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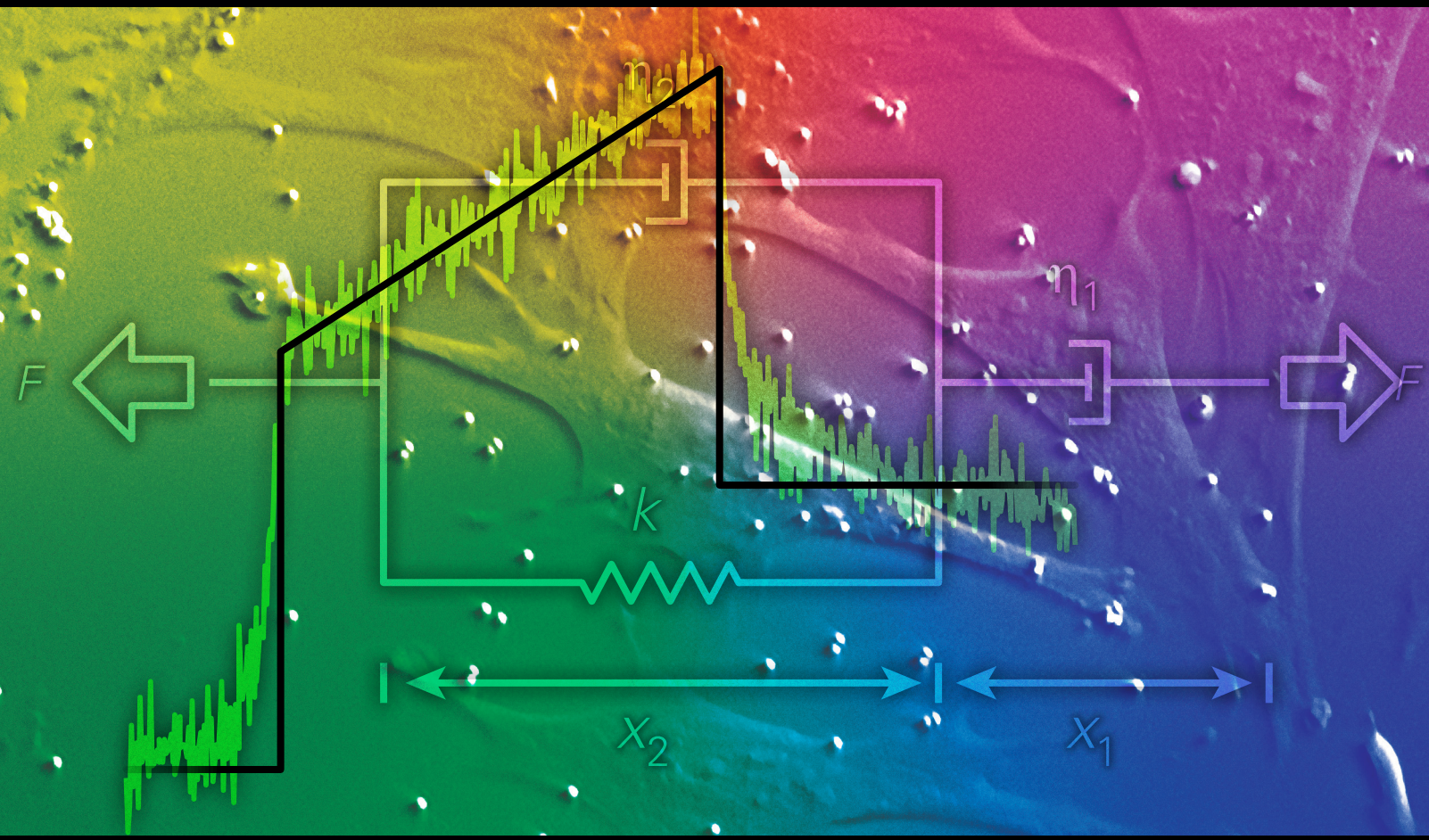
Introduction to Cell Mechanics and Mechanobiology



Christopher R. Jacobs
Hayden Huang
Ronald Y. Kwon

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Preface

In recent years, mechanical signals have become widely recognized as being critical to the proper functioning of numerous biological processes. This has led to the emergence of a new field called cellular mechanobiology, which merges cell biology with various disciplines of mechanics (including solid, fluid, statistical, computational, and experimental mechanics). Cellular mechanobiology seeks to uncover the principles by which the sensation or generation of mechanical force alters cell function. *Introduction to Cell Mechanics and Mechanobiology* presents students from a wide variety of backgrounds with the physical and mechanical principles underpinning cell and tissue behavior.

This textbook arose from a cell mechanics course at Stanford University first offered by two of us in 2005. Over several iterations, we taught from a set of course notes and chapter excerpts—having found no textbook to cover the necessary breadth of topics. Our colleagues had similar experiences teaching with the same adhoc approach, which convinced us of the need for a comprehensive instructional tool in this area. Another reason we felt compelled to write this text is that cell mechanics provides an excellent substrate to introduce many types of mechanics (solid, fluid, statistical, experimental, and even computational). These topics are traditionally covered in separate courses with applications largely focused on engineering structures. As authors, we have varied backgrounds, but share a common fondness for the insights mechanical engineering brings to cell biology.

Introduction to Cell Mechanics and Mechanobiology is intended for advanced undergraduates and early graduate students in biological engineering and biomedical engineering, including those not necessarily in a biomechanics track. We do not assume an extensive knowledge in any area of biology or mechanics. We do assume that students have a mathematics background common to all areas of engineering and quantitative science, meaning exposure to calculus, ordinary differential equations, and linear algebra.

The field of cell mechanics encompasses advanced concepts, such as large deformation mechanics and nonlinear mechanics. We do not expect our audience to have a strong background in the advanced mathematics of continuum mechanics. Our intent is to avoid graduate-level mathematics wherever possible. In our approach, the treatment of tensor mathematics—central to large deformation mechanics (common in cell mechanics)—poses unique difficulties. To show simplified mathematical derivations measuring mechanical parameters in the context of living cells, we present tensors “by analogy” as matrices, rather than introducing them in a fully rigorous fashion. For example, we skip index notation entirely. Admittedly, this approach may be less satisfying to mechanicians, which we also consider ourselves. However, we hope that the advantages of this approach will outweigh our oversimplifications.

The book is grouped into two parts: (I) Principles and (II) Practices. We have written the chapters to allow instructors flexibility in presentation, depending on the level of students and the length of the course. After introducing cell mechanics as

a framework in Chapter 1, we provide a review of cell biology in Chapter 2. The next four chapters establish the necessary concepts in mechanics with enough depth that the student attains a basic competency and appreciation for each topic. Chapter 3 covers solid mechanics—including rigid and deformable bodies as well as a short overview of large-deformation mechanics. Fluid mechanics (Chapter 4) is important for cell mechanics not only in cytoplasmic flow, but also as a physical signal that regulates cell mechanobiological behavior. Chapter 5 dives into statistical mechanics, with descriptions of energy, entropy, and random walks, common themes for understanding the aggregate behavior of systems composed of many objects. In Chapter 6, we describe experimental methods, an area that is always changing, but is essential in demonstrating how theory may be reconciled with actual experiments. These fundamentals in Part I are followed by cell mechanics proper in Part II. Chapters 7–9 begin with a discussion of an aspect of cell biology followed by analysis of the mechanics. We undertake polymer mechanics in Chapter 7 from a continuum and a statistical viewpoint and examine situations in which both need to be considered simultaneously. These tools are applied to individual cytoskeletal polymers as well as to other polymers such as DNA. Polymer networks are presented in Chapter 8, with a focus on the role of the cytoskeleton in regulating physical properties, such as red blood cell shape and limitations on cell protrusion lengths. Chapter 9 examines the bilayer membrane, from both the perspective of matter floating around within it (diffusion) as well as a mechanical perspective of bending and stretching. The last two chapters address mechanobiology. Chapter 10 is focused on cellular force generation and the related processes of adhesion and migration. Chapter 11 discusses the process of mechanosensing or mechanotransduction and intracellular signaling. These last chapters do not have as much rigorous mechanical engineering mathematics, but are an integral part of cell biomechanics.

