_1 = 2000\text{Pa}$, $E_2 = 20\text{Pa}$ and $\nu_1 = \nu_2 = 0.499$.

CHAPTER 6 CONCLUSIONS

6.1 CONCLUSIONS

6.1.1 General Summary

In this dissertation, the RBC deformability and membrane mechanical properties were investigated numerically and experimentally. For the experimental part, deformation of RBCs by optical laser under different loading conditions (radiation pressure, laser stretching and trapping in fluid flow) was studied respectively. Quantitative results were obtained for the relation between loading and deformation.

In the numerical side, different physical models were introduced to simulate the experimental results. In chapter 2, a physical model was proposed to analyze RBC deformation in Guck's experimental work by the optical stretcher. In chapter 3 and 4, RBC deformation in the experimental work by the optical tweezers were modeled and analyzed by two different mechanical models.

In order to make a decent quantitative comparison between the experimental data and numerical results, a new approach based upon the method of optimization was proposed. Through such a comparison, a reasonable range of the mechanical properties, either or both of shear stiffness and bending stiffness of RBC membrane, can be predicted. Different physical models indeed yield different predictions. The validity of the results was also assessed by the literature surveys for the mechanical properties of RBCs that were studied by different experimental techniques other than the optical traps. Their discrepancies are minor and the magnitude of values is in the same order. Thus, both the experimental and numerical work in this dissertation has the characters of reliability and reproducibility.

Finally, the interaction between cell/vesicle and fluid flow was simulated by the finite element method. The mechanical behaviors and deformation of an optically trapped cell/vesicle in fluid flow were analyzed by FEA with a 3D two-parameter hyperelastic material model; and the fluid field (velocity, pressure and drag force) around the deformed cell/vesicle was also numerically investigated. Furthermore, with a detailed parametric study on the 3D hyperelastic model, the Young's modulus of the membrane can be given a reasonable prediction on its magnitude.

Various results from different parts of this dissertation are summarized in the Table 6.1 as a concluding remark and reference as well.

6.1.2 Contributions

The novelties or contributions of this research work are outlined as below.

- 1) The force-strain response of erythrocytes was quantitatively assessed through optical trapping techniques. Numerical results of mechanical modeling are closely similar to the experimental observations.
- 2) An optimization method was firstly used to correlate the experimental data and numerical results so that reasonable values of the RBC membrane material properties (shear stiffness or bending stiffness, or both) can be predicted.
- 3) It was the first time that RBC membrane material properties were fairly precisely predicted by analyzing the experimental results of deformed RBCs trapped in the optical stretcher with the mechanical model in this thesis.
- 4) The fluid-vesicle interaction by optical trapping was first investigated by FEA. The numerical results show that the deformation of an optically trapped cell/vesicle in Stoke's flow (water) is caused by the combined effects of the optical trap. A reasonable prediction was given to the Young's modulus of RBC membrane

through a detailed parametric study on the 3D hyperelastic modeling by numerical tests.

6.2 RECOMMENDATIONS

The force capability of optical trapping in the experimental setup in this dissertation is from zero to tens of piconewtons (up to 100 pN). This will limit the measurement scope of RBC deformation. Since the experimental setup does not have the function of time measurement on the order of microsecond, the viscous effect of RBC membrane in stretching experiments cannot be detected. The prediction on RBC membrane material properties is strongly dependent on the physical models. The various models define different material properties. In some cases, these material properties are not comparable to each other.

These limitations can give some guidance for the further research work in the area of cell mechanics by considering possible improvements in both experiment design and physical modeling.

Table 6.1 A Summary of Predicted Mechanical Properties of Erythrocyte Membrane in this Dissertation

	Pamplona & Calladine's constitutive law	Evans & Skalak's constitutive law	Mooney-Rivlin
Radiation Pressure by optical stretching (Chap. 2)	—	Shear stiffness: 4.611×10^{-6} N/m Bending stiffness : $4.517 \times 10^{-18} \sim 1.506 \times 10^{-19}$ N·m	—
Optical stretching by optical tweezers (Chap. 3 & 4)	Shear stiffness: $4.2719 \sim 5.2537 \times 10^{-6}$ N/m Bending stiffness: $1.442 \times 10^{-18} \sim 9.573 \times 10^{-20}$ N·m	Shear stiffness: $2.3425 \sim 4.2893 \times 10^{-6}$ N/m Bending stiffness : $5.889 \times 10^{-19} \sim 2.422 \times 10^{-20}$ N·m	—
Optical trap in fluid flow (Chap. 5)	—	—	Young's modulus: 2000~3000 Pa

Appendix I-1

The following experimental pictures (a) and (b) come from Hotani's work, cited by Pamplona et al for their mechanical model establishment. (Pamplona, et al, 1996)

(a) A general view, showing two-, three- and four-lobed liposome shapes; the scale bar represents 25 μ m

(b) A sequence of pictures, spaced by 1/25 sec, showing stages in the development of a particular three-lobed conformation

Appendix I-2 Optical Tweezers

Introduction

Optical Tweezers are a technique that is built upon the principle that small particles can be trapped in the waist of a strongly focused laser beam. The trapping results from the fact that objects that are trapped in the focus of the laser beam experience a restoring force if they try to leave the high intensity volume.

Optical Tweezers can therefore be used for actively repositioning small objects with a precision that is better than the diffraction limit of modern optical microscopes. The technique works for a variety of micrometer-sized "objects", including living cells and bacteria.

Laser physics range a large field of science. A subfield within laser physics is optical trapping and an optical tweezers is an example of an optical trap.

