11 Summary

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The primary objective of this book was to bring together various points of view regarding cell mechanics, contrasting and comparing these diverse perspectives. This final short chapter summarizes the various models discussed in an attempt to identify commonalities as well as any irreconcilable differences.

A wide range of computational and phenomenological models were described for cytoskeletal mechanics, ranging from continuum models for cell deformation and mechanical stress to actin-filament-based models for cell motility. A concise review was also presented (Chapter 2) of numerous experimental techniques, which typically aim to quantify cytoskeletal mechanics by exerting some sort of perturbation on the cell and examining its static and dynamic responses. These experimental observations along with computational approaches have given rise to several often contradictory theories for describing the mechanics of living cells, modeling the cytoskeleton as a simple mechanical elastic, viscoelastic, or poroviscoelastic continuum, a porous gel, a soft glassy material, or a tensegrity (tension integrity) network incorporating discrete structural elements that bear compression.

With such remarkable disparity among these models, largely due to the diversity of scales and biomechanical issues of interest, it may appear to the uninitiated that various authors are describing entirely different cells. Yet depending on the test conditions or length scales of interest, identical cells may be viewed so differently as either a continuum or as a discrete collection of structural elements.

Experimental data are accumulating, and promising methods have been proposed to describe cell rheology. While there has been some convergence toward a range of values for the cytoskeletal shear modulus, the range remains large, spanning several orders of magnitude. This suggests either disparities in the measurement methods, considerable variability between cells or between cell types, or differences in the methods employed to interpret the data. A unique aspect of cellular mechanics is that active as well as passive characteristics need to be considered.

A variety of different approaches have been described to simulate cell or cytoskeletal stiffness. Likely there is not a single "correct" model; rather, one model may prove useful under certain circumstances while another model may be better suited in others. In part, the model of choice will depend on the length scale of interest. Cells contain a microarchitecture comprised of filaments ranging down to $\sim$10 nm in diameter

with separation distances of ∼100 nm. When considering whole-cell deformations, a continuum description may be appropriate; when the force probe is on the scale of an AFM tip, then details of the filament organization are almost certainly critical.

As a practical matter, it is important to determine what constitutive law best fits the observed structural behavior. While a linear elastic or even linear viscoelastic material description is sufficient to mimic certain observations, other more complex descriptions will almost certainly be needed to encompass a range of excitation frequencies and large deformations. These are just now being identified. There seems to be a growing consensus that the constitutive behavior of a cell corresponds to that of a soft glassy material (see Chapter 3) even though the underlying basis for this behavior is not yet clearly understood. Albeit lacking a fundamental understanding, these measurements and the relative simplicity of the generalized form that they exhibit provide at least two critical new insights. First is that the cell responds as though the relaxation times are distributed according to a power law, suggesting many relaxation processes at low frequencies but progressively fewer as frequency is increased. Second, cytoskeletal stiffness and friction or viscosity are interrelated, in that the same underlying principles likely govern both. Both stiffness and friction appear to be governed by a single parameter, the "effective temperature," that reflects the extent to which the material is solid-like or fluid-like. Bursac et al. (2005) speculate that this might relate to a process in which the cytoskeleton is "trapped" in a collection of energy wells but can occasionally "escape" utilizing, for example, either thermal or chemical (such as, ATP-derived) energy. In this connection, the effective temperature might be a measure of molecular agitation, reflecting the relative ability to escape. As appealing as these ideas might be, however, they remain to be fully demonstrated, and so remain intriguing speculation.

As Chapter 2 points out, while there appears to be some degree of convergence regarding the values and frequency dependence of viscoelastic parameters for the cytoskeleton, the results obtained remain somewhat dependent upon the method used to probe the cell. In publications as recent as this past year, values for cytoskeletal stiffness ranging from ∼20 Pa (Tseng et al., 2004) to 1.1 MPa (Marquez et al., 2005) have appeared, and the bases for these discrepancies still need to be resolved. In particular, as most (but not all) of the data on which the soft glassy material model is based are obtained from one measurement method (magnetic twisting cytometry), one still needs to exercise caution in making broad generalizations.