Given the varied backgrounds of our students and the interdisciplinary nature of the subject, we have attempted to provide some guidance on the treatment of variables and units. At the start of the book, we present a master list of all the variables used in the text that specifies exactly what each variable is used for in a particular chapter. We have retained the "contextual" usage in each chapter, accepted within each field, to prepare students for reading the literature. Three types of boxes supplement the main text: "Advanced Material" challenges readers to think critically and problem-solve; interesting and noteworthy asides are denoted as "Nota Bene"; "Examples" provide in-depth solved calculations and explanations. Each chapter concludes with a set of Key Concepts, Problems that can be used as homework sets, and Annotated References that guide students for further study.

Online Resources

Accessible from www.garlandscience.com/cell-mechanics, Student and Instructor Resource websites provide learning and teaching tools created for *Introduction to Cell Mechanics and Mechanobiology*. The Student Resources site is open to everyone, and users have the option to register in order to use book-marking and note-taking tools. The Instructor's Resource site requires registration; access is available to instructors who have assigned the book to their course. To access the Instructor's Resource site, please contact your local sales representative or email science@garland.com. Below is an overview of the resources available for this book. Resources may be browsed by individual chapters and there is a search engine. You can also access the resources available for other Garland Science titles.

For students:

- Computer simulation modules in two formats: ready-to-run simulations that simulate the mechanical behavior of cells and tutorial MATLAB modules on simulation of cell behavior with the finite element method.
- Color versions of several figures are available, indicated by the figure legend in the text.

- A handful of animations and videos dynamically illustrate important concepts from the book.
- Solutions to selected end-of-chapter problems are available to students.

For instructors:

- In addition to color versions of several figures, all of the images from the book are available in two convenient formats: Microsoft PowerPoint® and JPEG. They have been optimized for display on a computer. Figures are searchable by figure number, figure name, or by keywords used in the figure legend from the book.
- The animations and videos that are available to students are also available on the Instructor's Resource website in two formats. The WMV-formatted movies are created for instructors who wish to use the movies in PowerPoint presentations on computers running Windows®; the QuickTime®-formatted movies are for use in PowerPoint for Apple computers or Keynote® presentations. The movies can easily be downloaded to your personal computer using the "download" button on the movie preview page.
- Solutions to selected end-of-chapter problems are available to qualified adopters.

The origin of the book is rooted in teaching from sections of outstanding books by David H. Boal, Jonathon Howard, and Howard C. Berg. We thank Roger Kamm, Vijay Pande, and Andrew Spakowitz, who taught some of us at various times and have unselfishly shared course materials and handouts and, in the case of Dr. Kamm, unpublished drafts of his own textbook. With their permission, we have incorporated their approach to some topics in Chapters 4, 5, 7, 8, and 9 and adapted several problems into sections of our book. We are grateful for their amazing willingness to share their intellectual product in the name of improving the educational experience of students around the world. We also thank reviewers Roland R. Kaunas and Peter J. Butler, who shared notes from their own courses in cell mechanics. We are profoundly appreciative of the tireless work of those who have preceded us, without whom we never could have completed this task. We thank the additional reviewers of the book, Dan Fletcher, Christian Franck, Wonmuk Hwang, Paul Janmey, Yuan Lin, Lidan You, and Diane Wagner, for their valuable insight and critiques of our drafts. We are also grateful to Summers Scholl and the editorial and production teams at Garland who took a chance on three textbook neophytes and guided us unerringly through uncharted waters. Finally we are each deeply indebted to our families, including Roberta, Jolene, VH, YYH, LHH, Joyce, Melody, Tae, and Cynthia. Without your support, patience, and understanding—as this project took us away from you on so many nights and weekends—we never could have contemplated this undertaking, much less completed it.

Christopher R. Jacobs
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Ronald R. Kwon

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Detailed Contents

PART I: PRINCIPLES

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CHAPTER 1

Cell Mechanics as a Framework

Biological cells are the smallest and most basic units of life. The field of cell biology, which seeks to elucidate cell function through better understanding of physiological processes, cellular structure, and the interaction of cells with the extracellular environment, has become the primary basic science for better understanding of human disease in biomedical research. Until recently, the study of basic problems in cell biology has been performed almost exclusively within the context of biochemistry and through the use of molecular and genetic approaches. Pathological processes may be considered disruptions in biochemical signaling events. The regulation of cell function by extracellular signals may be understood from the point of view of binding of a molecule to a receptor on the cell surface. Basic cellular processes such as cell division are considered in terms of the biochemical events driving them. This emphasis on biochemistry and structural biology in cell biology research is reflected in typical curricula and core texts traditionally used for cell biology courses.

Recently, there has been a shift in paradigm in the understanding of cell function and disease primarily within the analytical context of biochemistry. In particular, it has become well established that critical insights into diverse cellular processes and pathologies can be gained by understanding the role of mechanical force. A rapidly growing body of science indicates that mechanical phenomena are critical to the proper functioning of several basic cell processes and that mechanical loads can serve as extracellular signals that regulate cell function. Further, disruptions in mechanical sensing and/or function have been implicated in several diseases considered major health risks, such as osteoporosis, atherosclerosis, and cancer. This has led to the emergence of a new discipline that merges mechanics and cell biology: cellular mechanobiology. This term refers to any aspect of cell biology in which mechanical force is generated, imparted, or sensed, leading to alterations in cellular function. The study of cellular mechanobiology bridges cell biology and biochemistry with various disciplines of mechanics, including solid, fluid, statistical, experimental, and computational mechanics.

The primary goal of this introductory chapter is to motivate the study of cell mechanics and cellular mechanobiology by: (1) demonstrating its role in basic cellular and pathological processes; and (2) showing how cell mechanics provides an ideal framework for introducing a broad mechanics curriculum in an integrated manner. We first present cell mechanics in the context of human disease by providing a survey of physiological and pathological processes that are mediated by cell mechanics and can be better understood through mechanical analyses. Next, we propose cell mechanics as an ideal substrate for introducing principles of solid, fluid, statistical, experimental, and even computational mechanics, and put forth the argument that cell mechanics may be the grand challenge of applied mechanics for the twenty-first century. Finally, we present a simple model problem: micropipette aspiration, in which a cell is partly "sucked" into a narrow tube by a vacuum. This example will help you develop a feeling for how cell mechanics is studied and demonstrate how a relatively

simple approach can give important insight into cell mechanical behavior (and how this behavior can dictate cellular function).

1.1 CELL MECHANICS AND HUMAN DISEASE

Most of our understanding of biomedicine, both in terms of health and disease, is biological or biochemical in nature. There are some exceptions, of course, such as the component of mechanics at the tissue or whole organism level when we think about fracture of a bone, soft tissue trauma, or surgical repair. Further, when you think of your senses that involve mechanics, such as hearing and touch, the fact that mechanically specialized cells are involved is unsurprising. By contrast, we do not typically think about mechanics of cells in relation to cancer, malaria, or viral infections—but they are related. What may be even more of a surprise is that many of the causes of human suffering involve cell mechanics to some degree or other.

For instance, the health of several tissues, particularly tissues of the skeleton (bone and cartilage) and of the cardiovascular system (the heart and arteries), is heavily dependent on mechanical loading, which in turn comes from physical activity and the environment (gravity). To be clear, we are not simply saying that these physiological systems have a mechanical function (bones support the body and the heart pumps blood)—which they do. We are also saying that these systems actively change and respond to changes in mechanical forces at the cellular level—bones will reinforce certain regions and actively degrade others. In this chapter we hope to convince the reader that mechanics is in fact involved in virtually every aspect of life, although its influence may be subtle or indirect.

Understanding human health and disease often requires an understanding of biomechanics and mechanobiology at the cellular level, for example:

- When bone cells do not experience proper mechanical stimulation, bone formation ceases and bone resorption is initiated. So, in prolonged space travel, where gravity is virtually nonexistent, astronauts face major bone loss, even with rigorous exercise regimens.
- In coronary artery disease, changes in the temporal and spatial patterns of fluid shear stress on endothelial cells are linked to the formation of atherosclerotic plaques.
- The pathogenesis of osteoarthritis occurs due to changes in physical loading that lead to altered mechanical signals experienced by chondrocytes.
- Lung alveolar epithelial cells and airway smooth muscle cells are regulated by cyclic mechanical stretch during breathing, and hypersensitization due to airborne pathogens that can lead to sustained hypercontractility, which in turn can cause asthmatic attacks.
- Infection can be initiated from mechanical disruption of the cell membrane by viruses delivering foreign genetic material. This is a serious problem—if we could deliver genes as easily as viruses, we could potentially cure many genetic diseases by having cells express the corrected version (of the mutated gene). But the cell membrane is actually an excellent mechanical barrier.
- Metastatic cancer cells must be able to migrate through tissue and attach at distant sites to spread. Why certain cancers appear to metastasize preferentially to particular locations is still a mystery.
- Mechanical stimuli regulate fibroblast behavior during wound healing. Further, there is a difference between “normal” wound healing, where the wound is grown over, and the development of scar tissue.
- Physical forces are also known to be a critical factor in the regulation of the tissue-specific differentiation of adult and embryonic stem cells. For example, it is thought that the beating of some mammalian embryonic hearts is more for shaping the

heart muscle rather than for functional pumping, given that the heart does not need to pump blood in any serious manner *in utero*.