A strongly focused laser beam has the ability to catch and hold particles (of dielectric material) in a size range from nm to μm . This technique makes it possible to study and manipulate particles like atoms, molecules (even large) and small dielectric spheres. A review on uses of optical tweezers can be found in (*A. Ashkin "Optical trapping and manipulation of neutral particles using lasers", Proc. Natl. Acad. Sci. USA, vol. 94, pp. 4853-4860, May 1997*).

Principle of Physics

Light has the ability to produce radiation pressure. When light is absorbed, reflected or refracted by dielectric material, tiny forces on the order of piconewtons are generated. For reference, several milliwatts of power corresponding to a bright laser beam gives

only a few piconewtons. However, a force of 10pN is sufficient to pull a bacterium such as E Coli through water ten times faster than it can swim. The force is proportional to the power of the laser.

Here is a ray optic representation of the gradient force valid for particles larger than the wavelength of the laser light. Parallel rays enter a small, refractile sphere from above and are bent because the sphere acts like a lens. Before entering the rays travel vertically with zero horizontal momentum. After deflection however, they pick up horizontal momentum. Since momentum is conserved, an equal and opposite momentum change is conveyed to the sphere. If the beam were uniform, the reaction forces would cancel and there would be no net sideways component. In a gradient however, the asymmetry in the light gives rise to an imbalance in the reaction forces and the object is pulled towards the brighter side. Near a diffraction-limited spot (i.e. for objects smaller than the wavelength of the laser light), however, the ray-optic picture breaks down and accurate calculations of the trapping forces on small particles become quite complex, beyond the scope of this discussion. Essentially, though, as light passes through polarizable material, it induces fluctuating dipoles, which then interact with the electromagnetic field gradient.

The following three diagrams describe the forces that can trap the particle. When light is scattered by an object, there is a scattering force which tends to push objects along the direction of light propagation, so in order to make an optical trap, the steepest possible light gradients are needed to ensure that the gradient force overcomes the scattering force, which is not necessarily in the direction of the light gradient. A light

Appendix I-2

gradient can be formed near any focus... the sharper the focus, the steeper the gradient.

A sufficiently steep three-dimensional light gradient can be achieved by focusing laser light to a diameter on the order of the laser wavelength.

To summarize, optical tweezers work by means of the gradient force of light. A sharp focal point is created using a laser, and when a small particle comes into contact with the focus it remains trapped. The particle is pulled towards the more intense light because it has a greater index of refraction than the surrounding medium, and momentum must be conserved. Optical tweezers have many uses, and the list is growing. Objects ranging from tens of nanometers to many micrometers in size can be manipulated by optical tweezers with laser powers ranging from several milliwatts up to several watts depending on the size of the object and the amount of radiation it can withstand.

If the object is placed below the center of the focus, the resulting force of the trap will act in the upward direction.

If the object is placed above the center of the focus, the resulting force of the trap will act in the upward direction.

If the object is placed to the right of the focus, the resulting force of the trap forces the object towards the center of the trap.

Appendix II

Following experimental pictures and graphs come from Guck's work. (Guck et al, 2000; Guck et al, 2001)

(a) Experimental images of a spherically swollen RBC trapped in the optical stretcher for increasing laser powers, $R_0=3.13\mu\text{m}$ and $W_0/R_0\approx 1.1$

(b) Surface stress profiles on the surface of a RBC trapped in the optical stretcher for $W_0/R_0=1.1$ between the laser beam waist radius W_0 and the cell radius R_0 ; and the peak stress $\sigma_0=1.38\text{ Pa}$ in this case

(c) The experimental data plots (symbols) of the longitudinal strain ϵ_l and the transverse strain ϵ_t against the peak stress σ_0 ; the solid line is a linear fitting curve from a pure linear elastic membrane theory.

APPENDIX III PROCEDURES OF ERYTHROCYTE STRETCHING EXPERIMENT

1 Red Blood Cell Preparation

The blood samples are collected from a mammalian source. Fresh blood was drawn from experimental white rats and stored in acid citrate dextrose (Sigma C3821) on ice or at 4°C in refrigerator. The fractionation and washing of red blood cells are directly conducted in centrifuge tubes.

- (1) A 100µl aliquot of original blood sample ($0.6\sim1.2\times10^7$ RBCs/µl) is transferred to a Sorvall SM-24 centrifuge tube by micropipette and diluted in 10ml of 150 mM PBS buffer solution (Sigma P4417).
- (2) Mix the sample thoroughly with buffer to begin the process of washing the red blood cells free of plasma proteins. An effective means of mixing is trituration. Trituration refers to repeatedly pulling liquid into a pipet and ejecting it, while keeping the tip immersed.
- (3) The diluted RBC suspension should be centrifuged immediately following trituration. Centrifugation at 600 xg brings down the red cells quickly. Ten minutes is more enough time to separate red cell pellet from dilute plasma supernatant.
- (4) After removing an aliquot of supernatant, the remaining liquid should be discarded and the pellet resuspended in isotonic buffer (to the 10 ml mark) by trituration, then re-centrifuged without spillage during centrifugation.
- (5) After two "spins," the buffy layer containing white blood cells should be lost, and platelets will not have spun down as quickly as red cells, so the pellet should consist almost exclusively of red blood cells.

The reason that one volume of blood sample is diluted and washed in 1000

volumes of isotonic buffer solution is that the red blood cells must be free of any adsorbed protein in order to guarantee the adhesion between RBCs and beads. Therefore, the RBCs have to be washed several times in a relative “huge” volume of PBS buffer without any protein (like bovine serum albumin). The washed, packed cells are diluted to $\sim 1 \times 10^5$ cells/ μl with 150mM PBS buffer solution, which will be used for incubation of RBCs and beads.

