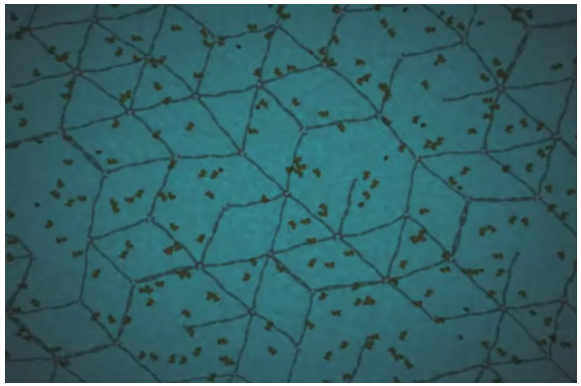
4. the cytoskeleton - network models



the inner life of a cell, viel & lue, harvard [2006]

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Biomechanics: Cell Research and Applications for the Next Decade

DENNIS DISCHER,[1] CHENG DONG,[2] JEFFREY J. FREDBERG,[3] FARSHID GUILAK,[4] DONALD INGBER,[5] PAUL JANMEY,[1] ROGER D. KAMM,[6] GEERT W. SCHMID-SCHÖNBEIN,[7] and SHELDON WEINBAUM[8]

[1]University of Pennsylvania, Philadelphia, PA, USA; [2]Pennsylvania State University, University Park, PA, USA; [3]Harvard School of Public Health, Boston, MA, USA; [4]Duke University, Durham, NC, USA; [5]Harvard Medical School, Boston, MA, USA; [6]MIT, Boston, MA, USA; [7]University of California, San Diego, San Diego, CA, USA; and [8]City College of New York, New York, NY, USA

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Abstract—With the recent revolution in Molecular Biology and the deciphering of the Human Genome, understanding of the building blocks that comprise living systems has advanced rapidly. We have yet to understand, however, how the physical forces that animate life affect the synthesis, folding, assembly, and function of these molecular building blocks. We are equally uncertain as to how these building blocks interact dynamically to create coupled regulatory networks from which integrative biological behaviors emerge. Here we review recent advances in the field of biomechanics at the cellular and molecular levels, and set forth challenges confronting the field. Living systems work and move as multi-molecular collectives, and in order to understand key aspects of health and disease we must first be able to explain how physical forces and mechanical structures contribute to the active material properties of living cells and tissues, as well as how these forces impact information processing and cellular decision making. Such insights will no doubt inform basic biology and rational engineering of effective new approaches to clinical therapy.

Keywords—Biomechanics, Cell, Mechanics, Rheology, Signaling, Force, Stress.

emerge through collective interactions within dynamically coupled regulatory networks. Systems Biology presently emphasizes information transfer,[17] but the three-dimensional geometries and physical forces that play so large a role in biological structure and function have yet to be fully taken into account. Indeed, without these biomechanical factors there would be no form, no function, no life.

Most diseases present as a complex genetic profile with multiple changes in molecular expression.[85,131] Nonetheless, a patient goes to the doctor's office often because of a mechanical defect in a tissue or organ: a new swelling or lump, pain due to nerve compression, stiffness that limits movement, edema caused by a leak of tissue bodily fluids, constricted blood flow or lymph flow, or obstructed airflow that restricts breathing. Cures and remedies are often judged successful by the patient only when such mechanical defects are remedied. In order to understand health-related and disease-related aspects of living systems—all of which work and

Write a one paragraph proposal for what you would like to do your final project. You will get some feedback on it, so you can use it as an opportunity to check whether any project ideas you may have are appropriate/acceptable. Some of last year's projects are given below. There are three options: (i) a literature review on a topic related to cell mechanics and/or mechanotransduction, (ii) a review of a topic on cell mechanics that was not covered in class, or (iii) a computational and/or theoretical analysis of some mechanical phenomenon related to cell mechanics. Papers in the past have generally been about 4-6 pages, two column, with about 3-5 figures and 8-12 references. Here are some examples of individual projects.

