Homework II - The cytoskeleton

due Thu 05/03/12, 12:50pm, edu-128

You can drop off late homework in a box in front of Durand 217. Please mark clearly with date and time @drop off. We will take off 10 points for each 24 hours late.

Problem 1 - Filament buckling

In class, we have discussed filament buckling of actin polymers. We have seen that the critical buckling force of filopodia

$$F_{\text{fil}} = \frac{\pi^2 EI}{[2\,L_{\text{fil}}]^2} = \frac{\pi^2 EI}{4\,L_{\text{fil}}^2}$$

can be increased by fascin which can create tightly crosslinked filament bundles.

- Make a table with actin filaments, intermediate filaments, and microtubules that summarizes: (i) Young's modulus E, (ii) the moment of inertia I, and (iii) the critical force F_{fil} at which each individual filament would begin to buckle. You can assume a filament length of an average generic cell radius of $L_{\text{fil}} = 5\mu m$.
- Explain why the critical buckling force F_{fil} can be increased by tight crosslinking through fascin.
- In class, we have assumed that there are about $n = 30$ actin filaments in one filopodium. Assume we do not know the exact number n. What is the minimum number of actin filaments to obtain a critical filopodia length of $L_{\text{fil}} = 5\mu m$ assuming that the actin filaments are (i) loosely assembled (4.2.9) and (ii) tightly crosslinked (4.2.12).
- How would the minimum number of required actin filaments n change if you assume a critical filopodia length of $L_{\text{fil}} = 2.5\mu m$?

Problem 2 - Forces on the cell membrane

To gain a better feeling for stresses that the cytoskeleton might induce on the cell membrane, this problem deals with the membrane pressure resulting from microtubules. Consider a generic spherical cell of radius 10μm with a tubulin heterodimer concentration of $C = n/V = 5\mu$M.

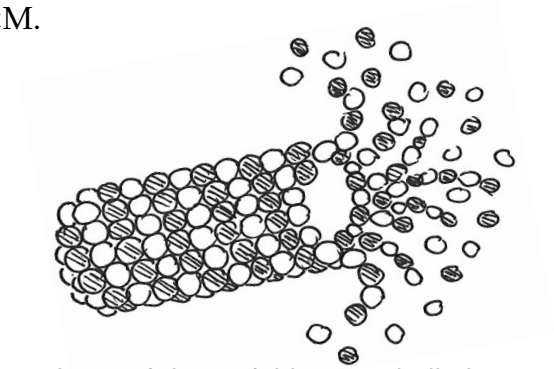

Figure. Microtubules are made up of rings of thirteen tubulin heterodimers which are 8 nm in diameter. The rings of tubulin heterodimers form a cylinder of thirteen protofilaments.

- Calculate the total length of microtubules that could be made from this amount of protein if each heterodimer is approximately 8 nm in diameter. Take into account that microtubules are made up of rings of thirteen tubulin heterodimers. Remember that 1μM = 1 μmol/liter and that 1 mol contains $6.02 \cdot 10^{23}$ heterodimers.
- Assume all microtubules connect the center of the cell with its membrane. What is the average membrane area per microtubule?
- Assume each microtubule generates a force of 10 pN. What is the average pressure exerted on the cell membrane if all microtubules were equally spaced?

The volume of a sphere is $V = 4/3\,\pi\,r^3$, the surface of a sphere is $A = 4\,\pi\,r^2$.

Problem 3 - Cell mechanics research

The recent manuscript *"Biomechanics: Cell Research and Applications for the Next Decade"* by Discher, Dong, Fredberg, Guilak, Ingber, Janmey, Kamm, Schmid-Schönbein and Weinbaum discusses the challenges for cell mechanics now and in the future.

- Read the manuscript carefully and summarize it with approximately 150 words.
- The authors have identified ten major accomplishments in cell mechanics. List all the ten accomplishments by title.
- Select your favorite past accomplishment and describe it in less than 100 words.
- The authors have identified seven major challenges for the future. List all the seven future challenges by title.
- Select your favorite future challenge and describe it in less than 100 words.

Problem 4 - Final project

Inspired by the recent manuscript on major accomplishments and challenges in cell mechanics,

- Identify a title for your final project.
- Identify five key words for your final project.
- Write a tentative abstract of approximately 150-200 words.

You will get feedback, so you can use this as an opportunity to check whether your project ideas are appropriate and acceptable. Basically, there are three major project categories: (i) a literature review on a topic related to cell mechanics or mechanotransduction, (ii) a review of a topic on cell mechanics that was not covered in class, and (iii) a theoretical/computational analysis of a mechanical phenomenon related to cell mechanics, for example, a parameter study using MATLAB or ANSYS.

Papers in the past have generally been about 4-6 pages long, 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
- Theoretical and experimental study of the penetration of the cell membrane
- Integrin and its role in mechanotransduction
- Finite element analysis of micropipette aspiration
- Mechanical stimulation of directed stem cell differentiation
- Models and mechanisms of leukocyte extravasion
- The swimming velocity of mammalian sperm

Final projects will be presented in 5-10 minute long presentations on Thursday 05/31/12 and Tuesday 06/05/12. Presentations will be graded by the entire class. Written project reports are due on Friday 06/08/12. You can work in teams of two, three, or four. If you decide to present your project together and hand in the same project report, it will have to be longer and you will all get the same grade.