2 Microbead Preparations

Silica microbeads are purchased from Bangs Laboratories (Fishers, IN). The physical characteristics of Silica Beads are shown in Table 3.1.

There is a common view that microspheres must be washed before they can be used by the customer. In this experiment, a key point to the spontaneous adhesion between beads and RBCs is that the microbeads and RBCs surface must be very clean, especially free of any adsorbed protein. Centrifugation is the most commonly used, and perhaps easiest, cleaning method, for microspheres larger than 300 nm in diameter. Following is the procedure of microbead washing.

- (1) Draw 20 μl original solution of microbeads (2.737×10^{10} beads/ml) by micropipette and transfer it into an appropriate centrifuge tube containing a certain amount (10ml) of ultrapure (deionized) water;
- (2) Centrifuge the microspheres at the appropriate G forces (1200 for larger than 800nm in diameter) for 15 minutes to clear the supernatant;
- (3) Remove and discard supernatant with transferring pipette after settling;
- (4) Re-trickle 10ml of deionized water into the centrifuge tube containing settled beads and shake up; repeat steps (2) and (3) two times;

Appendix III

-
- (5) After 3 times washing, the washed, packed beads are diluted to $\sim 2 \times 10^5$ beads/ μl with 2.5ml deionized water, which will be used for incubation of RBCs and beads.
 - (6) A small amount sample of the washed and diluted microbeads suspension should be drawn to observe in light microscope to see whether aggregation of beads takes place. Aggregation of microspheres may be caused in certain environments.
 - (7) If aggregation is found, sonication is a good method to break aggregation and following measures can be taken. Suspend the vial or tube containing the silica suspension in a sonic bath. Better sonication is achieved if the vessel containing the suspension is held above the floor of the sonic bath with a clamp, rather than resting on the bottom. The bath must also be filled to the proper level, which depends on the model.
 - (8) Sonicate for approximately 10 minutes, and then confirm that the microspheres are dispersed by viewing it in a light microscope. If clumps are visible, sonicate again for 10 minutes. Continue with 10 minute cycles until the microspheres are completely dispersed.

3 Incubation for the Adhesion between RBCs and Microbeads

In this experiment, the beads attached to the surface of a red blood cell will be trapped by optical laser beam and used like handles to stretch the RBC membrane. Therefore, the adhesion between RBCs and beads is one of the key points that guarantee the success of this experiment. After many times of trials, adhesion between RBCs and beads can be accomplished by following below procedures.

- (1) A **20 μl aliquot** of diluted RBC suspension ($\sim 1 \times 10^5$ cells/ μl) obtained by following the procedures described in Section 1 is co-mixed with **20 μl** of silica bead suspension ($\sim 2 \times 10^5$ beads/ μl) obtained by following the procedures described in

Appendix III

Section 2. The suspension of mixture is incubated at 4°C for 1 hour, allowing spontaneous and nonspecific adhesion of microbeads to RBC membrane.

- (2) After 1 hour's incubation, draw a small amount (1~2 μ l) of the mixture suspension diluted by an appropriate amount of PBS buffer and observe it in light microscope to make sure that adhesion between RBCs and beads has taken place.
- (3) If adhesion is observed, the suspension of mixture in step (1) should be diluted to $\sim 1 \times 10^3$ cells/ μ l by hypotonic buffer solution (10mM potassium phosphate, pH7.4, 75mM NaCl, i.e., 155mOsm/kg) (Henon, *et al*, 1999) to allow RBC to swell a spherical shape, and a small amount (~ 1 mg/ml) of BSA (Sigma A4503) is added to this suspension. This step should be done about 10~20 minutes ahead of starting optical stretch experiment of RBC.

In case of the failure of adhesion, the most possible reason is that the RBCs and microbrads are not clean enough because of imperfect washing. All procedures described in sections (1) ~ (3) should be repeated carefully. For RBCs, the centrifugation force cannot be too high; 600 x g is appropriate during washing. For silica beads, they should be carefully washed several times in ultrapure water. If it does not work, for instance, the beads should be washed in a "Piranha mixture" ($\text{H}_2\text{O}_2 + \text{H}_2\text{SO}_4$) and rinsed in DI water. But be careful that the beads can coagulate in an acid solution (and in a basic solution as well).

4 Optical tweezers

The optical tweezers used in this experiment is schematized in Figure.3.1 in Chapter 3. It is comparable to the one described by M. Dao *et al*. (Dao, *et al*, 2003). The whole equipment system was purchased from Cell Robotics Inc. in USA. The key component of this setup is a 1.5W diode pumped Nd: YAG laser source, which is

connected to an inverted microscope (Nikon optical microsystem). The laser beam with a wavelength of 1064nm is reflected by a dichroic mirror inside the microscope and focused on the observation point through an immersion objective (100X, 1.25 oil). To avoid possible damage to the cell, the wavelength of 1064nm was chosen to minimize water and hemoglobin absorption. Since the absorption coefficient for a 2mM hemoglobin solution is 0.2cm^{-1} for 1064nm wavelength (Svoboda, *et al*, 1994) and the estimated increment of local temperature for a red blood cell directly trapped in a 200mW laser beam focused over a $1\mu\text{m}^3$ region is about 3°C (Liu, *et al*, 1995), the laser beam was never directly focused on the cell, but on a bead attached on its surface. Thus, the actual heating of erythrocyte must be greatly decreased. In addition, the trap is located in the same plane as the observation plane of the microscope. At the converging waist of laser beam, the gradient force of electromagnetic field of laser beam is large enough to trap small dielectric objects.