- Predicting microtubules structure using molecular dynamics
- The primary cilium: A well-designed fluid flow sensor
- The tensegrity paradigm
- Mechanotransduction in hair cells Translating sound waves into neural signals
- Modeling cell membrane dynamics
- Dielectrophoresis properties and their microfluidic application: Cell concentrator
- Theoretical and experimental study of the penetration of the cell membrane
- Integrin and its role in mechanotransduction
- Finite element analysis of micropipette aspiration

ME339 FINAL PROJECT

THE ROLE OF CELL-CELL JUNCTIONS IN CARDIOMYOCYTE CONTRACTILITY AND MODIFICATIONS NEEDED IN MECHANICAL MODELS

Chelsey S. Simmons
Department of Mechanical Engineering, Stanford University
Stanford, California

ABSTRACT
As adult cardiomyocytes (CMs) cannot recover from damage due to pathologies like myocardial infarction, researchers are looking for ways to culture functional cardiomyocytes to repopulate diseased cardiac tissue. However, as damaged tissue may interfere with cell-cell communication, the role of cell-cell junctions in cardiomyocyte contractility must be considered. This paper examines current literature on CM contractility, evaluating first the role of the cytoskeleton in CM contractility and then the hypothesized role of cell-cell junctions. Additionally, the likelihood of adapting computational models to reflect abnormal conditions of cell-cell junctions is considered. Further examination of contractile force generation with modification of certain cytoskeletal and junctional proteins is recommended to facilitate incorporation into tissue-scale models and to understand the contribution of these various proteins to heart diease.

The Primary Cilium: A Well-Designed Fluid Flow Sensor

Bryan C. Petzold
Department of Mechanical Engineering, Stanford University
Stanford, California

The primary cilium is a highly specialized surface projection which extends from the apical surface of almost every vertebrate cell. After its initial discovery over 100 years ago, primary cilia were long overlooked and even purported by some to be extraneous genetic remnants from our evolutionary past. However, in the past decade, a wealth of evidence has begun to accumulate, indicating that cilia in various cell types act not only as mechanical and chemical sensors, but also play important roles in intracellular signaling and cell division[1]. Some have even suggested that cilia-related dysfunction may have an important role in modern human epidemics such as obesity, hypertension and diabetes[2]. One such link between cilia-related dysfunction and human disease that has been explored extensively involves the role of the primary cilia of renal epithelial cells as flow sensors. It is believed that a dysfunction in these cilia results in polycystic kidney disease (PKD), the most common inherited disease in the United States, with an estimated 600,000 current cases[3]. Numerous models have been proposed to explain the mechanotransduction mechanism which allows the primary cilia of renal epithelial cells to detect fluid flow, but many questions remain. Understanding the transduction mechanism and the features of the primary cilium which make it an ideal flow sensor will not only answer many interesting questions in biology and biomechanics, but could aid in the treatment of PKD and other diseases which are caused by cilia-related dysfunction.

Introduction to the Primary Cilium

The primary cilium is a long, cylindrical, microtubule-based structure which extends from the apical surface of most vertebrate cells, as shown in Figure 1. In general, cells only have a single primary cilium. Referred to as the axoneme, the main structural element of the primary cilium is a collection of nine circumferentially-arranged doublet microtubules encased by membrane continuous with the cell membrane[1]. These doublet microtubules extend from a structure known as a basal body within the cell, which links the base of the primary cilium to the cytoskeleton. The basal body consists of nine triplet microtubules, and two of the microtubules of each triplet form the axoneme of the primary cilium[1]. Further structural support is provided by the transitional fibers (alar shields), which add stability to the complex via attachment to the cell membrane[4]. In conjunction with a terminal plate at the end of the basal body, these transitional fibers also act as a protein filter, only allowing certain proteins to enter and exit the cilium[1]. At the far end of the cilium, the axoneme becomes more variable, but is typically composed of nine single microtubules[1]. Although cilia are not isolated from the cell by a membrane, it seems reasonable to consider them to be organelles due to their unique structure, their extreme location past the cell periphery, and the selectivity to protein movement across their boundaries resulting from the transitional fibers and the terminal plate.