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Biomechanics: Cell Research and Applications for the Next Decade

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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.

INTRODUCTION

In this post-genomic era, the challenge that all areas of biomedical research now face is to understand how the molecules that are expressed go on to fold, assemble and function within the context of living cells, tissues, and organs. Just as challenging is the question of how complex biological characteristics subsequently 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 move as multi-molecular collectives—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.[50,71] Such insights will inform not only basic biology but also rational engineering of effective new approaches to clinical therapy. Here we discuss key obstacles and major opportunities confronting the field of biomechanics, as well as implications for the future of science, engineering, and healthcare.

HISTORICAL BACKGROUND

In the first half of the 20th century, D'Arcy Thompson proposed that mechanical forces act as causative agents during tissue morphogenesis. At a time when the molecular basis of viscosity was being

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This is a white-paper developed by the Cell Mechanics working group of the US National Committee on Biomechanics.

developed by Einstein,[28] some of the earliest quantitative evidence of non-Newtonian viscosity emerged from studies of biological fluids Even though the reality of molecules, as distinct from colloidal particles, was still being contested, the unusual mechanical properties of cytoplasm led to visionary proposals that the cell contained a system of molecular filaments (reviewed in Trepat *et al.*[119]).

Clinicians recognized quite early the central importance of physical forces in physiological control. Well-known examples include the effects of inspiratory pressure on lung function, hemodynamic shear stress on vascular remodeling, compression on bone generation, and tension on skin aging. There is now the recognition of pivotal roles played by physical forces in genetic and cellular regulation, as well as in developmental control.[42] Interest in biomechanics has since grown exponentially and now includes researchers in a wide range of biological disciplines including molecular biophysics, cell biology, developmental biology, genetics and physiology, as well as mechanical engineering, materials science and nanotechnology.

CURRENT STATUS OF THE FIELD

Even if they are not presented in the context of biomechanics, clinical therapies and cues to treat disease often rely directly upon biomechanics.[56] Some are ancient, such as a mechanical support of the skin with a bandage in venous ulcers. Modern examples include stents, ventilators, and vasodilators/constrictors. A recent and intriguing example is the vacuum-assisted closure sponge[100]; application of cyclic suction to a non-healing wound is more effective at healing than are two other FDA-approved therapeutics: platelet derived growth factor (PDGF) or a tissue engineered implant with stem cells. Foams are used to close blood vessels during uncontrolled angiogenesis and venous ulcerations. Across a broad spectrum of disorders, better understanding of the biophysical basis of cellular mechanotransduction seems likely lead to new drug-based and nanotechnology-based therapeutics. Some heart arrhythmias will almost certainly come to be treated with inhibitors of stress-sensitive ion channel, for example.

Microscopic changes in cell mechanics and extracellular matrix structure are expected to dysregulate molecular mechanisms of mechanotransduction—which is the process by which cells sense mechanical signals and convert them into chemical responses.[4,5,42] Examples include numerous developmental abnormalities (e.g., osteogenesis imperfecta) that result from altered matrix mechanics, enhanced cancer cell metastasis within the microvasculature that result from hydrodynamic flow-mediated tumor cell adhesion,[67,114] loss of lung elasticity in emphysema,[77,98,116] excessive narrowing of airways in asthma,[38] increased wall stiffness in hypertension, enhanced rigidity and adhesion of red cells to the endothelium in malaria and sickle cell disease,[65,73,94] and abnormal cellular mechanotransduction in deafness as well as polycystic kidney disease. There are numerous other examples in virtually all areas of medicine and surgery.[56] Even in embryonic development and cancer, it is physical forces, material flows, and differences in cellular mechanics that provide essential inputs in the program that drives cell sorting, differentiation, growth and angiogenesis.[58,89] In these cases, biomechanics underlies the abilities of the cell, tissue or organ to carry out normal functions in health or to malfunction in disease.

PAST ACCOMPLISHMENTS IN CELL MECHANICS

An essential aspect of biomechanics emerging from many lines of evidence is that cells are not only exposed to forces, stresses, and tensions, but that they also actively generate their own. This and additional determinants are summarized in a few pertinent examples.

Cardiovascular Cell Mechanics and Microcirculation

One of the most thorough analyses of the mechanical properties of living cells has been carried out on the mammalian red blood cell, which is a uniquely simple structure with predominantly two components—a membrane with bending and shearing properties that are dependent upon strain, strain rate, and strain history, and a cytoplasm that in the normal red cell is predominantly a Newtonian viscous fluid.[16] Quantitative passive biomechanical models were developed that serve to predict red cell motion and deformation in a large number of *in vivo* situations.[44,45,101,113] A key element of these models was the recognition that under the influence of membrane tension the lipid bilayer preserves membrane area within narrow limits. Discrete network models of the red blood cell membrane are increasingly taking into account the particular load-bearing functions of specific proteins (e.g., flexible spectrin springs, and actin protofilament nodes) as well as the key role of prestress for shape stability.[121] Newly developed constitutive models for the red cell membrane show the full power of biomechanical analysis not only as a starting point for prediction of whole cell and cell suspension behavior but also as a reference for molecular models

of cell membranes derived from the crystal structure of its constituents.