5 Erythrocyte Stretching Experiments

After carefully preparations of RBCs and beads, and the incubation for adhesion, a sample suspension solution containing with spherically swollen erythrocytes bound with microbeads was transferred to the chamber assembled on the mobile platform. All observations and measurements were made at room temperature in laboratory environment ($T \approx 25^\circ\text{C}$).

- (1) Immediately before introducing the RBC sample solution, the assembled chamber should be incubated with a buffer containing BSA (100mg/ml); and in most experiments, the bottom coverglass of the chamber should also be silanized with silanization solution, i.e., 5% dichlorodime (Sigma, 85126) (Svoboda, *et al*, 1992).
- (2) After transferring, RBCs with microbeads in the sample suspension in the chamber

should be allowed to spontaneously settle down for 3~5 minutes to allow one of the beads bound on the surface of a RBC to stick to the bottom coverglass surface. However, the time of settling cannot be too long, otherwise two beads possibly both stick to the coverglass

- (3) After carefully observation, select a RBC bound with two microbeads nearly in the diametrical position. Among of them, one is stuck to the bottom coverglass, another is free to move.
- (4) Setting a certain value to the laser power, switch on the optical tweezers and trap the free microbead. Adjust the platform to move at quite slow speed controlled by computer; at the meantime, a CCD camera will take pictures of the deformed RBC during stretching at certain frequency per second. At certain critical point, the trapped bead will escape from optical trap. The shape of deformed RBC at this moment is the maximum corresponding to the present laser power.
- (5) Changing the laser power and repeating the above experimental processing, the pictures of different maximum deformation of a RBC corresponding to their individual values of laser power can be obtained. After image processing, the pictures of deformed cells can be quantitatively analyzed.

APPENDIX IV

Governing Equations Based on Pamplona and Calladine's Constitutive Law

Pamplona and Calladine (Pamplona, et al, 1993; Pamplona, et al, 1996) considered the phospholipid bi-layer as a shell structure at the continuum level and assumed no relative sliding between layers during deformation. Their constitutive model was adapted from the Love-Kirchoff hypothesis in the classic theory for thin-shells. In other words, the model accounts both the bending and shear effects of the phospholipid bi-layer. Based on such assumptions, several important mechanical mechanisms governing the deformation, including resultant tensile forces (force/length, T_s, T_θ), bending moments (force, M_s, M_θ) and transverse shear (force/length, Q_s, Q_θ) across the layer thickness for any infinitesimal element of a membrane are taken into account as shown in Fig. 4.1(c). For an axisymmetric liposome, the transverse shear is independent of θ . Moreover, the principal direction coincides with the circumferential (θ) and meridian (s or φ) directions.

With application of the constitutive relations (3.12), (3.13) mentioned in Chapter 3 and the relation (4.16) and with non-dimensionlization by the same definition of dimensionless variables in Chapter 4, different governing equations can be obtained.

Governing Equations:

$$\frac{dR^*}{ds^*} = \frac{\cos \varphi \sin s^*}{R^*} \quad (1)$$

$$\frac{dZ^*}{ds^*} = \frac{\sin \varphi \sin s^*}{R^*} \quad (2)$$

Appendix IV

$$\frac{d\varphi}{ds^*} = \frac{\kappa_s^* \sin s^*}{R^*} \quad (3)$$

$$\frac{d\kappa_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[-Q_s^* - \frac{\kappa_s^* \sin \varphi}{R^*} + \frac{\sin \varphi \cos \varphi}{R^{*2}} \right] \quad (4)$$

$$\frac{dQ_s^*}{ds^*} = \frac{\sin s^*}{R^*} \left[-\left(T^* + C \frac{\sin s^*}{R^*} \right) \kappa_s^* - \left(T^* + C \frac{R^*}{\sin s^*} \right) \frac{\sin \varphi}{R^*} - \frac{Q_s^* \cos \varphi}{R^*} \right] \quad (5)$$

$$\frac{dT^*}{ds^*} = \frac{\sin s^*}{R^*} \left[\kappa_s^* Q_s^* + C \left(\frac{R^*}{\sin s^*} - \frac{\sin s^*}{R^*} \right) \frac{\cos \varphi}{R^*} \right] - C \cdot \frac{\cos s^*}{R^*} + C \cdot \frac{\cos \varphi \sin^2 s^*}{R^{*3}} \quad (6)$$

$$\frac{dT}{ds} = \frac{\sin s}{R} \left[+C \left(\frac{R}{\sin s} - \frac{\sin s}{R} \right) \cdot \frac{\cos \varphi}{R} + \kappa \cdot Q \right] - C \cdot \frac{\cos s}{R} + C \cdot \frac{\cos \varphi \cdot \sin^2 s}{R^3} \quad (7)$$

Boundary Conditions:

With the same approach of analysis, following boundary conditions can be obtained.

At $s=s_0=\sin^{-1} \delta$

$$R^*(s_0^*) = \delta^* \quad (8)$$

$$Z^*(s_0^*) = 0 \quad (9)$$

$$\varphi(s_0^*) = \varphi_0 \quad (10)$$

$$\kappa_s^*(s_0^*) = \kappa_{s0}^* \quad (11)$$

$$Q_s^*(s_0^*) = Q_{s0}^* \quad (12)$$

$$T^*(s_0^*) = \frac{F^*}{2\pi\delta^* \sin \varphi_0} - \frac{Q_s^* \cos \varphi_0}{\sin \varphi_0} - C \quad (13)$$

and at $s^* = \frac{\pi}{2}$,

$$\varphi(\pi/2) = \pi/2 \quad (14)$$

$$Q_s^*(\pi/2) = 0 \quad (15)$$

$$T^*\left(\frac{\pi}{2}\right) = \frac{F^*}{2\pi R^*(\pi/2)} - C \frac{1}{R^*(\pi/2)} \quad (16)$$

APPENDIX V

The following pictures come from Hochmuth and Mohandas' work (Hochmuth, et al, 1972).