Depending on the species, primary cilia of renal epithelial cells typically vary between 2-20 μm in length *in vivo*[5]. However, lengths up to 30 μm have been observed *in vitro*[3]. In addition, studies involving mice renal epithelial cells measured primary cilia 2-3 μm long and 0.2 μm in diameter on average[3]. Since microtubules have an outer diameter of ~30 nm, this relatively small diameter indicates that nearly half the volume of a primary cilium is occupied by the microtubules alone[3].

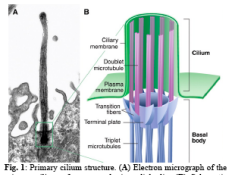


Fig. 1: Primary cilium structure. (A) Electron micrograph of the primary cilium of a canary brain radial glia. (B) Schematic showing structure of the basal body and primary cilium. Adapted from Singla *et al.* (2006)[1].

Mechanisms of Neuron Repair and Stem Cell Neural Differentiation:

Neural Stem Cell Grafts and Current Advances in Spinal Cord Injury Research

José Solomon

***Abstract*—Neural tissue regeneration and neuronal stem cell differentiation are discussed within the context of spinal cord injuries. The neuron is the fundamental element of the spinal cord and nervous system, and its functioning characteristics as well as the process of synaptic transmission are introduced to understand the impact of spinal cord injury. Three techniques are reviewed that address the issue of neural tissue regrowth from varying prospectives. The methods are interelated and demonstrate the potential role of neuron stem cell grafting in resolving severe spinal cord injury.**

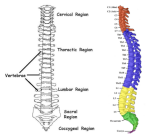
***Index Terms*—Neuron stem cell differentiation, axon ion discharge, synaptic transmission, spinal cord injury.**

TABLE I
Regions of Trauma and Resulting Neurological Impact

C_1 - C_3	Loss of all motor skills
C_4	Minimal upper body control, very weak
C_5	Regular shoulder and bicep control, no hand or wrist sensation/control
T_2-T_9	Good midsection control, little to no lower body sensation/control.
S_1-S_3	Some lower body motor control

I. Introduction

Spinal cord injuries represent a pervasive and often severe source of neurological trauma. The spinal cord represents the connection bridge between the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS normally refers to the brain and spinal cord itself, where as the PNS is the vast network of nerves that permeates throughout the body. The human spinal cord consists of five main regions, as illustrated in Figure 1(a), each of which has a varying number of vertebrae, shown in Figure 1(b). The vertebrae have a set configuration network of nerves which they protect, and thus the physiological impact of a spinal cord injury is directly correlated to the specific vertebrae effected.



(a) Spinal cord vertebrae regions (b) Vertebrae configuration

Fig. 1. The spinal cord and the five primary vertebrae regions, [9]