- Post-birth, brain development and angiogenesis all centrally involve cells' ability to interact with their dynamic mechanical environment.
- Cardiovascular diseases such as hypertension and heart failure often result from long-term mechanical influences. Indeed, cardiac hypertrophy is one of the most common responses to changes in forces. The distinction between healthy hypertrophy (resulting from exercise) versus pathological hypertrophy (resulting from poor health) is still not well understood.
- The fundamental cellular processes of membrane trafficking, endocytosis and exocytosis (the ways in which a cell engulfs or expels substances, respectively), microtubule assembly and disassembly, actin polymerization and depolymerization, dynamics of cell–matrix and cell–cell adhesions, chromosome segregation, kinetochore dynamics (such as DNA motion during cell division), cytoplasmic protein and vesicle sorting and transport, cell motility, apoptosis ("programmed cell death"), invasion (motion of a cell to where it is not usually located), and proliferation and differentiation (specialization of a cell to a phenotype with a particular function) are all regulated, at least in part, by mechanical forces.

In the sections that follow, we examine a few of these examples in more detail.

Specialized cells in the ear allow you to hear

At its most basic level, hearing is a process of transduction (transduction being the conversion of a signal from one type to another). A physical signal in the form of sound (pressure) waves is converted into electrical impulses along a nerve. Mechanotransduction (transduction in which the incoming signal is mechanically based) occurs in the ear via a specialized cell called the inner ear hair cell. This cell has small hairs called *cilia* (singular: cilium) extending from the apical (top) surface of the cell into the lumen of the cochlea. Sound in the form of pressure waves caused by vibrations of the inner ear bones travels through the fluid in the cochlea.

Investigators have recently deduced the remarkable mechanism of transduction in the hair cell. Filaments (fibers) of the cytoskeletal protein actin were identified linking the tip of one cilium to the side of an adjacent cilium (**Figure 1.1**). The actin filaments are anchored to proteins that span the cell membrane and form small holes or pores known as channels. These channels are normally closed, but, when open, permit the passage of small ions (in the case of hair cells, calcium

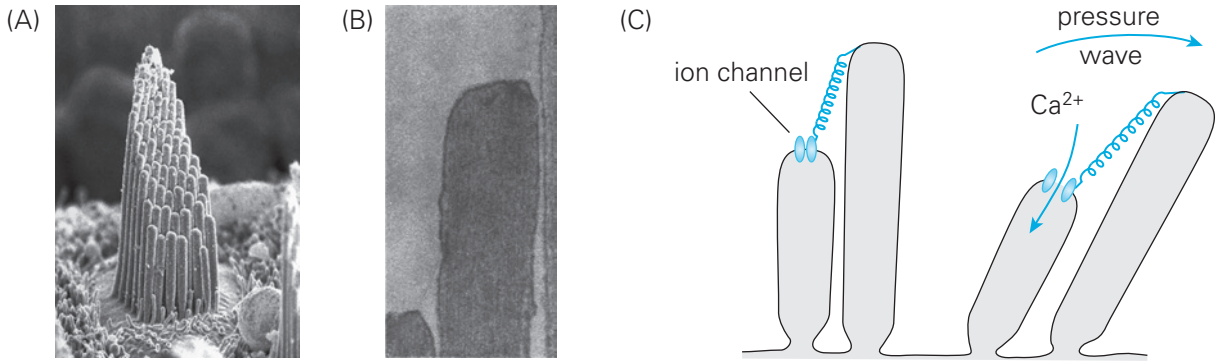

Figure 1.1 Hearing occurs via mechanotransduction by the inner ear hair cell. (A) The bundle of cilia extending from the apical surface of the cell is deflected by pressure waves in the cochlear lumen. (B) Tiny actin bundles called tip links are stretched as the cilia deflect due to the pressure wave. (C) The tip links are attached to calcium channels or pores that open and allow calcium into the cell where it eventually leads to a nerve impulse. (A, Courtesy of Dr. David Furness; B, from, Jacobs RA, Hudspeth AJ (1990) *Symp. Quant. Biol.* 55, 547–561. With permission from Cold Spring Harbor Press.)

ions) along their concentration gradient. In the resting state, the cell keeps its internal calcium level extremely low (<1 mM) relative to the calcium concentration outside the cell. When sound is transmitted to the inner ear, the vibrations cause the cilia to deflect, which in turn stretches the actin filaments. This stretching creates tension that is transmitted to the channels, causing them to open. So, when the channel is opened, calcium flows down its concentration gradient, and the intracellular calcium concentration increases. The kinetics of signaling proteins inside the cell are altered by this change in concentration, and a cascade of biochemical events is initiated that eventually leads to a depolarization of the cell and a nerve impulse.

As you might imagine, mechanics is very important in this process. The cilia need to have the right mechanical characteristics to stand upright, but remain flexible enough that they can be deflected by sound waves. The actin tip links need to be strong enough to open the channel and to have the appropriate polymer mechanics behavior so that they are stretched by cilium deflection, but are not affected by thermal noise (recall that these are very small objects, so the soup of molecules floating around will periodically collide with them, and can generate some forces that need to be ignored). In this text, our goal is to build a foundation and present a framework so that you can consider these questions effectively.

Hemodynamic forces regulate endothelial cells

Blood vessels are not passive piping for the blood. They are very responsive and are constantly changing their radius (via vascular tone or under the influence of vasodilators and vasoconstrictors) and leakiness. The cells lining these vessels are called *endothelial* cells (or collectively, the *endothelium*). Endothelial cells are very responsive to mechanical forces generated by the circulatory system, including the shear from flow, stretch from the distension of the (larger) vessels, and transmural pressure differences (pressure differences between the inside of the vessel and outside). The response of the endothelial cells is varied—they can change shape to align their long axis in the direction of flow, alter their internal structure (the cytoskeleton and adhesive plaques), and release a variety of signaling molecules. These actions help maintain blood flow and homeostasis (maintenance of physiological conditions at some baseline), and there is strong evidence that pathophysiological changes (such as atherosclerosis) occur in regions where mechanical signaling is disrupted.

To keep bone healthy, bone cells need mechanical stimulation

Physical loading is critical for skeletal health. Indeed, one of the most important factors in keeping bone healthy is for it to receive normal mechanical stimulation. When bone is not loaded it is said to be in a state of partial disuse, perhaps owing to a sedentary lifestyle, or complete disuse, which might occur because of bed rest or during long-duration spaceflight. In these extreme latter cases bone loss has been documented to occur at rates as high as 1–2% of total bone mass per month. Bone loss puts people at increased risk of fracture, even when trauma is absent or relatively low. These *fragility* or *osteoporotic* fractures can be devastating both to individuals and as a public health issue, costing billions of dollars annually. In fact, one-half of all women and one-quarter of all men older than 50 today will experience an osteoporotic fracture in their lifetime. Hip fractures are the most devastating result of low bone mass, and for most patients the first step in a downward spiral of lost ambulation, lost independence, institutionalization, and secondary medical morbidity and mortality. Shockingly, within 1 year of a hip fracture, 50% of patients will be unable to walk unaided, 25% will be institutionalized, and 20% will have died.

The good news is that physical loading on your bone from staying active will protect you from losing bone, although some activities appear to be better than others. Ballet is better than swimming, presumably because of the impact loading involved. In fact, it has been shown that high-level athletes can actually build bone specifically in regions of the skeleton that experience higher loading during their sport. Despite its critical importance for human health and its status as a compelling scientific question, the mechanism that allows bone cells (*osteocytes* and *osteoblasts* primarily) to sense and respond to loading by coordinating the cellular response remains basically unknown. It has been suggested that the sensing mechanism might involve the cytoskeleton, focal adhesions, adherens junctions, membrane channels, and even the biophysical behavior of the membrane itself. Indeed there is evidence for each of these and many others, so it seems likely that several cellular sensors exist, perhaps forming a redundant system.