While some of the models appear quite disparate, there are some significant similarities. The cellular solids and biopolymer (Chapter 8) theories differ in terms of how the individual elements in the structure resist deformation, with the cellular solids model considering these to be beams subject to bending, and the biopolymer theory treating them as entropic chains that lose configurational entropy as the material is stretched. Recent studies (Gardel et al., 2004) are beginning to reconcile these differences and, perhaps not surprisingly, are finding that both descriptions might apply depending upon the concentrations of actin and cross-linkers and the state of stress in the material. Neither of these models, however, can be readily connected to the observed behavior as a soft glassy material.

Another microstructural model is based on the concept of tensegrity (Chapter 6), and is most closely related to the cellular solids model in that cytoskeletal structure

is defined by an interconnected network of elastic elements. The key distinction here, though, is that stiffness is conferred not by the stiffness of the individual elements, but rather primarily by the baseline stresses they support. These stresses are imposed either by cell adhesions to extracellular structures or by internal members such as microtubules that are in compression. In either case, the elastic properties of the elements take on secondary importance, provided they are sufficiently stiff to undergo relatively small changes in length under normal stress.

In a sense, the continuum descriptions (Chapters 4, 5 and 10) are for the most part independent of behavior at the microstructural level, and simply make use of constitutive laws that can either be based on experiments or derived directly from one of these microstructural models. Consequently, while the continuum models can be useful in describing how deformations or stresses distribute throughout the cell, they provide no information on the deformations at the microscale (that is, within the individual elements of the matrix), and are entirely dependent on information contained in the constitutive relation.

Although this one text could not possibly capture all the work being done on cell mechanics in that it represents a broad spectrum of these activities, it should immediately become clear that one fruitful direction for future research is in the modeling of dynamic processes – cell migration, phagocytosis and division. In fact, with only a few exceptions (notably the work described in Chapters 7, 9, and 10) the cell is treated as a traditional engineering material, meaning one with properties that are time invariant. Cells, on the other hand, are highly dynamic in that their cytoskeletal structures are constantly changing in response to a variety of external stimuli including, especially, external forces. Consequently, each time we probe a cell to measure its mechanical properties, we may alter those same properties. One exception to this statement is the use of the Brownian motions of intracellular structures to infer stiffness, but these measurements are still being refined; as currently implemented, they are subject to some degree of uncertainty. Still, this represents an important direction for research, and we are sure to see refinements and wider use of these nonintrusive methods in the future.

While advances in cell mechanics are considerable, many open questions still remain. Mechanotransduction, the active response of living cells to mechanical signals remains an active area of investigation. It is well known that living cells respond to mechanical stimulation in a variety of ways that affect nearly every aspect of their function. Such responses can range from changes in cell morphology to activation of signaling cascades to changes in cell phenotype. Mechanotransduction is an essential function of the cell, controlling its growth, proliferation, protein synthesis, and gene expression.

Despite the wide relevance and central importance of mechanically induced cellular response, the mechanisms for sensation and transduction of mechanical stimuli into biochemical signals are still largely unknown. What we know is that living cells can sense mechanical stimuli. Forces applied to a cell or physical cues from the extracellular environment can elicit a wide range of biochemical responses that affect the cell's phenotype in health and disease.

Various mechanisms have been proposed to explain this phenomenon. They include: changes in membrane fluidity that act to increase receptor mobility and

lead to enhanced receptor clustering and signal initiation (Haidekker et al., 2000); stretch-activated ion channels (Hamil and Martinac, 2001); mechanical disruption of microtubules (Odde and co-workers, 1999); and forced deformations within the nucleus (Maniotis et al., 1997). Constrained autocrine signaling, whereby the strength of autocrine signaling is regulated by changes in the volume of extracellular compartments into which the receptor ligands are shed, is yet another mechanism (Tschumperlin et al., 2004). Changing this volume by mechanical deformation of the tissues can increase the level of autocrine signaling.