As another component of whole blood is the white blood cell, which are the basis of immune surveillance and inflammation (Fig. 1). Several generations of biomechanical models[25,31,112] have found use in prediction of cell–cell interactions in the microcirculation,[23] and similar models have been developed for endothelium, platelets, and metastatic tumor cells. These passive mechanical models were developed with a view towards the microcirculation, the cardiovascular system, and their modeling was based on fundamental principles of mechanics.

By integration of whole-cell viscoelastic mechanical models with traditional biofluid mechanics it has been possible to predict a considerable number of microvascular events.[101] Biomechanical analyses of different cell types in the circulation has yielded a new level of understanding of cell interactions in the circulation, and it is now possible to predict cell behavior in narrow vessels, such as capillaries. Models have been developed in several organs (e.g., lung, heart, skeletal muscle, connective tissue) that quantitatively predict basic aspects of organ perfusion.[39,115] In addition, there is increasing evidence to suggest that key steps in vascular development are under the control of biomechanical forces. Fluid shear stress may play a central role in the development of the first blood vessels in the yolk sac and the heart, and influences remodeling of the embryonic primary capillary plexus.[6,54] Blood pressure controls the development of arterioles[120] and cytoskeletal forces that capillary cells exert on extracellular matrix control angiogenesis during embryonic lung development.[81]

A major new area of research in endothelial cell mechanics is the role of the thin 150–500 μm thick layer of membrane bound macromolcules that ubiquitously coats the inner surface of all blood vessels.[122,128] Despite the striking fragility of this layer in response to light or chemical enzymes, it provides several vital functions necessary for life. It provides the molecular sieve that determines the oncotic forces necessary for the movement of water into and out of our microvessels,[78,126] provides a protective barrier that prevents adhesive molecular interaction between red and endothelial cell membranes,[126] allows white cells to roll freely through our circulation preventing the penetration of leukocyte microvilli except in areas of inflammation,[103] and appears to be the critical layer in the transmission of fluid shear stress into the cytoskeleton.[103,129] Recent experiment have shown that the integrity of the layer is necessary for flow alignment under fluid shear[118,132] and the regulation of eNOS release.[34]

Effects of Fluid Shear Stress on Vascular Function and Dysfunction

Disturbed or turbulent flow is widely recognized as being a leading cause of atherosclerosis. By manipulating fluid flow above them, underlying vascular cells can be transformed from normal to abnormal phenotypes. Ever since the earliest observations that endothelial cells orient themselves in the direction of fluid shear, a large body of evidence has come forward to show that just about all endothelial cell functions—from morphology, to signal transduction and gene expression—can be influenced by the

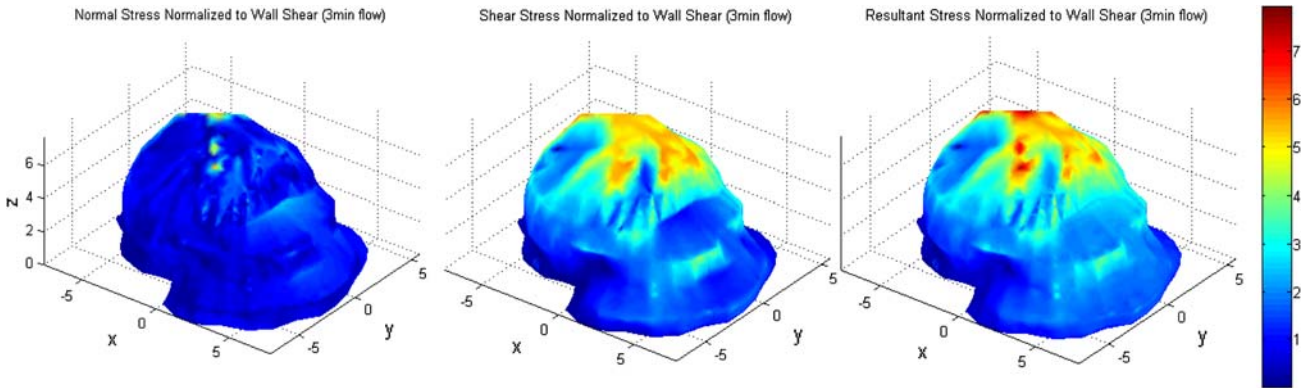

FIGURE 1. Distribution of normalized fluid stresses acting on the membrane of a migrating leukocyte with active cytoplasmic projections (pseudopods). The cell is attached to a flat glass surface and exposed to a constant shear stress. The fluid shear stress was determined by solution of the equation of motion for a Newtonian fluid (plasma) with non-slip condition on the cell membrane and on the substrate. All stress values are normalized by the applied shear stress (2.2 dyn/cm^2) on the cell substrate away from the cell. The cell shape was reconstructed from a three-dimensional stack of confocal images with a fluorescent membrane label. Su, SS: Fluid Stress on the Surface of a Migrating Leukocyte in a Flow Field and the Involvement of Formyl Peptide Receptor in Its Mechanotransduction. Ph.D. Thesis. Department of Bioengineering, University of California San Diego, 2007.