The cells that line your lungs sense stretch

During respiration, the lung is exposed to constant oscillatory stresses arising from expansion and contraction of the basement membrane. These mechanical signals are postulated to play an important role in maintaining normal lung function and morphology. Stretch regulates pulmonary epithelial cell growth and cytoskeletal remodeling, as well as secretion of signaling molecules and phospholipids. These mechanical loads may be increased, for example, when a patient is subjected to mechanical ventilation. The physiological consequences of altered cellular function in response to such perturbations in mechanical loading are not yet fully understood.

Pathogens can alter cell mechanical properties

Malaria provides an interesting example of subtle mechanical alterations at the cellular level. Malaria is a mosquito-transmitted parasite that infects *red blood cells* (RBCs). Because the parasite resides in the RBCs during a large part of its lifetime, it is generally protected from the immune system. Because infected RBCs can be destroyed by the spleen, the parasite causes the infected RBC to increase its stickiness by inducing the expression of adhesive surface proteins on the RBC membrane. This allows the RBC to stick to the vessel walls and avoid being filtered in the spleen. Because there are many variations of this class of malaria surface proteins, the immune system is slow to adapt and remove these infected RBCs. As you can imagine, there has to be some deftness in the change in adhesion so that the cells will tend to stick a bit more, but not so much more that all the blood clumps together. Indeed, one effect of having stickier RBCs is that occasionally there will be an accumulation of RBCs in smaller blood vessels, resulting in a hemorrhage.

Other pathogens can use cell mechanical structures to their advantage

Bacteria of the genus *Listeria* act similarly by hiding within cells to evade the immune system. To invade other cells, the bacteria take over part of the cell's *actin* machinery (part of the cell *cytoskeleton*). Actin is polymerized to form fibers within the cell to provide structure and anchorage. The bacteria "sit" on the tip of the growing actin polymer and wait for a polymer to grow long enough for the bacteria to be pushed out of the cell and into an adjacent cell. Once the host is infected, the bacteria can spread throughout the host's body without ever exposing themselves to the immune system. For this mechanism to work, the bacteria must be able to achieve sufficient force to break through two cell membranes. The bacteria have to be able to "know" where to sit on the actin filament to be propelled—this is a source of active investigation for use in generating molecular machines.

Cancer cells need to crawl to be metastatic

Cancer metastasis is the process whereby an individual cancer cell(s) detaches from the main tumor, enters the bloodstream, reattaches at some new location, exits the blood vessel, and starts growing in its new location. Metastasis causes most cancer deaths, but many aspects of this process have yet to be fully understood. Cell migration is a critical component that is mediated by mechanical processes such as adhesion and intracellular force generation. Changes to aspects of these processes (such as the migratory speed of the cells) are generally tied to the long-term prognosis of the cancer, but the ways in which this occurs are not well understood. Adhesion may not only be important for allowing cancer cells to migrate but also for them to home in on a particular location. Not all tumor cells metastasize the same way; certain tumors will preferentially metastasize to specific regions or tissues. Whether this is due to selective adhesion at the preferred sites or diminished survival at other sites is not clear.

Solid tumors, as a whole, also exhibit altered physiology. Not only do the cells within tumors exhibit increased ("out-of-control") division rates, but tumors can redirect blood flow to allow themselves to grow faster. Further, many primary solid tumors tend to be stiffer than the surrounding tissues, even though they generally originate from the same tissue mass. Whether this increased stiffness alters cancer cell function through a mechanosensing function is not known, but it does have a practical use—many superficial (i.e., close to the skin) tumors can be detected by performing a self-examination, by feeling for a "lump" or "bump" that is somewhat harder compared to the surrounding tissue.

Viruses transfer their cargo into cells they infect

Nota Bene

Membrane fusion is a chemical process by which a pore is introduced into the cell membrane at the point where the membranes of the virus and the target cell are fused together. The mechanism underlying this is not understood well and may not be as mechanically dependent as endocytosis. But a certain degree of adhesion and membrane bending will invariably have to occur.

When a cell is invaded by a virus, the viral cargo of genetic material must be introduced into the cell. There are two mechanisms by which this can occur: endocytosis or membrane fusion. In the case of the former, the binding of proteins (called *ligands*) on the surface of the virus to proteins (called *receptors*) on the surface of the cell initiates a process called receptor-mediated endocytosis. In this process, the virus is enveloped by the cell, allowing it to deliver its genetic cargo and replicate. This process relies on a coordinated sequence of mechanical events, including adhesion, membrane pinching, and generation of cytoskeletal force that may provide potential targets for therapeutics aimed at inhibiting viral invasion. In addition, given the highly efficient means by which viruses invade cells, there is interest in understanding these mechanical processes for purposes of biomimicry, such as virus-based methodologies for nanoparticle delivery into cells.

1.2 THE CELL IS AN APPLIED MECHANICS GRAND CHALLENGE

In the twentieth century, the state of the art for applied mechanics was structural analysis on the large scale. Amazing achievements were realized in construction, such as high-rise buildings and beautiful bridges. Architecture was allowed to move beyond bulky stone and brick to elegant steel and glass. Transportation was revolutionized with cars and trains and modern aircraft representing outstanding examples of highly efficient structures that could only be created once their mechanical behavior had been analyzed in detail—engines, streamlining, brakes, lift, power, heat, etc., all having to be characterized and then applied together. Mechanics also played a major role in allowing people to reach the moon and explore the planets. Mechanics was and is key to military advances (missile technology, armor, advanced planes and drones, robotics, etc.). However, the theories and analysis required to design and build these impressive structures are, to a great extent, mature. For instance, much of car body design is based on computational analysis and not on the development of new laws or principles. Although

applied mechanics experienced great growth in the past, more recently it has undergone some degree of contraction.

Comprehensive mechanical analysis of a cell is extremely complex. There are one-dimensional linear elements in the cytoskeleton and two-dimensional curved shells in the cell membrane. There are also three-dimensional solids and enormous potential for pressure effects and fluid–solid interaction. Indeed, the overall cell is part–solid, part–liquid, something we call *viscoelastic*. Not only that, but the properties of the cellular "substance" change depending on the frequency with which forces are applied. On top of this, cellular structures are so small that thermal and entropic effects can play an important role in their mechanics, often requiring the analytical framework of statistical mechanics to understand their behavior. Thus, in terms of difficult challenges in applied mechanics with potential for critical new advances and fundamental insight, it is hard to imagine a more compelling problem than the cell. The overall picture we wish to present to you is that much can be explained using basic mechanics, but there is still much to be done using only slightly more advanced mechanical analysis.

Computer simulation of cell mechanics requires state-of-the-art approaches

Just as cell mechanics is a compelling challenge in applied mechanics, it is also a difficult, but rewarding, challenge in computational mechanics. For example, multi-scale modeling involves coupling a large-scale simulation with another simulation representing microscopic behavior. For cells one might simulate the behavior of individual actin and tubulin polymers and couple that to models of cytoskeletal networks or even the whole cell. The full mechanical behavior of the cell is a synthesis of solid, fluid, and statistical mechanics such that there is an opportunity for multi-physics formulations. There is also potential for fluid-structure problems, contact, even nonlinear material models. There is hardly an area within advanced computational mechanics that does not have application within cell mechanics.

1.3 MODEL PROBLEM: MICROPIPETTE ASPIRATION

We conclude this chapter with a simple model problem to give you a first glimpse into approaches for investigating cell mechanics and what sort of understanding we can gain from these analyses. Micropipette aspiration was one of the first methods used to examine cellular behavior and is responsible for some remarkably important and surprising insights into cellular behavior. Micropipette aspiration involves relatively simple instrumentation, and the experimental analysis can be very straightforward. The introduction of micropipette aspiration here is meant to foreshadow the level of abstraction and rigor that will follow throughout the text.

What is a typical experimental setup for micropipette aspiration?

Some of the earliest mechanical measurements of cell membranes were made using micropipette aspiration experiments. These measurements were based partly on the concept that cells were pouches with fluid interiors (use of RBCs eliminated the problem of the nucleus, because RBCs have none). The versatility of micropipette aspiration and ease of interpretation of experimental results continue to make this an important experimental approach for studying the mechanics of cells (and not just RBCs). As we will see, these experiments not only allow one to make measurements of cell membrane mechanical properties, but they also provide insight into the mechanical behavior of whole cells.