(a) Hochmuth and Mohandas's Experiment Setup, a Flow Channel

Appendix V

(b) RBC deformation in flow channel as line attachment

(c) RBC deformation in flow channel as point attachment

REFERENCES

- Ashkin, A., 1998, Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime, *Methods in cell biology*, Vol.55, 1
- Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E. and Chu S., 1986, Observation of a single-beam gradient trap for dielectric particles, *Optics Letters*, Vol.11, 288
- Ashkin, A., J. M. Dziedzic, 1987, "Optical Trapping and Manipulation of Viruses and Bacteria," *Science*, Vol.235, 1517
- Bakker Schut, T. C., Hesselink G., Grroth, B. G., Greve, J., 1991, Experimental and Theoretical Investigations on the Validity of the Geometrical Optics Model for Calculating the Satbility of optical Traps, *Cytometry*, Vol.12, 479
- Barthes-Biesel, D. and J. M. Rallison, 1981, Time dependent deformation of a capsule freely suspended in linear shear flow, *Journal of Fluid Mechanics*, Vol.113, 251
- Barthes-Biesel, D., Anna Diaz, and Emmanuelle Dhenin, 2002, Effect of constitutive laws for two-dimensional membrane on shear flow-induced capsule deformation, *Journal of Fluid Mechanics*, Vol.460, 211
- Barthes-Biesel, D., H. Sgaier, 1985, "Role of Membrane Viscosity in The Orientation and Deformation of A Spherical Capsule Suspended in Shear Flow," *Journal of Fluid Mechanics*, Vol.160, 119
- Bausch, A. R., Ziemann, F., Boulbitch, A. A., Jacobson, K. and Sackmann E., 1998, Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry, *Biophysical Journal*, Vol.75, 2038
- Bennett, V. and D. M. Gilligan, 1993, the spectrin-based membrane skeleton and micron-scale organization of the plasma membrane, *Annual Review Of Cell Biology*, Vol.9, 27
- Bozic, B., S. Svetian, and B. Zeks, 1997, Theoretical analysis of the formation of membrane microtubes on axially strained vesicles, *Physical Review E*, Vol.55, 5834
- Chang, K. S., 1993, Experimental studies of the deformation and breakup of a synthetic capsule in steady and unsteady simple shear flow, *Journal of Fluid Mechanics*, 250, 609
- Chang, K. S., Olbricht, W. L., 1993, Experimental Studies of the Deformation of a Synthetic Capsule in Extensional Flow, *Journal of Fluid Mechanics*, Vol.250, 587

References

-
- Chien, S. and L. A. Sung, 1990, Molecular basis of red blood cell membrane rheology, *Biorheology*, Vol.27, 327
- Christophe Queguiner, and D. Barthes-Biesel, 1997, Axisymmetrical motion of capsules through cylindrical channels, *Journal of Fluid Mechanics*, Vol.348, 349
- Chu, S., 1991, Laser manipulation of atoms and particles, *Science*, Vol.253, 861
- Cokelet, G. R., and H. J. Meiselman, 1968, Rheological comparison of hemoglobin solutions and erythrocyte suspensions, *Science*, Vol.162, 275
- Cranston, H. A., C. W. Boylan, G. L. Carroll, S. P., Sutura Williamson J. R., Gluzman I. Y., and Krogstad D. J., 1984, Plasmodium falciparum maturation abolishes physiologic red cell deformability, *Science*, Vol.223, 400
- Dao, M., C. T. Lim and S. Suresh, 2003, Mechanics of the Human Red Blood Cell Deformed by Optical Tweezers, *Journal of the Mechanics and Physics of Solids*, Vol.51, 2259
- De Hass, K. H., Blom, C., van den Ende, D., Duits, M. H. G., Mellema, J., 1997, Deformation of Giant Lipid Bilayer Vesicles in Shear Flow, *Physical Review E*, Vol.56 (6), 7132
- Denk, W., and W. W. Webb, 1990, Optical measurements of picometer displacement, *Applied Optics*, Vol.29, 2382
- Dharmadhikari, J. A., S. Roy, A. K. Dharmadhikari, S. Sharma, and D. Mathur, 2004, Torque-generating malaria-infected red blood cells in an optical trap, *Optics Express*, Vol.12, 1179
- Dong, C., X. X. Lei, 2000, "Biomechanics of Cell Rolling: Shear Flow, Cell-Surface Adhesion, and Cell Deformability," *J. Biomechanics*, Vol.33, 35
- Dvies, P. F., 1995, Flow-mediated endothelial mechanotransduction, *Physiological Reviews*, Vol. 75, 519
- Evans E. A. and Skalak R., 1980, Mechanics and Thermodynamics of Biomembranes, CRC Press, Boca Raton
- Evans, E. A., 1973, New Membrane Concept Applied to the Analysis of Fluid Shear- and Micropipette-Deformed Red Blood Cells, *Biophysical Journal*, Vol.13, 941
- Evans, E. A., Waugh, R. and Melnik, L., 1976, Elastic area compressibility modulus of red cell membrane, *Biophysical Journal*, Vol.16, 585