relatively modest forces generated by fluid shear stress. Today we recognize that just about all cell types inside and outside the cardiovascular system respond to fluid shear stress.[82] Even cells that are much more primitive than mammalian cells, like dinoflaggelates (red tide) in the ocean, respond to modest fluid shear stress.[14] Responses to similar shear stresses are cell-type specific, however. For example, endothelial cells elongate in the direction of flow but vascular smooth muscle cells elongate in a direction perpendicular to the direction of fluid shear, as do endothelial cells derived from heart valves. Cardiac myocytes, by contrast, respond by changing the beating rate of their sarcomeres without significant change in cell shape; migrating leukocytes show a dramatic changes in cell shape. There are also responses of cells to normal stress, but tend to require higher stress levels and produce a more selective response. As described below, mechanisms by which cells sense fluid shear stress is a very active area of study. The fact that the stimulus is the shear stress itself, rather than a chemical whose local concentration is affected by fluid flow, is now well established. Recent evidence suggest that cells may utilize existing receptors (e.g., G-protein coupled receptors, integrin membrane adhesion receptors),[72] a feature that may underlie the cell-specific response encountered in mechanotransduction and may suggest that biochemical pathways (e.g., via agonists and antagonists) may also be responsive to fluid shear stress. The interaction of membrane receptors with the glycocalyx and cytoskeletal structures may contribute to amplification mechanisms.

Development and Verification of Effective Surface Tension as a Mechanism for Cell Sorting

The active segregation of mixed cell types into multiple homogenous structures that lead to specific organs is a hallmark of early development. As early as the 1960's embryologists such as M. Steinberg proposed that cells spread and sort according to adhesion and surface tension, with multiple demonstrations of how organizational sorting could occur through a hierarchy of physical forces.[36,125] Modern molecular biology has identified at least some of the proteins and signaling pathways that are used for sorting, but the physical principles remain the underlying predictive mechanisms by which cell sorting occurs.[36] For example, the organization of endocrine cells in pancreatic islets is established through a series of morphogenetic events involving cell sorting, migration, and re-aggregation processes for which intercellular adhesion is thought to play a central role.[19,60,109]

Shape-dependent Control of Cell Fate Switching

The importance of cell shape and cell deformation for control of cell growth and function was first recognized almost 30 years ago.[35] Since that time it has become clear that mechanical distortion of cells resulting from physical interactions between cells and their extracellular matrix adhesions can regulate their responsiveness to soluble cues, and thereby govern switching between different fates, including growth, differentiation and apoptosis, as well as control directional cell motility.[15,30,88,111] Lineage switching of the human mesenchymal stem cell can even be controlled by either physically distorting the cells[75] or simply by varying the mechanical compliance of the extracellular matrix.[30]

Effect of Extracellular Matrix Stiffness and Tractions on Cell Structure and Function

Studies from the 1980's and earlier[49] showed that cells apply traction forces to the networks or the surfaces to which they are bound (Fig. 2). The inability of many solid tissue cell types to grow on liquid or very soft surfaces has long been used as a diagnostic to detect transformed cells. The importance of matrix stiffness for control of cell shape and function had thus been hinted at for some time, but the effects were most definitively demonstrated by Y.-L. Wang in the 1990's with studies of fibroblasts and epithelial cells on collagen-coated polyacrylamide gels of varied flexibility.[3,49] Traction force microscopy methods were subsequently developed to quantify this response.[20]

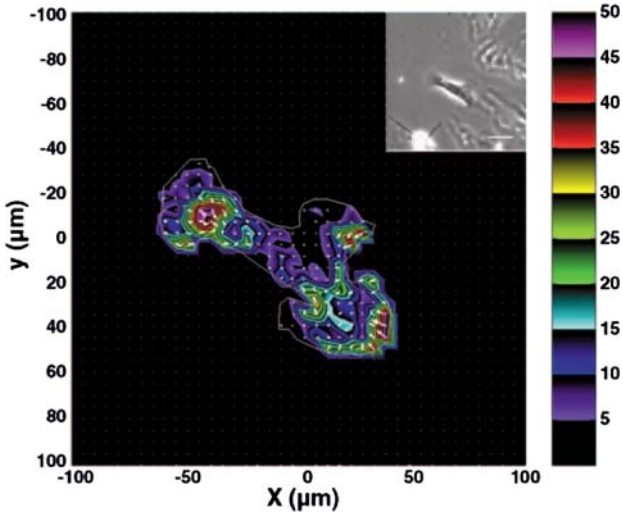

FIGURE 2. Traction microscopy image: Traction stress field exerted by a rat pulmonary microvascular endothelial cell upon its substrate. Inset: Phase contrast image at reduced magnification. Scale: Shear stress in Pascals. Adapted from An *et al.*[1]