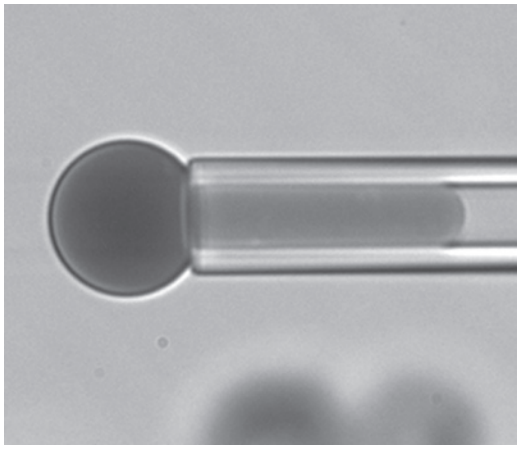

Figure 1.2 Red blood cell being drawn into a micropipette. (Courtesy of Richard Waugh, University of Rochester.)

A micropipette is a rigid tube (usually glass) that tapers to a diameter of several micrometers at the tip (near the tip, the diameter is constant). It is hollow all the way through its length and a suction (negative) pressure is applied to the interior (the *lumen*). If the end is brought in proximity to a cell while suction is applied, a seal will form, and the cell will be drawn into the micropipette, forming a protrusion (**Figures 1.2 and 1.3**). The negative pressure can be applied in a variety of ways. One way is to apply the suction by mouth—this method actually provides a lot of control, and researchers commonly do this when forming the seal.

Nota Bene

Micropipette aspiration is harder in winter. Early investigators conducting micropipette experiments found that the drift was much worse in the winter. Why? The evaporation rate at the top of the fluid reservoir was faster because the air tends to be drier in winter and the evaporation rate was therefore higher. Contemporary engineering tools have made this reservoir-associated problem obsolete.

Another common way is to connect the micropipette to tubing that runs to a water-filled reservoir with controllable height. In this case, decreasing the height of the fluid surface in the reservoir relative to the height of the fluid surface in the dish in which the cells are cultured creates a suction pressure within the micropipette. In theory, the minimum suction pressure that can be applied is determined by the minimum change in height of the fluid reservoir that can be achieved (typically on the order of ~0.01 Pa). In practice, the resolution is worse (usually on the order of ~1 Pa), owing to drift caused by water evaporating from the reservoir. Typically, the maximum pressure that can be applied is on the order of atmospheric pressure, resulting in a wide range of forces, from ~10 pN to ~100 nN.

Once the cell is drawn into the micropipette, the morphology of the cell relative to the pipette can be divided into three regimes, as in **Figure 1.4**. The first regime is when the length of the protrusion of the cell into the pipette L_{pro} is less than the radius of the pipette R_{pip}, or $L_{pro}/R_{pip} < 1$. The second regime is when the protrusion length is equal to the pipette radius, or $L_{pro}/R_{pip} = 1$, and the protrusion is hemispherical. The third regime is when $L_{pro}/R_{pip} > 1$, and the protrusion is cylindrical with a hemispherical cap. The radius of the hemispherical cap is R_{pip}, since the radius of the protrusion cannot change once the hemispherical cap is formed.

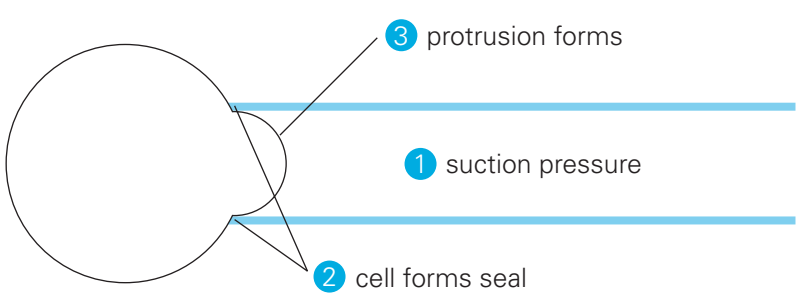

Figure 1.3 A micropipette aspiration experiment. The micropipette tip is placed in the proximity to the cell, and suction pressure is applied. A seal forms between the cell and the micropipette, forming a cell protrusion into the micropipette.

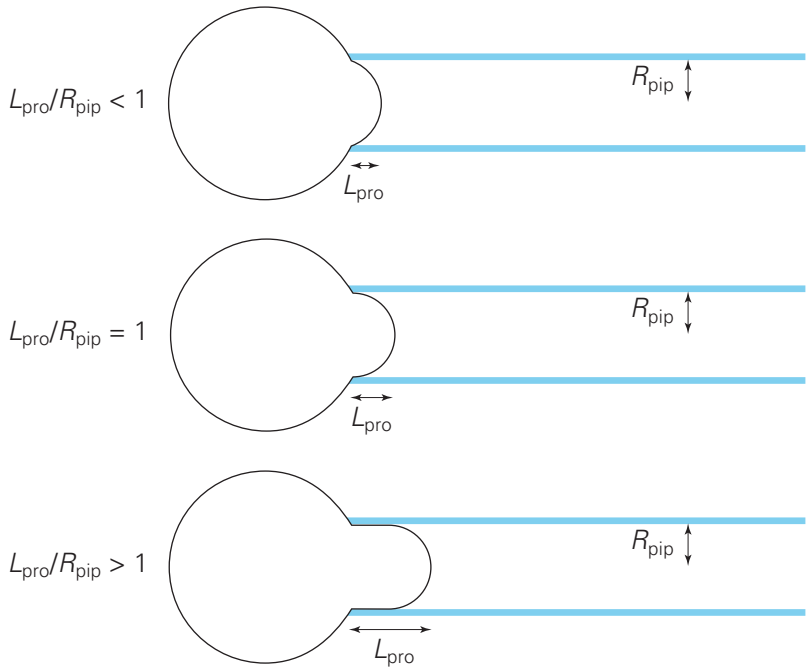

Figure 1.4 Three regimes of cell aspiration into a micropipette. (Upper) The length of the protrusion of the cell into the pipette is less than the radius of the pipette, or $L_{pro}/R_{pip} < 1$. (Middle) $L_{pro}/R_{pip} = 1$. In this case, the protrusion is hemispherical. (Lower) $L_{pro}/R_{pip} > 1$. The protrusion is cylindrical with a hemispherical cap of radius R_{pip}.

At this point one should be able to see that geometrically the radius of the protrusion of the cell in the first regime is larger than R_{pip}.

The liquid-drop model is a simple model that can explain some aspiration results

When researchers performed early micropipette aspiration experiments on cells such as *neutrophils* (a type of white blood cell), they noticed that after the micropipette pressure exceeded a certain threshold, the cells would continuously deform into the micropipette (in other words, the cells would rapidly "rush into" the pipette). This observation led to the development of the *liquid-drop model*. In this model, the cell interior is assumed to be a homogeneous Newtonian viscous fluid, and the surrounding membrane is assumed to be a thin layer under a constant surface tension, and without any bending resistance. Further, it is assumed that there is no friction between the cell and the interior walls of the pipette.

Surface tension has units of force per unit length, and can be thought of as the tensile (stretching) force per unit area (*stress*) within the membrane, integrated through the depth of the membrane. For example, if a membrane of thickness d is subject to a tensile stress σ that is constant through its depth, then we can model the membrane as having a surface tension of $n = \sigma d$. If the membrane is very thin compared to the radius of the cell, we can ignore the membrane thickness for analysis and rely exclusively on the surface tension n (**Figure 1.5**).

Why is this model called the liquid-drop model? A drop of cohesive liquid (such as water) suspended in another less cohesive fluid (such as air) has a thin layer of water molecules at the surface of the drop, and, because of the imbalance of intermolecular forces at the surface, packs together and causes surface tension. In brief, every molecule exerts, on average, an attractive force on every other molecule. So, a molecule deep within the drop is pulled in every direction about equally and has no net average force on it. However, a molecule at the surface is pulled with a net force into the "bulk" of the drop, resulting in a spherical shape for the drop and some resistance to deformation at the surface, which we call

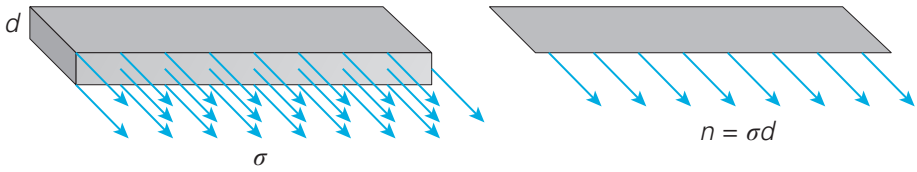

Figure 1.5 A membrane of thickness *d* subject to a tensile stress σ that is constant through its depth can be modeled as infinitely thin with a surface tension of $n = \sigma d$.

surface tension. This surface tension is what allows some types of insects to walk on the surface of water.