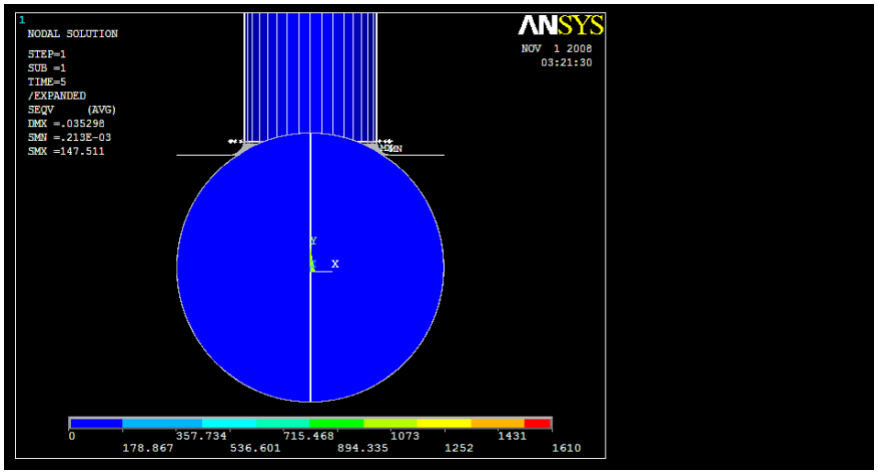
Although there is a certain randomness in the specific effects of a spinal cord injury that stems from an individual's age and the nature of the impact, a general guideline can be arrived at that directly correlates the injured vertebrae with the most likely resulting region of sensory/mobility loss. Some specific correlations are given in Table 1. As shown in the table, injuries in the cervical region normally tend to lead to major mechanical and sensory feedback loss, affecting a larger range of nerve networks than lower spinal regions since it is situated directly at the root of the brain-spinal cord interface. As the region of spinal trauma moves further down the length of the spinal cord, the effected region is reduced in scope.

Spinal cord injuries themselves can be classified into two general categories: **complete** and **incomplete**, [9]. In a **complete** injury, the damage is for the most part permanent and the effected individual usually exhibits little to no recovery of motor or sensory feedback. In **incomplete** injuries, the effects of the injury tend to be of a temporary nature and the individual may exhibit a full recovery. Two examples of individuals who experienced complete spinal cord injury are presented in Figure 2.

(a) Christopher Reeves: C2 fracture (b) Dan James: C3-C4 fracture, [11]

Fig. 2. Examples of complete spinal cord injury

example: finite element simulation of pipette aspiration

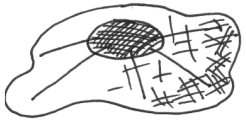


zubin huang [2007]

from molecular level to cellular level

assuming we know the mechanical properties of the individual filaments, what does that actually tell us about the assembly of filaments that we find in the cell?

- could we then predict the **stiffness of the overall assembly**?
- how does the filament microstructure affect **cytoskeletal properties**?
- how can we calculate the **macroscopic network properties** from the individual microscopic filament properties?



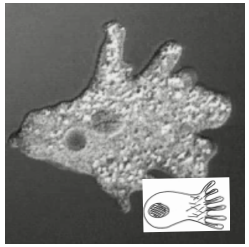
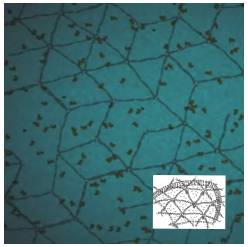
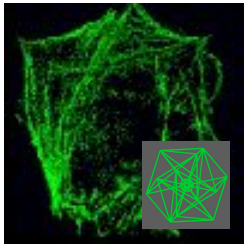
elements of the cytoskeleton
microtubules
intermediate filaments
actin filaments

Figure 4.1: The cytoskeleton provides structural stability and is responsible for forces during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

from molecular level to cellular level

three examples

- **fiber bundle model** for filopodia
- **network model** for red blood cell membranes
- **tensegrity model** for generic cell structures

microstructural arrangement of actin

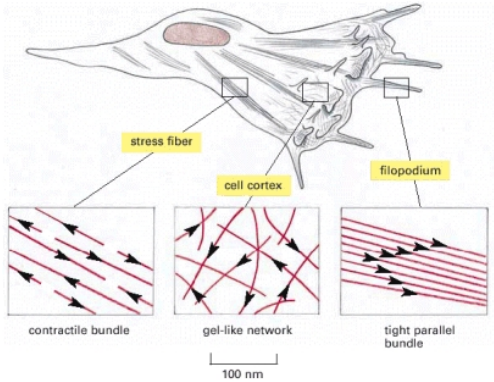


Figure 4.2.1. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments.