References

-
- Evans, E. and Young A., 1989, Apparent viscosity and cortical tensional of blood granulocytes determined by micropipette aspiration, *Biophysical Journal*, Vol.56, 151
- Flugge, W., 1973, *Stresses in Shells*, 2nd, p.324, Springer Verlag New York, Heidelberg, Berlin
- Foo, J. J., K. K. Liu, V. Chan, 2004, "Viscous Drag of Deformed Vesicles in Optical Trap: Experiments and Simulations," *American Institute of Chemical Engineers*, Vol. 50 (1), 249
- Foo, J. J., Liu, K. K. and Chan, V., 2003, Thermal effect on viscously-deformed liposome in a laser trap, *Annals of Biomedical Engineering*, Vol. 31 (3), 354
- Fung, Y. C. B., 1972, in: *Biomechanics: Its objectives and foundations*, 181, Prentice-hall, Englewood Cliffs, N. J.
- Fung, Y. C., 1993, *Biomechanics: Mechanical Properties of Living Tissues*, Springer-Verlag, New York
- Fung, Y. C., W. C. O. Tsang, P. Patitucci, 1981, High-resolution data in the geometry of red blood cells, *Biorheology*, Vol.18, 369
- Gibson, L. J., M. F. Ashby, 1982, "The Mechanics of Three-Dimensional Cellular Materials," *Proceedings of the Royal Society of London, series A*, Vol. 382, 43
- Green, A. E., and J. E. Adkins, 1970, *Large Elastic Deformations*, the Oxford University Press, 2nd, London
- Greulich, K. O., 1997, "Micromanipulation by Light in Biology and Medicine: The Laser Microbeam and Optical Tweezers," Birkhuser, Boston
- Grover, S. C., R. C., Gauthier and A. G. Skirtach, 2000, Analysis of the behaviors of erythrocytes in an optical trapping system, *Optical Express*, Vol.7, 533
- Guck, J., Ananthakrishnan, R., Moon, T. J., Cunningham, C. C., Kas, J., 2001, The Optical Stretcher: A Novel Laser Tool to Micromanipulate Cells, *Biophysical Journal*, Vol.81, 767
- Guck, J., R. Ananthakrishnan, T. J. Moon, C. C. Cunningham, and J. Kas, 2000, Optical Deformability of Soft Biological Dielectrics, *Physical Review Letters*, Vol.84, 5451
- Gudi, S. R., Lee, A. A., Clark, C. B., Frangos J. A., 1998, Equibiaxial strain and strain rate stimulate early activation of G proteins in cardiac fibroblasts, *American Journal of Physiology*, Vol. 274: C1424

References

-
- Gudi, S. R., Nolan, J. P., Frangos, J. A., 1998, Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition, *Proceedings of the National Academy of Sciences, USA*, Vol.95, 2515
- Hammer, D. A., and S. M. Apte, 1992, "Simulation of Cell Rolling and Adhesion on Surfaces in Shear Flow: General Results and Analysis of Selectin-Mediated Neutrophil Adhesion," *Biophysical Journal*, Vol. 63, 35
- Hansen, J. C., R. Skalak, S. Chien and A. Hoger, 1997, Influence of network topology on the elasticity of the red blood cell membrane skeleton, *Biophysical Journal*, Vol.72, 2369
- Hansen, J. C., R. Skalak, S. Chien, A. Hoger, 1996, An Elastic Network Model Based on the Structure of the Red Blood Cell Membrane Skeleton, *Biophysical Journal*, Vol.70, 146
- Hawkey, C. M., P. M. Bennett, S. C. Gascoyne, M. G. Hart, and J. K. Kirkwood, 1991, Erythrocyte size, number and haemoglobin content in vertebrates, *British Journal of Haematology*, Vol.77, 392
- Hecht, E., 2001, Optics, 4th ed., Addison Wesley; New York, Chapter 2
- Henon, S., G. Lenormand, A. Richert and F. Gallet, 1999, A New Determination of the Shear Modulus of the Human Erythrocyte Membrane Using Optical Tweezers, *Biophysical Journal*, Vol.76, 1145
- Hochmuth, R. M. and N. Mohandas, 1972, Uniaxial loading of the red-cell membrane, *Journal of Biomechanics*, Vol.5, 501
- Hochmuth, R. M., 1987, Properties of red blood cells, In: Skalak R., Chien S. (Eds.), *Handbook of Bioengineering*, McGraw-Hill Book company, New York, 12.1-12.17
- Hochmuth, R. M., 2000, Micropipette aspiration of living cells, *Journal of Biomechanics*, Vol. 33, 15
- Hochmuth, R. M., Waugh, R. E., 1987, Erythrocyte membrane elasticity and viscosity, *Annual Review of Physiology*, Vol.49, 209
- Hofmann, U. G., C. Rotch, W. J. Parak, M. Radmacher, 1997, "Investigating the Cytoskeleton of Chicken Cardiocytes with the Atomic Force Microscope," *Journal of Structural Biology*, Vol.110, 84
- Hotani, H., 1984, Transformation Pathways in Liposomes, *Journal of Molecular Biology*, Vol.178, 113