The Law of Laplace can be applied to a spherical cell

By modeling the cell as a liquid drop, we can analyze micropipette aspiration experiments using the *Law of Laplace*, which relates the difference in pressure between the inside and outside of a thin-walled pressure vessel with the surface tension within the vessel wall. It can be derived from a simple analysis using a *free body diagram*, a topic discussed in detail in Chapter 3. Consider a spherical thin-walled vessel with radius R, a pressure of P_i inside the vessel, and a pressure of P_o outside the vessel (**Figure 1.6**).

If we cut the sphere in half, then there are two equal and opposite resultant forces acting on the cut plane. The first is due to pressure, and it is calculated as $F_p = (P_i - P_o)\pi R^2$. The second resultant force is due to surface tension on the wall. If the surface tension is given by n, then the resultant force due to surface tension F_t is s $F_t = n2\pi R$ (the surface tension multiplied by the length of the edge exerting such tension, which would be the circumference of the circle). Setting $F_p = F_t$, we arrive at the Law of Laplace,

$$P_i - P_o = \frac{2n}{R}. \tag{1.1}$$

Note that we set the forces equal because we assume that the droplet is not accelerating. In this case, Newton's second law implies that the forces due to pressure and surface tension must sum to zero. Because they act in opposite directions, the forces are equal.

Nota Bene

History of Young and Laplace. The Law of Laplace is also known as the Young–Laplace equation in honor of Thomas Young, who made initial qualitative observations of the curvature of liquid menisci in 1804, and Pierre-Simon Laplace, who later introduced the mathematical formalism. It is also sometimes called the Young–Laplace–Gauss equation.

Micropipette aspiration experiments can be analyzed with the Law of Laplace

We can use the Law of Laplace to analyze micropipette aspiration experiments by relating suction pressure to the morphology of the cell as it enters the pipette. Consider the configuration in **Figure 1.7**, where P_{atm} is the pressure of the environment, P_{cell} is the pressure within the cell, P_{pip} is the pressure within the pipette, R_{cell} is the radius of the cell outside the pipette, R_{pip} is the radius of the pipette,

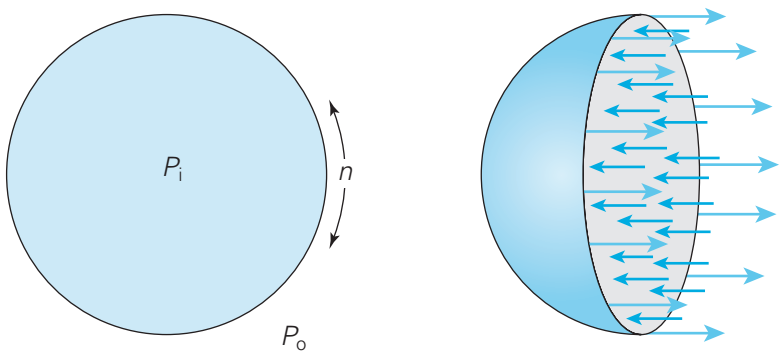

Figure 1.6 A spherical vessel with an inner pressure exceeding the outer pressure balanced by a membrane tension *n* (left) and the resulting free-body diagram (right).

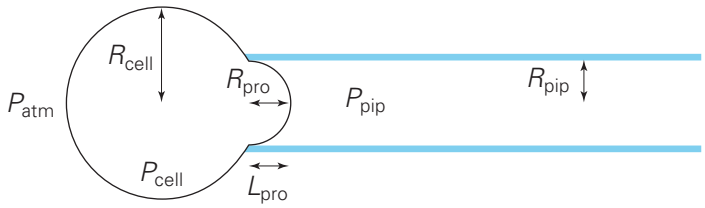

Figure 1.7 Schematic depicting quantities used in analyzing a micropipette aspiration experiment.

R_{pro} is the radius of the protrusion, L_{pro} is the length of the protrusion into the micropipette and R_{pip} is the radius of the micropipette.

For the portion of the cell that is not in the micropipette, from Equation 1.1 we know that

$$P_{cell} - P_{atm} = \frac{2n}{R_{cell}}, \tag{1.2}$$

where n is the surface tension of the cell. For the protrusion, or the portion of the cell in the pipette,

$$P_{cell} - P_{pip} = \frac{2n}{R_{pro}}. \tag{1.3}$$

Combining equations 1.2 and 1.3, we obtain

$$P_{atm} - P_{pip} = \Delta P = 2n\left(\frac{1}{R_{pro}} - \frac{1}{R_{cell}}\right), \tag{1.4}$$

which relates the difference between the pressure in the surroundings and in the pipette with the radius of the cell inside and outside of the pipette for a cell with a given surface tension. Note that we assume the surface tension is constant throughout the cell, even in the "crease" region where the cell contacts the micropipette, and in the protrusion area and the cell membrane outside the micropipette.

How do we measure surface tension and areal expansion modulus?

One can now easily measure surface tension from Equation 1.4. Once the cell is drawn into the micropipette such that the protrusion is hemispherical ($L_{pro} = R_{pip}$), then the radius of the protrusion also equals the radius of the pipette, $R_{pro} = R_{pip}$. Here,

$$\Delta P = 2n\left(\frac{1}{R_{pip}} - \frac{1}{R_{cell}}\right). \tag{1.5}$$

The pressure ΔP is controlled by the user, and R_{pip} is also known. The cell radius R_{cell} can be measured optically under a microscope, allowing one to calculate the surface tension n. Evans and Yeung performed this experiment using different micropipette diameters and found a surface tension of (~35 pN/μm) in neutrophils. This tension was found to be independent of the pipette diameter, which supports the validity of the technique. We will see in the next section that surface tension does not stay exactly the same if the cell is further deformed.

For a liquid drop, the surface tension will remain constant as it is aspirated into a micropipette. In reality, cells do not behave like a perfect liquid drop. This is because the cell membrane area increases as it is aspirated, resulting in a slight increase in surface tension. The increase in tension per unit areal strain is given

by what is called the *areal expansion modulus*. Needham and Hochmuth quantified the areal expansion modulus in neutrophils by aspirating them through a tapered pipette (**Figure 1.8**). Applying progressively higher pressures, caused the cell to advance further into the taper, increasing its surface area while maintaining constant volume (maintaining constant volume is a condition called *incompressibility*). The radii at either end of the cell, R_a and R_b, the total volume V, and the *apparent* surface area A were measured from the geometry. By apparent we mean that the surface area of the cell is approximated to be smooth, and we ignore small folds and undulations. The surface tension was calculated using the Law of Laplace as

$$\Delta P = 2n\left(\frac{1}{R_a} - \frac{1}{R_b}\right). \tag{1.6}$$

The "original" radius R_o of the cell was calculated from the volume (assuming the volume remained constant) as $V = 4/3\pi R_o^3$, allowing the "original" or undeformed apparent surface area to be calculated as $A_o = 4\pi R_o^2$. The areal strain $(A - A_o)/A_o$ and surface tension could therefore be found for the same cell as the pressure was increased, and the cell advanced through the taper. The surface tension was plotted as function of areal strain, and the data was fitted to a line. The areal expansion modulus was found from the slope of the line and was calculated to be 39 pN/μm. Extrapolating the fit line to zero areal strain resulted in a resting surface tension of 24 pN/μm in the undeformed state.