alberts, johnson, lewis, raff, roberts, walter [2002]

assembly of crosslinked actin filaments

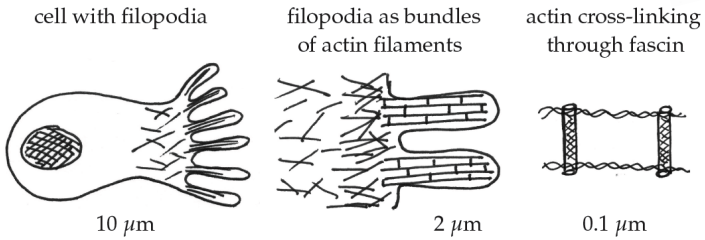


Figure 4.3. Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 um in diameter. they can easily extend up to 1.5um. they typically polymerize and depolymerize at rates of approximately 10 um/min. the mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown.

pushing the envelope

simplified model for cell locomotion

- protrusion ... polymerization at the leading edge of the cell
- attachment ... formation of focal adhesions to link the cell to the surface
- retraction ... contraction of stress fibers to retract the rear of the cell

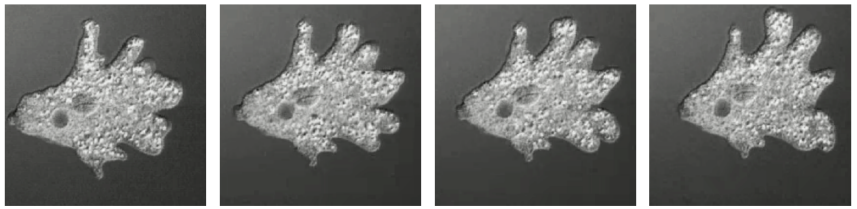
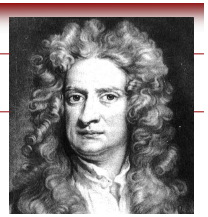


Figure 4.4: Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organelles move in complex patterns within the cell,

alberts, johnson, lewis, raff, roberts, walter [2002]

pushing the envelope - critical length



Newton's third law: actio = reactio

$$F_{\text{fil}} \doteq F_{\text{mem}}$$

$$F_{\text{fil}} = \frac{\pi^2\, EI}{[2\,L]^2} = \frac{\pi^2\, EI}{4\,L} \qquad F_{\text{mem}} \approx 5\,\sqrt{n}\, r_{\text{act}}\,\text{pN/nm}$$

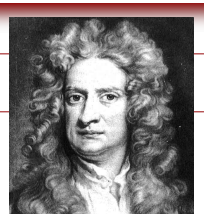
$$\frac{\pi^2\, EI}{4\,L_{\text{crit}}^2} = 5\,\sqrt{n}\, r_{\text{act}}\,\frac{\text{pN}}{\text{nm}} \qquad \text{thus} \qquad L_{\text{crit}} = \frac{\pi}{2}\,\sqrt{\frac{EI}{5\,\sqrt{n}\, r_{\text{act}}\,\text{pN/nm}}}$$

$E = 1.9 \cdot 10^9\ \text{N/m}^2$=1.9 GPa $\quad r_{\text{act}} = 2.5 \quad$ moment of inertia I

- loose assembly
- tightly crosslinked

4.2 fiber bundle model for filopodia 13

case I - loosely assembled actin filaments



$$L_{\text{crit}} = \frac{\pi}{2}\,\sqrt{\frac{EI}{5\,\sqrt{n}\, r_{\text{act}}\,\text{pN/nm}}}$$

moment of inertia I

$$I = n\, I_{\text{act}} \qquad \text{with} \qquad I_{\text{act}} = \frac{\pi\, r_{\text{act}}^4}{4}$$