Finally, others have proposed conformational changes in intracellular proteins along the force-transmission pathway, connecting the extracellular matrix with the cytoskeleton through focal adhesions, as the main mechanotransduction mechanism (see Kamm and Kaazempur-Mofrad, 2004 for a review). In particular, the hypothesis that links mechanotransduction phenomena to mechanically induced alterations in the molecular conformation of proteins has been gaining increased support. For example, certain proteins that reside in 'closed' conformation can be mechanically triggered to reveal cryptic binding sites. Similarly, small conformational changes may also change binding affinity or enzyme activity. For example, when protein binding occurs through hydrophobic site interactions, a conformational change could modify this function and potentially disrupt it totally. Force transmission from the extracellular matrix to the cell interior occurs through a chain of proteins, located in the focal adhesion sites, that are comprised of an integrin–extracellular matrix protein bond (primarily vitronectin and fibronectin), integrin-associated proteins on the intracellular side (paxillin, talin, vinculin, and others), and proteins linking the focal adhesion complex to the cytoskeleton. Stresses transmitted through adhesion receptors and distributed throughout the cell could cause conformational changes in individual force-transmitting proteins, any of which would be a candidate for force transduction into a biochemical signal. The process by which changes in protein conformation give rise to protein clustering at a focal adhesion or initiate intracellular signaling, however, remains largely unknown (Geiger et al., 2001).

External stresses imposed on the cell are transmitted through the cytoskeleton to remote locations within the cell. To understand these stress distributions requires knowledge of cytoskeletal rheology, as governed by the structural proteins, actin filaments, microtubules, and intermediate filaments. For example, a simplified picture can be painted of the cytoskeletal rheology that is limited to actin filaments and actin cross-linking proteins living in a dynamic equilibrium. These cross-links constantly form and unbind at rates that are largely influenced by the forces borne by the individual molecules. Cytoskeletal rheology would then be determined at the molecular scale by the mechanics and binding kinetics of the actin cross-linking proteins, as well as by the actin matrix itself (Gardel et al., 2004). To understand the phenomena related to mechanotransduction in living cells and their cytoskeletal rheology, the mechanics and chemistry of single molecules that form the biological signaling pathways that act in concert with the mechanics must be examined.

Another largely open question in the field of cytoskeletal mechanics is related to the cell migration and motility that is essential in a variety of biological processes in health (such as embryonic development, angiogenesis, and wound healing) or disease (as in cancer metastasis). As discussed in Chapter 9 and 10, the process of cell motility or

migration consists of several steps involving multiple mechanobiological signals and events starting with the leading edge protrusion, formation of new adhesion plaques at the front edge, followed by contraction of the cell and the release of adhesions at the rear (see Li et al., 2005 for a recent review). A host of mechanical and biochemical factors, namely extracellular matrix cues, chemoattractant concentration gradients, substrate rigidity, and other mechanical signals, influence these processes. Many unanswered questions remain in understanding the signaling molecules that play a key role in cell migration, and how they are regulated both in time and 3D space. It is largely unknown how a cell actively controls the traction force at a focal adhesion or how this force varies with time during the cell migration.

To understand the mechanobiology of the cell requires a multiscale/multiphysics view of how externally applied stresses or traction forces are transmitted through focal adhesion receptors and distributed throughout the cell, leading subsequently to conformational changes that occur in individual mechanosensing proteins that in turn lead to increased enzymatic activity or altered binding affinities. This presents both a challenge and an opportunity for further research into the intrinsically coupled mechanobiological phenomena that eventually determine the macroscopic behavior and function of the cell.

Because no one method has emerged as clearly superior in describing the mechanics and biology of the cell across all cell types and physical conditions, this might reflect the need for new approaches and ideas. We hope that this monograph has inspired new researchers with fresh ideas directed toward that goal. Perhaps the biggest question that still remains is whether it is at all possible to construct a single model that is universally applicable and can be used to describe all types of cell mechanical behavior.

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