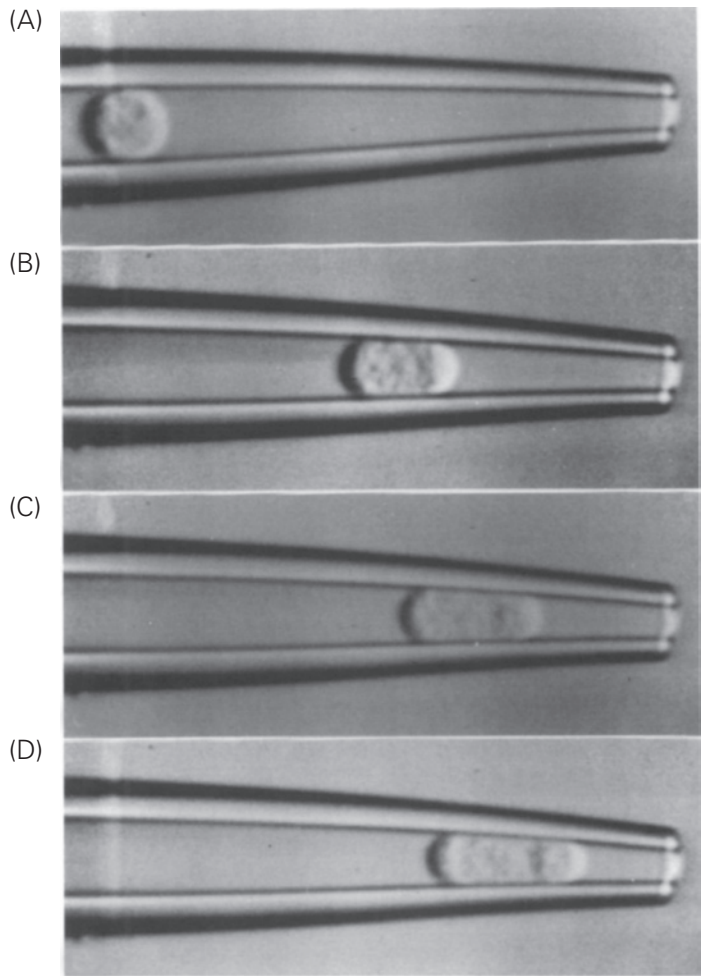

Figure 1.8 Cell being aspirated within a tapered pipette. The radius of the pipette opening is 4 μm. (A) A cell was aspirated into the tapered pipette and allowed to recover to its resting spherical shape. A positive pipette pressure was then applied, and the cell was driven down the pipette. Final resting configuration after (B) ΔP = 2.5 Pa, (C) ΔP = 5.0 Pa, and (D) ΔP = 7.5 Pa. (Adapted from, Needham D & Hochmuth RM (1992) *Biophys J*. 61, 1664–1670.)

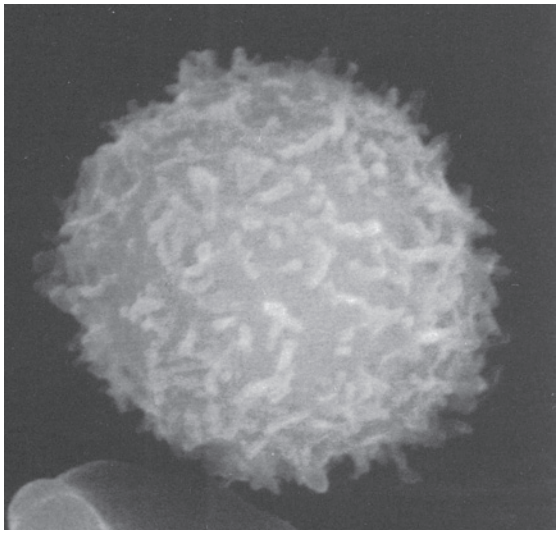

Figure 1.9 Electron micrograph of a neutrophil. Ruffles in the membrane can be clearly seen. (Adapted from, Needham D & Hochmuth RM (1992) *Biophys J*. 61, 1664–1670.)

Why is this tension in the undeformed state important in neutrophils? Remember that neutrophils circulate in the blood and therefore need to squeeze through small capillaries with diameters smaller than the cells themselves, similar to RBCs. As neutrophils squeeze through capillaries, the shape of the cells transform from a sphere into a "sausage" (i.e., a cylinder with hemispherical caps at both ends). Because the cells contain mostly fluid, they are usually incompressible and so must maintain constant volume during this shape change. The surface area of the "sausage" is greater than a sphere of the same volume, and the increase in surface area grows larger as the radius of the "sausage" decreases. However, we will see later that biomembranes are quite inextensible. So how do neutrophils undergo this increase in surface area when squeezing through small blood vessels?

As can be seen in **Figure 1.9**, neutrophils contain many microscopic folds in their membrane. What this means is that their "apparent" surface area is much less than the actual surface area of the membrane if one were to take into account all the folds and ruffles. The folds allow neutrophils to substantially increase their apparent surface area without actually increasing the surface area of the membrane, as long as the folds are not completely smoothed out. The tension within the cortex of these cells has a crucial role: it allows the cells to have folds and ruffles in the membrane while maintaining their spherical shape.

Why do cells "rush in"?

Remember that the liquid-drop model was developed in large part in response to the observation that some cells would "rush in" after applying any pressure greater than the critical pressure at which $L_{pro} = R_{pip}$. Why do liquid drops do this? Remember that Equation 1.4 is a relation that must be satisfied for equilibrium. Suppose we apply the critical pressure such that $L_{pro} = R_{pip}$, and then we increase ΔP. Let us examine what happens to the terms on the right-hand side of Equation 1.4. We already learned that n is constant for liquid drops, and approximately constant for neutrophils as they are aspirated. The radius of the protrusion, R_{pro}, will also remain constant, because the radius of the hemispherical cap will be equal to R_{pip} for any $L_{pro} > R_{pip}$. R_{cell} cannot increase, meaning that the volume of the cell remains essentially constant over the time of the experiment. We therefore have increased the left-hand side of Equation 1.4 with no way to increase the right-hand side. The result is that equilibrium cannot be satisfied. This produces an instability, and the result is the cell will rush into the pipette.

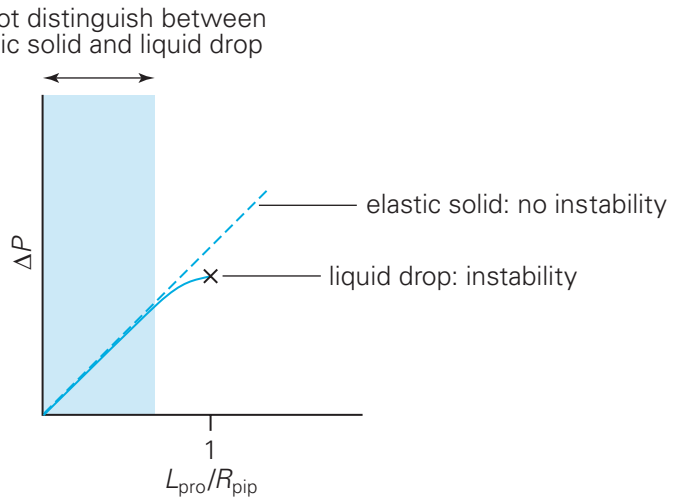

Figure 1.10 ΔP as a function of $L_{pro} = R_{pip}$ for a cell that behaves like an elastic solid (dotted line) and a cell that behaves like a liquid drop (solid line). When $L_{pro} = R_{pip} = 1$, an instability occurs for the cell that behaves like a liquid drop, and the cell rushes into the micropipette. By contrast, a cell that behaves like an elastic solid will not have this instability.

Cells can behave as elastic solids or liquid drops

Micropipette aspiration is an extremely versatile technique for measuring the properties of membranes. It can apply a wide range of forces, and the experiments are conducive to mechanical analysis. In addition to making mechanical measurements of membranes, micropipette aspiration experiments play perhaps an even more important role in understanding cell mechanics, in that they easily allow insight into the fundamental mechanical behavior of different cell types. For example, when researchers performed experiments on endothelial cells or chondrocytes, they found that they would not rush into the pipette, even after $L_{pro} = R_{pip}$. Why? Put simply, these cells do not behave like a liquid drop. Subsequent experiments and analyses showed that their mechanical behavior is much more like that of an elastic solid, so it will not have this instability. Therefore, by observing whether a particular cell does or does not rush into the pipette after $L_{pro} = R_{pip}$, one can easily distinguish whether its mechanical behavior is more like a liquid drop or an elastic solid. Note that this simple method for classification is only possible if the critical pressure is exceeded. If the experiment is terminated before $L_{pro} = R_{pip}$, then one cannot (as easily) distinguish between elastic solid and liquid-drop behavior, as can be seen in **Figure 1.10**.