Cell tension generated by nonmuscle myosins described first in the 1960/1970's and the level of prestress (isometric tension) in the cytoskeleton have consistently proven key to the sensitivity of cells to matrix elasticity; but the myosins only 'pull the trigger' that initiates downstream signal trajectories, which continue to be obscure.[12] It is nonetheless clear that in determining cell morphology, transcriptional programs, and cell fate, the stiffness of a cell's substrate and cell traction forces provide as much input as do chemical messengers.[30,55] Also, stretching of the cytoskeleton alone is sufficient to initiate biochemical signaling.[83,84,99]

Mechanotransduction by Primary Cilia and Other Elongated Structures

In the ear and kidney there are specialized cellular projections that serve as sensors of mechanical force. Auditory sensation is mediated by acoustic forces applied to the tips of stereocilia which are communicated to the transduction channels regulating the transport of Ca^{++} by gating springs that are stretched when the hair bundle is displaced towards its tall edge.[59] More recent studies have shown that there are two distinct mechanisms for transducer adaptation, one acting on a time scale of 1 ms or less in addition to the slower mode of 10 ms or more first identified in the pioneering work of Hudspeth and co-workers.[59] As a result, two models of hair cell adaptation have been proposed, an active motor associated with a tension generating system that slowly slips down an actin-myosin network and a calcium closure mechanism that acts on a shorter time scale.[53]

In the kidney, two possible mechanical sensors of flow have been proposed. In the proximal tubule the fundamental mystery, since it was first observed four decades ago,[102] is glomerular tubular balance, which is the ability of the brush border epithelium to sense the filtered load that enters the tubule and reliably reabsorb 2/3 of this flow independent of the glomerular filtration rate. Guo *et al.*[47] proposed that the 4000 microvilli per cell act as a flow sensor that responds collectively to the bending moment produced by the fluid shear stress acting on microvilli tips. This hypothesis was quantitatively confirmed by the experiments of Du *et al.*[27] using a mathematical model for the hydrodynamic forces acting on brush border microvilli. In the cortical collecting duct there are two cell types, principal and intercalated cells, with the former secreting K^+ and the latter regulating pH. Praetorius and Spring[93] have proposed that principal cells have primary cilia which regulate intracellular calcium. In both cell types it has been difficult to determine whether it is flow or stretch that leads to the abrupt increase in intracellular calcium[69] and, in principal cells, whether the flow sensor is the primary cilia. In addition, defects in the structure/function of the primary cilia in polycystic kidney disease appear to be due to the stunting of these cellular projections.[68] Biomechanical models have contributed greatly to the interpretation of these experiments.

The Musculoskeletal System

Bone cells (osteocytes) live in a rigid mineralized tissue. Accordingly, one of the most intriguing problems in mechanotransduction is how such cells are able to sense mechanical loading associated with locomotion. Bone is widely recognized to atrophy in the weightlessness of space or prolonged bed rest, for example, and fractures resulting from osteoporosis are one of the most costly health care risks in an aging population. Wolff recognized more than a century ago that trabecular bone adapts to loading by developing an architecture that closely paralleled the principal stresses in bone tissue.[130] Although Piekarski and Munro[91] postulated that bone cells receive their nutrition though load-induced fluid flow in the lacunar-canalicular system, there is little connection between this hypothesis and the remarkable observation by Rubin and Lanyon[97] that bone maintenance requires nothing more than a few cycles per day of mechanical loading that produce in excess of 1000 microstrain. Weinbaum *et al.*[127] hypothesized that the fluid shear stress acting on cell processes was the activating signal. If the pericellular space contains a matrix with sieving properties that exclude albumin, their theoretical model then predicts that fluid shear stress would be comparable in magnitude to that acting on vascular endothelial cells even though dimensions of these pores are two orders of magnitude smaller than that of capillaries. This highly non-intuitive prediction conforms to flow culture experiments of Reich and Frangos[96] and numerous subsequent investigators.[48,133,124]

More broadly, tissues of the musculoskeletal system show exquisite sensitivity to their biomechanical environment, and it is now clear that mechanical forces serve as a regulatory factor not only in remodeling of bone, but also muscle and cartilage. Disuse of joints leads to cartilage atrophy, whereas overuse and abnormal loading are associated with degenerative joint diseases such as osteoarthritis.[46] These processes appear to be mediated at the cellular level through complex interactions between biomechanical factors, soluble mediators, and genetic programming. Thus a further understanding of such mechanisms regulating cell behavior under physiologic or pathologic conditions could provide new insight into the development

of physical or pharmacologic therapies for mechanically based musculoskeletal diseases such as osteoporosis, osteoarthritis, and muscular dystrophy/atrophy.