$E = 1.9 \cdot 10^9\ \text{N/m}^2$=1.9 GPa $\quad r_{\text{act}} = 2.5$

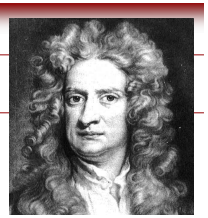
$$L_{\text{crit}} = \frac{\pi}{2}\,\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\,\sqrt{n}\;3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{1/4}$$

$n = 30$ filaments $\qquad L_{\text{crit}} = 0.416\,\mu\text{m}$

much too low - disagrees with observations of 2um

4.2 fiber bundle model for filopodia 14

case II - tightly crosslinked actin filaments



$$L_{\text{crit}} = \frac{\pi}{2}\,\sqrt{\frac{EI}{5\,\sqrt{n}\, r_{\text{act}}\,\text{pN/nm}}}$$

moment of inertia I

$$I = \frac{\pi\, r_{\text{fil}}^4}{4} = n^2\,\frac{\pi\, r_{\text{act}}^4}{4} \quad \text{with } r_{\text{fil}} = \sqrt{n}\, r_{\text{act}}$$

$E = 1.9 \cdot 10^9\ \text{N/m}^2$=1.9 GPa $\quad r_{\text{act}} = 2.5$

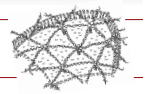
$$L_{\text{crit}} = \frac{\pi}{2}\,\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n^2\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\,\sqrt{n}\;3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{3/4}$$

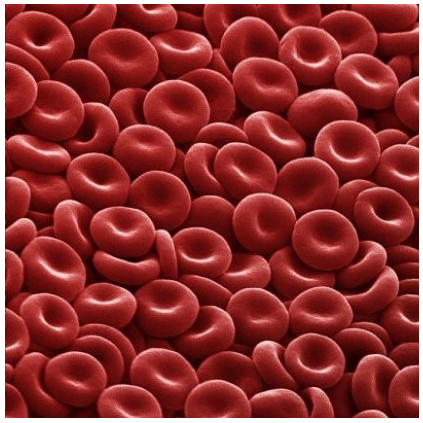
$n = 30$ filaments $\qquad L_{\text{crit}} = 2.278\,\mu\text{m}$

better model - agrees with observations of 2um

4.2 fiber bundle model for filopodia 15

red blood cells





erythrocytes, red blood cells are essential to deliver oxygen to the body via the blood flow through the circulatory system. they take up oxygen in the lungs and release it while squeezing through the body's capillaries. adult humans have about 2-3 10^{13}, 20-30 trillion, red blood cells comprising about a quarter of the total amount of cells in the human body.

4.3 network model for red blood cells 16

red blood cells

human mature red blood cells are **flexible biconcave disks** that lack a cell nucleus and most organelles. typical human erythrocytes have a disk diameter of 6–8μm, a thickness of 2μm, a volume of 90fL, and a surface of 136μm^2. they can swell to spherical shape of 150fL, without membrane distension. the membrane of the red blood cell plays a key role in regulating **surface deformability**, **flexibility**, and adhesion to other cells. these functions are highly dependent on its composition. the red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer consisting of lipidic main constituents and transmembrane proteins; and the membrane skeleton, a **structural network of proteins** located **on the inner surface** of the lipid bilayer.

red blood cells

Metabolic remodeling of the human red blood cell membrane

YongKeun Park[a,b], Catherine A. Best[c], Thorsten Auth[d,e], Nir S. Gov[f], Samuel A. Safran[e], Gabriel Popescu[g], Subra Suresh[b,h,1], and Michael S. Feld[a,1]

[a]G. R. Harrison Spectroscopy Laboratory and [b]School of Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; [c]Harvard-Massachusetts Institute of Technology Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139; [d]College of Medicine and [g]Quantitative Light Imaging Laboratory, Department of Electrical and Computer Engineering, Beckman Institute for Advanced Science & Technology University of Illinois at Urbana-Champaign, Urbana, IL 61801; [e]Forschungszentrum Jülich, Institute for Solid State Research, 52425 Jülich, Germany; and [f]Department of Materials and Interfaces and Department of Chemical Physics, Weizmann Institute of Science, P.O. Box. 26, Rehovot 76100, Israel