References

-
- Hubner, S., Couvillon, A. D., Kas, J. A., Bankaitis, V. A., Vegners R., et al., 1998, Enhancement of phosphoinositide 3-kinase (PI 3-kinase) activity by membrane curvature and inositol-phospholipid-binding peptides, *European Journal of Biochemistry*, Vol. 258, 846
- Hulst, H. C., 1957, *Light Scattering by Small Particles*, P224-226, Dover Publications, Inc., New York,
- Ingber, D. E., J. A. Madri and J. D. Jamieson, 1981, Role of basal lamina in neoplastic disorganization of tissue architecture, *Proceedings of the National Academy of Sciences of the USA*, Vol. 78: 3901
- Janmey, P. A., U. Euteneuer, P. Traub and M. Schliwa, 1991, Viscoelastic properties of vimentin compared with other filamentous biopolymer networks, *The Journal of Cell Biology*, Vol. 113, 155
- Konig, K., 2000, "Laser Tweezers and Manipulation Microscopes in the Life Science," *Histochemistry and Cell Biology*, Vol.114, 79
- Landau, L. D., and E. M. Lifshitz, 1959, *Theory of Elasticity*, Pergamon Press, New York, 56
- Lardner, T. J., P. Pujara, 1978, "On the Contact Problem of a Highly Inflated Spherical Nonlinear Membrane," *Journal of Applied Mechanics*, Vol.45, 202
- Lee, J. C-M., Discher, D., 2001, Deformation-Enhanced Fluctuations in the Red Cell Skeleton with Theoretical Relations to Elasticity, Connectivity, and Spectrin Unfolding, *Journal of Biomechanics*, Vol.81, 3178
- Lei, X., M. R. Lawrence, C. Dong, 1999, "Influence of Cell Deformation on Leukocyte Rolling Adhesion in Shear Flow," *Journal of Biomechanical Engineering*, Vol.121, 636
- Lelievre, J. C., Bucherer, C., Geiger, S., Lacombe, C., Vereycken, V., 1995, Blood cell biomechanics evaluated by the single-cell micromanipulation, *Journal de Physique III France*, Vol.5, 1689
- Len Fisher, 1993, Force between biological surfaces, *Journal of the Chemical Society Faraday Transactions*, Vol.89, 2567
- Lenormand, G., Hénon, S., Richert, A., Siméon, J., Gallet, F., 2001, Direct Measurement of the Area Expansion and Shear Moduli of the Human Red Blood Cell Membrane Skeleton, *Biophysical Journal*, Vol.81, 43
- Lim C.T., Dao M., Suresh S., Sow C.H., Chew K.T., Large deformation of living cells using laser traps, *Acta. Materialia*, Vol.52 (7), 1837-1845, 2004

References

-
- Lin, K. L., L. Lopez and J. D. Hellums, 1973, Blood flow in capillaries, *Microvascular Research*, Vol.5, 7
- Liu Y., D. K. Cheng, G. J. Sonek, M. W. Berns, C. F. Chapman, and B. J. Tromberg, 1995, Evidence for localized cell heating induced by infrared optical tweezers, *Biophysical Journal*, Vol.68, 2137
- Liu, K. K., D. R. Williams, and B. J. Briscoe, 1996, Compressive deformation of a single microcapsule, *Physical Review E*, Vol.54, 6673
- Liu, Y., Cheng, D. K., Sonek, G. J., Berns, M. W., Chapman, C. F., Tromberg, B. J., 1995. Evidence for Localized Cell Heating Induced by Infrared Optical Tweezers, *Biophysical Journal*, Vol.68, 2137
- Malek, A. M., Izumo, S., 1996, Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress, *Journal of Cell Science*, Vol.109, 713
- Miao, L., U. Seifert, M. Wortis, and H. G. Dobereiner, 1994, Budding transitions of fluid-bilayer vesicles: the effect of area-difference elasticity, *Physical Review E*, Vol. 49, 5389
- Mills J.P., Qie L., Dao M., Lim C.T., Suresh S., Nonlinear Elastic and Viscoelastic Deformation of the Human Red Blood Cell with Optical Tweezers, *Mechanics and Chemistry of Biosystems*, Vol.1(3), 169-180, 2004.
- Nemoto, S., Togo, H., 1998, Axial Force Acting on a Dielectric Sphere in a Focused Laser Beam, *Applied Optics*, Vol.37 (27), 6386
- Pamplona, D. C., and C. R. Calladine, 1993, The Mechanics of Axially Symmetric Liposomes, *Journal of Biomechanical Engineering*, Vol.115, 149
- Pamplona, D. C., Calladine, C. R., 1996, Aspects of the Mechaincs of Lobed Lipsomes, *Journal of Biomechanical Engineering*, Vol.118, 482
- Parker, K. H. and C. P. Winlove, 1999, The Deformation of Spherical Vesicles with Permeable, Constant-Area Membranes: Application to the Red Blood Cell, *Biophysical Journal*, Vol.77, 3096
- Platt, O. S., 1995, the sickle syndrome, in: Haldin, R. I. Lux, S. E. Stossel, T. P. (Eds.), *Blood: Principles and practice of hematology*, J. B. Lippincott, Philadelphia, 1592~1700
- Pozrikidis, C., 2003, *Modelling and Simulation of Capsules and Biological Cells*. Chapman & Hall/CRC Press, New York, Chap. 1 and 2