Cell Stretch, Bronchospasm, and Asthma

Of all known bronchodilatory agents or drugs, the single most potent is a simple deep inspiration. In asthma, however, this innate protection agency fails, and this failure has been implicated as a proximal cause of excessive airway narrowing.[38] We now have insights into this fundamental bronchodilatory mechanism: a deep inspiration stretches the airway smooth muscle cell and causes its contractile machinery and cytoskeletal scaffolding to fluidize.[86,119] We understand less well the failure of the airway smooth muscle cell to fluidize in the spontaneous asthmatic attack, but this failure is now known to involve transition of the airway smooth muscle cell to a state in which its cytoskeletal network becomes frozen in a stiff glassy phase, and involves the modulation of that transition within the airway smooth muscle (ASM) cell by MAP kinase pathways,[26] remodeling of the ASM contractile apparatus and its cytoskeletal scaffolding,[52,105] increase of ASM mass,[62] and by remodeling of connective tissues within the surrounding airway wall.[86]

Stretch and Mechanoprotection

Cells exhibit a repertoire of strategies to protect themselves against damage caused by imposed mechanical stretch—mechanoprotection. The best known strategy is reinforcement and resulting cytoskeletal stiffening that results from activation of stress-activated ion channels, the small GTPase Rho, and myosin-dependent focal adhesion assembly, as well as tensegrity-based interactions within the microfilament–intermediate filament–microtubule lattice of the deep cytoskeleton.[74,123] Recent studies suggest that the cell can deploy another strategy for mechanoprotection that is based on cytoskeletal fluidization.[9,86,119] In cells resident in organs that routinely undergo large stretch (heart, gut, lung), fluidization implies dramatically augmented mobility of macromolecules which, ordinarily, is thought to be markedly retarded due to molecular crowding, caging, and assembly.[22,43,70,79] Hence, molecular crowding and cell stretch are now understood to have potent but opposite compensatory effects. Finally, prompt fluidization followed by slow resolidification provides the freedom for the cell to move and reorganize its contractile units, stress fibers, and adhesion processes in response to mechanical stress or other stimuli.[18]

Mechanotransduction

Cells are constantly pulling on their surroundings in order to probe and adjust to their mechanical microenvironment.[95] Stem cell differentiation was already mentioned as strongly influenced by the substrate stiffness.[30] Recent evidence suggests that a number of molecules and cellular structures are involved: stress-activated ion channels, transmembrane proteins that mediate cell-matrix or cell–cell contacts, focal adhesion complexes, membrane lipids, glycocalyx proteins, and also G-protein coupled receptors can all serve as mechanosensors and transducers.[57] Thus, receptors that traditionally have been thought of as being under the control of biochemical agonist and antagonist molecules, might also respond to fluid shear or substrate strain by a conformational change to initiate intracellular signaling events.[8] However, activation of these receptor signaling cascades by mechanical cues may produce entirely different physiological responses depending on the overall deformation state of the cell and cytoskeleton, and the physical context of the tissue and organ in which the cell normally experiences mechanical stresses.[57]

CHALLENGES FOR THE FUTURE

What Determines Stiffness and Other Constitutive Physical Properties of the Cell?

To answer this question, top-down reductionist vs. bottom-up integrative approaches presently define a divide. Bottom-up reductionist approaches[2,92] are sometimes rooted in the traditional viscoelastic paradigm. At the level of fundamental constituents and their use in reconstituted systems *in vitro*, cell-derived materials and molecules are terrifically rich because, among other things, they are well-defined, they can have motors and crosslinkers that provide contraction and change on/off rates, and they can be manipulated precisely.[13,16,106] Additionally, like living cells these materials can get around limits set by thermal forces and the fluctuation–dissipation theorem.[80] Nonetheless, current understanding of cell-derived materials and their major molecular components have thus far failed to account for integrated cell physical properties, such as stiffness, in most cases even to within orders of magnitude.[41] In certain limiting cases,[21] or if the networks are prestressed,[41] physical properties comparable to those observed in cells can be approximated, but mechanism remains unclear. As such, constitutive models in cell mechanics are available today, but with limited ability to predict intracellular biological events. From a top-down perspective, by contrast, there has emerged a striking analogy between the dynamics of

the intact living cell and that of the universal but relatively featureless pattern of inert soft glassy materials such as foams, pastes and colloids.[32,61,119] Glassy dynamics cannot predict what will happen to matrix dynamics if a particular protein is mutated, but suggest that dynamics of cytoskeletal proteins generically, and transitions between fluid-like vs. solid-like behavior in particular, are governed by free energy barriers incorporated into a rough energy landscape.[37] Integration of the competing paradigms of viscoelasticity vs. glassy dynamics, along with the central role of cytoskeletal prestress,[117] into a single unified framework has become a major challenge in cell biophysics,[107,108] but promising inroads have been suggested in a recently proposed model of a 'glassy wormlike chain'.[61,104] Whether similar concepts apply to the observed power law rheology of the chromatin-packed nucleus remains unclear, but there are intriguing differences in nuclear mechanics between embryonic stem cells and committed cells.[87]