Edited by Zdeněk P. Bažant, Northwestern University, Evanston, IL, and approved November 25, 2009 (received for review September 18, 2009)

The remarkable deformability of the human red blood cell (RBC) results from the coupled dynamic response of the phospholipid bilayer and the spectrin molecular network. Here we present quantitative connections between spectrin morphology and membrane fluctuations of human RBCs by using dynamic full-field laser interferometry techniques. We present conclusive evidence that the presence of adenosine 5′-triphosphate (ATP) facilitates nonequilibrium dynamic fluctuations in the RBC membrane that are highly correlated with the biconcave shape of RBCs. Spatial analysis of the fluctuations reveals that these non-equilibrium membrane vibrations are enhanced at the scale of spectrin mesh size. Our results indicate that the dynamic remodeling of the coupled membranes powered by ATP results in non-equilibrium membrane fluctuations manifesting from both metabolic and thermal energies and also maintains the biconcave shape of RBCs.

red blood cells

Metabolic remodeling of the human red blood cell membrane

YongKeun Park[a,b], Catherine A. Best[c], Thorsten Auth[d,e], Nir S. Gov[f], Samuel A. Safran[e], Gabriel Popescu[g], Subra Suresh[b,h,1], and Michael S. Feld[a,1]

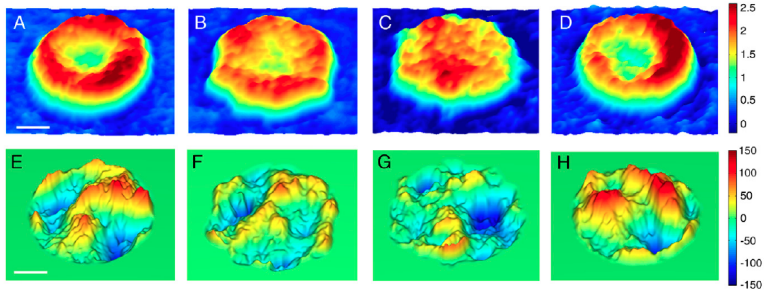
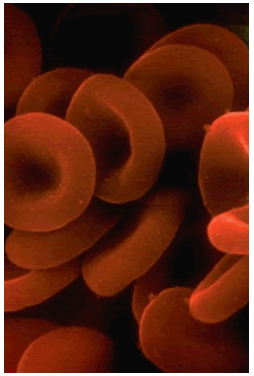
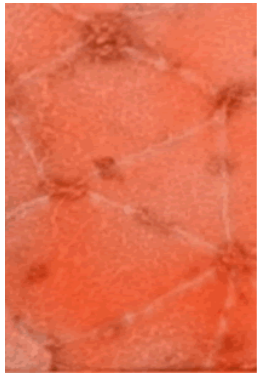
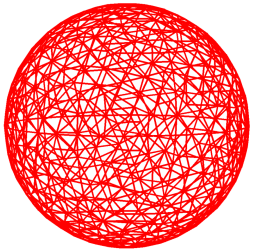


Fig. 1. Effects of ATP on morphology and dynamic fluctuation in RBC membrane. Topography of a healthy RBC, (*A*) of an ATP-depleted RBC (irreversible-ATP group), (*B*) of an ATP-depleted RBC (metabolic-ATP group), (*C*, and of a RBC with recovered ATP level (+ATP group), (*D*) resp. (*E–H*) Instantaneous displacement maps of membrane fluctuation in the Fig. 1*A–D*, resp. The scale bar is 2 μm. The colorbar scales are in μm and nm, resp.

red blood cells







the human red blood cell membrane skeleton is a network of roughly 33,000 protein hexagons that looks like a microscopic geodesic dome