Key Concepts

- The study of cellular mechanobiology bridges cell biology and biochemistry with various disciplines of mechanics, including solid, fluid, statistical, experimental, and computational mechanics.
- A wide variety of devastating human diseases such as osteoporosis, heart disease, and even cancer involve cell mechanics in a fundamental way.
- Cell mechanics is an excellent substrate for introducing students to a wide variety of cutting-edge approaches in mechanics.
- Understanding the mechanical behavior of cells presents a grand challenge in theoretical, computational, and experimental mechanics.
- Micropipette aspiration is an early and straightforward approach for investigating cell mechanics. By modeling the cell as a liquid drop, we can analyze micropipette aspiration experiments using the Law of Laplace.
- Liquid-drop cells exhibit an instability when the radius of the aspirated protrusion is equal to the radius of the pipette. At this point the cell cannot resist increased pressure and rushes into the pipette.
- By observing the movement of cells within the micropipette at the instability point, one can distinguish two types of cellular behavior cells: those whose behavior is dominated by membrane tension (similar to a liquid drop), and those that act as a continuum solid.

Problems

1. As we have seen, when a liquid drop–type cell such as a neutrophil is subjected to micropipette aspiration, it becomes unstable. This occurs when the radius of the protrusion is equal to the radius of the pipette. For a cell whose behavior is better approximated by a continuum such as a chondrocyte, do you think there is a maximum pressure that can be exerted on the cell? What configuration would the cell be in at this point?

2. In our analysis we ignored the friction between the inside of the pipette and the cell wall. Is this a reasonable assumption? Why? How might friction affect the results of an aspiration experiment if it were large?

3. The classic micropipette aspiration experiment can be modified to address the instability of a cell with liquid-drop behavior so that membrane behavior can be measured. This is done with a tapered pipette (**Figure 1.8**). With this approach the pipette radius changes along its length. Derive the relationship between membrane tension and the pressure in the micropipette assuming that the two sides of the cell have radii of R_a and R_b and pipette pressures are P_a and P_b.

4. There are other structures similar to cells that can be treated with the liquid-drop model. The bronchial passages of the lungs terminate in small spherical sacs known as alveoli. There are around 150 million alveoli in your lungs. During respiration the alveoli are filled and emptied through the action of the diaphragm and intercostal muscles in the chest wall. During inhalation the pressure outside the alveoli can drop by up to 200 Pa. The layer of cells that line the alveoli is constantly hydrated. So, we can model them as a bubble of air surrounded by water, assuming that the surface tension of water is 70 dyn/cm. With this information, what is the radius of the alveoli? In actuality, the radius of an alveoli is about 0.2 mm. They can be this small because the epithelial cells secrete a protein called *surfactant* that reduces the water surface tension. If fact, there is a developmental condition known as infant respiratory distress syndrome (IRDS) that occurs when insufficient surfactant protein is produced. What must the surface tension be to keep the alveoli inflated?

5. Consider two adjacent alveoli and the end of a bronchus such that air can pass easily between them as well as to the outside. Assume that they have the same radius and that they are in a state of equilibrium with respect to pressure and surface tension. What would happen if a small volume of air moved from one alveolus to the other? What must be true of surfactant as a result?

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CHAPTER 2

Fundamentals in Cell Biology

When thinking about cell mechanics, it is easy to get caught up in models and mathematics, and thinking about cells as somewhat exotic material that can be subjected to the same sort of testing as is performed on inert metals and plastics. However, an equally important part of cell mechanics is the study of how mechanics interacts with the biological behavior of cells. The former is termed "biomechanics"; the latter is increasingly referred to as "mechanobiology," as mentioned in Chapter 1. The former is meant to emphasize biomechanics as the subdiscipline of mechanics that considers the mechanical properties of biological structures and mechanobiology as the subdisipline of biology that is focused on how mechanics regulates biological processes, or how biological processes generate and regulate physical forces. Oftentimes this distinction is artificial and may not be useful, as some of the most fascinating problems cannot be understood without considering biology and mechanics with equal depth and rigor.

Just as our treatment of cell biomechanics is built on a firm understanding of the fundamental principles of mechanics, to appreciate mechanobiology fully we must take a step back and form an understanding of the fundamentals of biology. This must include not only the underpinnings of cell and molecular biology, but also some of the modern experimental techniques that allowed these insights to be made. Our goal is not only to provide you with enough biology background to understand cell mechanics, but to allow you to read and understand the basics of modern biology scientific publications.

Modern biology is undergoing a revolution of understanding that began in 1953 with Watson and Crick's determination of the double-helix structure of DNA. This has led to the sequencing of the human genome, modern genetics, and, in an amazingly short period of time in terms of the history of science, to dramatic advances in biology. This explosion of knowledge, known as the molecular revolution, has given rise to the field of molecular biology sometimes referred to as *modern biology*. J. Craig Venter, one of the pioneering founders of the high-throughput approaches to the study of genes and their regulation (who also participated via Celera in the Human Genome Project), declared this to be the opening of the "Century of Biology." Many have speculated that the discoveries currently being made will lead to the next wave of social change following the Industrial Revolution and the Information Revolution.

2.1 FUNDAMENTALS IN CELL AND MOLECULAR BIOLOGY

Formal writing in biology is heavy on terminology, in part due to the need to be precise in descriptions while being succinct. Let us say you develop a hairline fracture on one of your ribs. If the location of the fracture is closer to the sternum, we call it a *medial* fracture (closer to the "middle" of the body). If it is closer to your sides/arms/shoulders, we call it a *lateral* fracture. The terms medial and lateral

Table 2.1 The monomer subunits and resulting polymers that make up the biochemical constituents of the cell.

Monomer	Polymer	
nucleic acid	RNA, DNA	genes
amino acid	peptide, protein	gene products
fatty acid	lipid	not coded by genes
sugar	polysacharide, carbohydrate	not coded by genes

(along with other terms) can be used to quickly describe locations without having to reference specific sites, much like north and south can be used if you are not familiar with local landmarks.

In a similar way, there are a few terms that recur in biomedical writing with which you should be familiar. We may not use them all in this book, but the diligent student will want to get to know these terms. Some common ones are:

- **Cell culture:** the process of extracting live cells from biological tissue, and the maintenance and growth of those cells, typically *in vitro*, to study the physiological behavior of the cells under controlled conditions.
- ***In vitro*:** in a laboratory dish; not inside an organism; literally, "in glass".
- ***Ex vivo*:** sometimes interchanged with *in vitro*, but occasionally referring to entire tissues that are cultured in a laboratory dish.
- ***In vivo*:** inside a living organism, typically but not always at the natural location.
- ***In situ*:** inside a living organism, in the natural location. Growing an ear in a mouse's back would be an *in vivo* but not an *in situ* experiment.
- **Amino acids:** the fundamental building blocks (monomers) of proteins.
- **DNA:** deoxyribonucleic acid, the "hard copy" of genes and their regulatory components.
- **RNA:** ribonucleic acid, the "working copy" of genes that assembles proteins.
- **Gene:** sequence of DNA that encodes a protein.
- **Promoter:** sequence of DNA that regulates when a particular gene is expressed. Typically, but not always, near the gene being regulated.
- **Probe:** A fragment of DNA or RNA that is complementary to a specific target sequence. The probe is able to bind to the target in a process called *hybridization*.

Nota Bene

Inorganic carbon compounds. There are several carbon compounds that are inorganic. Diamond and graphite are obvious examples. However, there are no hard-and-fast rules to make the distinction.

Many cellular components are polymers, assembled from subunits called monomers (Table 2.1). The major exception to this is the most abundant constituent of cells, water, which makes up roughly 70% of a cell's mass. Inorganic ions are another exception and are critical to cell metabolism and signaling ("organic" compounds have carbon, "inorganic" compounds do not). These components are vital for the non-inert response of cells to mechanical forces. In some cases, biological polymers form the physical structure of the cell, and the biomechanical characterization of the cell relies on the properties of these polymers. In other cases, the molecules are responsible for signaling. Such molecules can be important for signaling in response to mechanical stresses (mechanosignaling), which we discuss further in Chapter 11.

Proteins are polymers of amino acids

In general, proteins consist of many (> 50) amino acids. Short chains of amino acids or fragments of proteins are called peptides. Each amino acid shares a common backbone structure of an amino group (H_2N), a carbon atom, and a carboxyl group (COOH) (Figure 2.1). The central carbon, known as the α-*carbon*, attaches to a side chain. The diversity of charges, sizes, and interactions of these side chains