How to Measure Stiffness and Forces More Accurately and Non-invasively

Developments in ultrasound, MRI, and other methods might make it possible to determine frequency and/or strain rate dependent viscoelastic properties in whole tissues without disrupting resident cells. Spatial resolution at the cellular scale within intact tissues is not so clearly on the horizon, but concepts of super-resolution in optics and other imaging fields could provide opportunities. Effective methods exist to measure details of mechanical strain and strain-rates for localized cytoplasmic regions.[76,110]

Impacts on New Biomaterials and Artificial Organs

Chemical synthesis and degradation as well as issues such as cell attachment and solute diffusion have been principal design criteria for tissue replacements and bioreactors to date. A growing field, termed "Functional Tissue Engineering," has sought to further emphasized the role of biomechanical factors in the repair and regeneration of tissue.[10] In particular, a further understanding of the ability of cells to perceive and respond to their mechanical environment is necessary within the context of engineered tissue replacements. At the cellular and subcellular level, compliance and such features as cytoskeletal rheology, strain-stiffening, and fluidization, and their interaction with scaffolds and other artificial extracellular matrices will likely need to be worked into the operating principles of tissue engineers.

Biomechanical Therapeutics and Diagnostics

Investigation of the role of cell mechanics in inflammatory cardiovascular cell activation is still in an early state. This is a field with considerable opportunities, since inflammation is now being recognized as common to many human diseases, including aging, cancer,[41,42] and a large number of chronic and acute diseases. One of the hallmarks of inflammation is the activation of endothelial cells, leukocytes and platelets with local actin polymerization in form of pseudopod formation. These events serve as ready diagnostic targets, as signposts to trace the origins of inflammation, and as therapeutic targets. Inflammation is associated with cell interactions that include leukocyte attachment to endothelium, homotypic cell interactions, and highly regulated cell interactions to specific tissue structures (e.g., lymphocytes to high endothelium in lymph nodes). Increasing evidence shows that pseudopod formation is controlled by a group of proteins that controls not only the shape of the actin polymers to be formed, but also the location and rate of the polymerization, its attachment to integrins, and specific actions by myosin-like motions with actin polymers.

Leukocyte rolling and attachment to the endothelium[24,63] have become the subjects of extensive analysis (Fig. 3), but less studied are mechanisms for migration across the endothelium, mechanisms for endothelial pore formation, and mechanics of extracellular matrix proteins such as the basement membrane, which appears to be a relatively strong mechanical structure

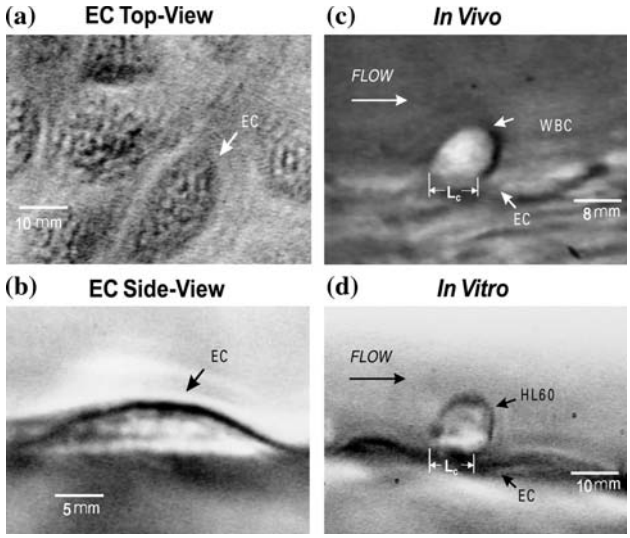

FIGURE 3. ***In vitro*** **flow assays yield images obtained from both top-view and side-view of an adherent cell under flow conditions compared with** ***in vivo*** **images.**[11,64]

that requires proteolytic breakdown in inflammation. Migration of cells in the interstitial space, as a coordinated event between pseudopod formation/retraction and controlled membrane attachment/detachment, is largely unexplored at the whole cell level; few predictive theories have been advanced and tested. The role of the extracellular matrix and basement membrane in tumors is also beginning to attract increased attention from a biomechanics point view because changes in matrix structure and mechanics can promote tumor formation.[89] Other important biomechanical events in inflammation are mechanisms that control mechanical attachment of adhesion molecules in cell layers, such as the endothelium or the epithelium in the skin or in the intestine.

Preservation of barrier properties is one of the key mechanisms to minimize the inflammatory cascade as well as cancer metastasis.[66,90] Separation of cell sheets (e.g., interendothelial pores) or pore formation inside a cell cytoplasm (e.g., transendothelial pores) are controlled by biomechanical events including the effects of fluid shear stress and anchoring by inter-endothelial adhesion molecules, like VE-cadherin.

Another key event to minimize the inflammatory cascade is the transport in the lymphatic system. Although mechanical contributions to lymphatic function have been long recognized, these responses have never been quantified or defined at the molecular biophysical level. The fundamental function of the lymphatic system is still open to speculation and disagreement, and therefore biomechanics needs to play a central role to identify its modus operandi, including transport of antigens, viruses, cellular reactions to antigen presenting cells, cell contact, and apoptosis.