References

-
- Rachel Elaine Mahaffy, 2000, the quantitative characterizations of the viscoelastic properties of cells and polymer gels, Ph.D. thesis, University of Texas at Austin, U.S.
- Radmacher, M., M. Fritz, C. M. Kacher, J. P. Cleveland and P. K. Hansma, 1996, Measuring the viscoelastic properties of human platelets with the atomic force microscope, *Biophysical Journal*, Vol.70, 556
- Ragsdale, G. K., J. Phelps, K. Luby-Phelps, 1997, "Viscoelastic Response of Fibroblasts to Tension Transmitted through Adherent Junction," *Biophysical Journal*, Vol.73, 2798
- Rand, R. P., 1964, Mechanical Properties of the Red Cell Membrane: II. Viscoelastic Breakdown of the Membrane, *Biophysical Journal*, Vol.4, 303
- Ranjan, M., Gerald Lim H. W. and Michael W., 2002, Echinocyte Shapes: Bending, Stretching and Shear Determine Spicule Shape and Spacing, *Biophysical Journal*, Vol.82, 1756
- Reissner, E., 1969, On Finite Symmetrical Deflections of Thin Shell of Revolution, *Journal of Applied Mechanics*, Vol.36, Trans. ASME 91, Series E, 267
- Rotsch, C., K. Jacobson, M. Radmacher, 1999, "Dimensional and Mechanical Dynamics of Active and Stable Edges in Motile Fibroblasts Investigated by Using Atomic Force Microscopy," *Proceedings of the National Academy of Sciences*, Vol.96, 921
- Sandford, M. R. and Shipman, J. S., 1972, Two-Point Boundary Value Problems: Shooting Methods. American Elsevier Pub. Co, New York
- Sato, M. K., N. Nagayama, N. Kataoka, M. Sasaki, and K. Hane, 2000, "Local Mechanical Properties Measured by Atomic Force Microscopy for Cultured Bovine Endothelial Cells Exposed to Shear Stress," *Journal of Biomechanics*, Vol.33, 127
- Sato, M., M. J. Levesque and R. M. Nerem, 1987a, An application of the micropipette technique to the measurement of the mechanical properties of cultured bovine aortic endothelial cells, *Journal of Biomechanical Engineering*, Vol. 109, 27
- Sato, M., M. J. Levesque and R. M. Nerem, 1987b, Micropipette aspiration of cultured bovine aortic endothelial cells exposed to shear stress, *Arteriosclerosis*, Vol. 3, 276
- Secomb, T. W., R. Skalak, N. Ozkaya, and J. F. Gross, 1986, Flow of Axisymmetric Red Blood Cells in Narrow Capillaries, *Journal of Fluid Mechanics*, Vol.163, 405
- Shelby, J. P., J. Ganeshan, P. K. Rathod, and D. T. Chiu, 2003, A microfluidic model for

References

-
- single-cell capillary obstruction by falcipuram-infected erythrocyte, *Proceedings of the National Academy of Sciences*, Vol.100, 14618
- Shen, B., 1989, Ultrastructure and function of membrane skeleton, in *Red Blood Cell Membranes: Structure, Function, Clinical Implication*, Marcel Dekker, New York, 261
- Shojiro Nemoto and Hiroyoshi Togo, 1998, Axial Force Acting on a Dielectric Sphere in a Focused Laser Beam, *Applied Optics*, Vol.37, No.27, 6386
- Skalak, R., 1973, Modeling the Mechanical Behavior of Red Blood Cells, *Biorheology*, Vol.10, 229
- Skalak, R., A. Tozeren, R. P. Zarda, and S. Chien, 1973, Strain energy function of red blood cell membranes, *Biophysical Journal*, Vol.13, 245
- Sleep, J. A., Wilson, D., Simmons, R. M., Gratzner, W. B., 1999. Elasticity of the Red Cell Membrane and its Relation to Hemolytic Disorders, An Optical Tweezers Study, *Biophysical Journal*, Vol.77, 3085
- Sokabe, M., Naruse, K., Sai, S., et al., 1997, Mechanotransduction and intracellular signaling mechanisms of stretch-induced remodeling in endothelial cells, *Heart Vessel Supply*, Vol.12, 191
- Sutera, S. P., V. Seshadri, P. A. Croce, and R. M. Hochmuth, 1970, Capillary Blood Flow II. Deformable Model Cells in Tube Flow, *Microvascular Research*, Vol.2, 420
- Svoboda, K., Block, S. M., 1994, Biological Applications of Optical Forces, *Annual Review of Biophysics & Biomolecular Structure*, Vol.23, 247
- Svoboda, K., Schmidt, C. F., Branton, D., Block, S. M., 1992. Conformation and Elasticity of the Isolated Red Blood Cell Membrane Skeleton, *Biophysical Journal*, Vol.63, 784
- Thoumine, O., A. Ott, 1997, Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation, *Journal of Cell Science*, Vol.110, 2109
- Timoshenko, S. P., Woinowsky-Krieger, S., 1959, *Theory of Plates and Shells*, 2nd. Ed., McGraw-Hill, New York, 320
- Ugural, A. C., 1999, *Stresses in Plates and Shells*, 2nd, 395, McGraw-Hill, New York
- Wang, N., J. P. Butler and D. E. Ingber, 1993, Mechanotransduction across the cell surface and through the cytoskeleton, *Science*, Vol.260, 1124

References

- Waugh, R. E., and R. M. Hochmuth, 1995, Mechanics and deformability of hematocytes, In: Bronzino, J. D. (Ed.), *Biomedical Engineering Handbook*, CRC Press Inc, Boca Raton, 474
- Waugh, R., E. A. Evans, E. A., 1979, Thermoelasticity of Red Blood Cell Membrane, *Biophysical Journal*, Vol.26, 115
- Waugh, R.E., Song, J., Svetina, S., Zeks, B., 1992, Local and Nonlocal Curvature Elasticity in Bilayer Membranes by Tether Formation from Lecithin Vesicles, *Biophysical Journal*, Vol.61, 974
- Weiner, S., Traub, W., 1991, Organization of crystals in bone, In: Suga, S., Nakahara, H. (Eds.), *Mechanisms and Phylogeny of Mineralisations in Biological Systems*. Springer, Tokyo, 247
- Zahalak, G. I., W. B. McConnaughey and E. L. Elson, 1990, Determination of cellular mechanical properties by cell poking, with an application to leukocytes, *Journal of Biomechanical Engineering*, Vol.112, 283
- Zarda, P. R., S. Chien, and S. Skalak, 1977, Elastic deformations of red blood cells, *Journal of Biomechanics*, Vol.10, 211