From Genomics to Biomechanics

There is ample evidence that many biological processes surrounding growth and disease are governed by mechanics. Mechanics, in turn, is concerned with three-dimensional shapes, dynamics of deforming bodies, forces, and energies. Mechanics is based on fundamental physical principles that give it the unique ability to predict and anticipate events on many different scales. Given a full genomic blueprint today and given a good deal of homology between proteins among many cell and animal types, we have no formulation of physical laws from which we might predict stem cell fate in a given niche or microenvironment, let alone the shape of the encoded organism or its motions.[50] Progress is being made on mechanics of chromatin, tension-dependent controls of chromosome movements, and biomechanics of RNA polymerases, but our limited understanding of protein folding and unfolding,[7,8,51] molecular crowding,[22,29,70] and the thermodynamics of small open systems that reside far from thermodynamic equilibrium[50,71] remain important barriers.

Three-dimensional Matrices

Cell biology is in the early phase of moving from cells cultured on flat rigid 2D substrates to cells grown within 3D compliant substrates, a state far more physiologic for most cells. Associated biological knowledge and methodological approaches require much further development. Transport issues need to become an integral part of compliant 3D tissue matrices, as layers of metabolically active cells will consume and create gradients of oxygen as well as other metabolites. Interstitial velocities that arise from cell motion might be small, but shear stresses on interstitial cells might well reach physiologically relevant levels and shape interstitial structures.

Many soluble factors such as growth factors interact with matrix and their homogeneity seems unlikely; growth factor expression even appears to depend on matrix elasticity.[54] Heterogeneity in 3D cultures could couple to stress generation of cells and seems likely to have biologically significant effects on key pathways that include oxygen sensitive transcription factors. Cells in interstitial spaces are not only subject to such soluble factors (e.g., cytokines) but also are simultaneously subject to solid molecular attachments (e.g., via integrins) as well as to fluid shear stresses, with sensors that include G-protein coupled receptors and stretch-sensitive ion channels. How these multiple cues are integrated by cells is a challenging facet of ''Biomechanics Inside.''

Funding: Obstacles and Opportunities

Paradoxically, a major obstacle surrounding funding in biomechanics may have stemmed from the central dogma of molecular biology, in which the gene is held to be at the apex of a causal cascade of highly specific signaling pathways, and a physical picture in which this cascade of events plays out according to the rules of dilute solution biochemistry. Within such a conceptual framework, physical forces are easily dismissed as being merely end-effects that reside far downstream of initiating causal events. Related funding obstacles have been concerns surrounding causality and specificity; physical force, after all, is innately nonspecific in both its causes and its effects. Specific genes and signals do indeed control cell-based generation of physical forces, but, as described above, there is now a growing recognition that physical forces, cell shape, cytoskeletal tension, and cell deformation—all of which are nonspecific—act to control signal transduction, gene

expression, and stem cell fate.[15,40,88,111] Therefore, the framework of linear and specific causal cascades playing out according to the rules of dilute solution chemistry, with genes at the top of the cascade and physical forces near the bottom, is now being replaced by the notions that the cell interior is a crowded chemical space[22,70] that is much closer to the solid state than the fluid state,[33,119] and a framework in which there plays out a web of causality in which cell structure, physical forces, and epigenetic factors are seen to play indispensable roles.

Taken together, these obstacles comprise a creative tension that highlights the need for fuller integration of quantitative cell mechanics into normative biology. Because cell mechanics has application to all human diseases, its inclusion into health related research efforts represents a major opportunity.

CONCLUSION

Despite remarkable progress to date, the challenges outlined above suggest that the greatest discoveries in cell mechanics are yet to come. It remains unclear how to augment basic principles of mechanics (conservation of mass, momentum, and energy) with a set of principles embracing basic biological paradigms so as to unify mechanics with cell biology, development, and molecular biology. With suitable resources and genomic data, we should one day be able to predict quantitatively and from first principles the way a cell divides, differentiates, matures, and migrates. We should be able to predict how a cell generates and incorporates itself into an extracellular matrix, as well as the shapes it assumes. Biology could thus become a truly predictive and mechanistic science in which forecasts of multifactorial processes impacting living systems—in groups as well as at the individual level—could be used not only to make informed decisions but also to improve health by creating new effective drugs and inexpensive replacement tissues.

Nowhere is our current limited ability to predict biological processes better visible than at the level of individual cells, i.e., in cell biomechanics. Few cell shapes or material properties have ever been predicted based on fundamental principles of mechanics. We will have to learn how to understand the mechanics of single proteins and lipid membranes, groups or modules of proteins and lipid structures, and integrate these into predictions for whole cell behavior.[17] These opportunities are rich and enduring. There are many immediate opportunities to study the role of cell biomechanics in reproduction, growth and tissue repair, in numerous organ systems such as orthopedic and cardiovascular mechanics, as well as in a long list of diseases, from the malformations of primary genetic defects to inflammation and eventual cell death.

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