

Chapter 2

Complex Fluids and Soft Structures in the Human Body

Paula A. Vasquez and M. Gregory Forest

Abstract The human body is a composite of diverse materials able to perform specific functions. Few of these materials are simple liquids or solids; rather they share both liquid-like and solid-like properties. The material world between liquids and solids is unlimited and exploited by Nature to form complex fluids and soft structures with properties that are tuned to perform highly specialized functions. This chapter will briefly summarize the diversity of materials in the human body, and then drill deeper into one complex fluid (mucus, which coats every organ in the body) and one soft structure (an individual cell), and their remarkable properties. Some progress in characterizing these materials and modeling their functional properties, by others and our research group, will be presented with the take home message that we are in the early stages of interpreting experimental data and building predictive models and simulations of biological materials.

1 Introduction

This review chapter takes a *multidisciplinary* point of view toward complex fluids, or soft matter, in the human body. This multidisciplinary approach is required since the remarkable properties and functions of biological complex fluids are hard to resolve from a singular disciplinary approach. Examples of disciplines encountered in the study of biological fluids are systems biology, molecular biology, applied mathematics, medical biology, chemistry, physics, computer science, and several aspects of engineering. In this chapter, we highlight two biological fluids, lung mucus and single cells, and use them to illustrate the challenges faced in faithfully modeling their function and behavior.

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A fundamental goal of systems biology is to understand the mechanisms underlying material performance, e.g., clearance of mucus from lung airways, intracellular organization during phases of a cell cycle, and motility of cells in one environment versus another. What these mechanisms have in common is that they are emergent processes from the collective behavior of many (perhaps thousands) molecular components. The challenge in systems biology and molecular biology is to describe the dynamic network that results from the complex interactions among molecular constituents. Such interactions dictate material properties and functions, such as the distribution of mucins, other proteins and ions that determine the flow and diffusive transport properties of mucus, how dynein motors control spatial extent and polarity of the mitotic spindle, and how intracellular structures, activating and deactivating proteins, and remodeling processes conspire to achieve cell motility.

The applied mathematical goal is to capture the molecular constituents and their interactions in a modeling and simulation toolkit that reproduces collective, organized behavior and thereby reveals biological mechanisms. An understanding of how mechanisms work is prerequisite to understanding failure and strategies to recover from compromised functionality. Systems biology has traditionally averaged over molecular details, positing continuum-scale balance laws for observable macroscopic properties. From a bottom-up approach, coarse-graining methods, such as projection onto moments of distributions for molecular constituents, often lead to continuum equations that resemble and validate continuum-scale models and give molecular meaning to their coefficients. These connections between molecular kinetic and continuum models have been studied for many model complex fluid systems within the statistical physics literature. For polymers, the books by Bird, Curtiss, Armstrong, and Hassager [1], Beris and Edwards [2], Larson [3], and Rubinstein and Colby [4] give excellent treatments.

A medical biology challenge is to detect and quantify the sources of disruptions in normal material functionality, e.g., a genetic defect that disrupts an ion channel in cystic fibrosis or DNA damage that leads to a runaway cascade in a cell cycle. Once understood, these insights explain symptoms (e.g., dehydrated lung mucus, proliferation of cell division), point to diagnostics for disease progression, and focus medical treatment on the sources of compromised function.

The engineering and clinical challenge is to design health solutions to restore function, aided by validated models and predictive simulations to test outcomes of alternative therapies. The solution could likewise be molecular (e.g., drugs specific to cellular pathways, gene therapy, DNA repair) or systems level (e.g., a percussive therapy), or both.

Each challenge above provides research opportunities in complex biological fluids for experimental and theoretical scientists. The aim of this chapter is to give young researchers an insight into fascinating aspects of biological complex fluids through the lens of our experiences with colleagues spanning all of the above disciplines. Subsequent chapters in this book will focus and go into significant detail on:

- Diverse biological fluids
- Methods to experimentally probe their behavior
- Observations and data afforded by advanced instrumentation
- Progress in theory, modeling, and direct numerical simulation

There are open problems as far as one can foresee in the quest to understand, measure, characterize, model, or predict biological soft matter behavior, a point that will become clear throughout this and other chapters. Two materials are discussed in this chapter as already mentioned: mucus and a single cell. *Mucus* is a barrier complex fluid, coating every human organ, whose components and properties are considered to be stationary on short time scales (perhaps hours depending on the organ). A *single cell* is an assembly of diverse structural components that are locally in time (perhaps minutes) spatially organized yet undergoing continuous activation by molecular events that maintain a living cell as a nonstationary complex fluid mixture.

We will highlight lung mucus in particular, a remarkable functional material that is continuously forced toward the larynx by coordinated cilia and asymmetric air drag from breathing and cough, and other mechanisms like chest cavity pumping in tapered, deformable airways and surfactant gradients in the deep lung airways. Lung mucus has a dual role: to trap and to clear airborne pathogens, and it is biochemically tuned to perform both tasks. While lung mucus constituents are locally stationary, airway mucus in healthy humans is in a state of continuous forcing at diverse length scales, frequencies, and force scales by the clearance mechanisms noted just above. In this way, transport of mucus is dictated by its nonequilibrium properties, and we have yet to determine thresholds of nonlinear behavior from all physiological forcing conditions and airway geometries. In this chapter we will briefly discuss nonlinear viscoelastic behavior in the context of lung mucus and we refer the reader to Chap. 6 for a more detailed discussion of nonlinear viscoelastic behavior. While Chap. 6 addresses metrics of nonlinearity in macrorheology, a microrheological alternative that can be applied to microliter volumes to characterize mucus and substructures inside of cells is still needed.

Chapter 3 addresses active microrheology, which we also touch upon below to explore whether a single cilium is capable of forcing airway mucus into a nonlinear response regime. Chapters 7 and 8 are related to cilia-mucus interactions in that cilia can be viewed as “swimmers” that penetrate and pass through mucus during the power stroke. A fundamental challenge remains to understand the mechanism for the transfer of energy spent in a cilium stroke cycle into transport of the mucus layer: is it simply a momentum transfer, or is there a deformation of the microstructure that tugs on the entangled polymer network and pulls on the mucus like a carpet layer? For medical biologists and clinicians, it is critical to understand that mucus changes, biochemically and functionally, over time scales of disease progression and what those changes are, to guide potential remedies and therapies. We will address this issue in more detail below.

Living cells are inherently active materials, to be distinguished from other biological materials such as mucus, which are passive materials undergoing active forcing at free interfaces. The molecular machinery inside a living cell is constantly working (e.g., molecular motors, polymerization and depolymerization, biochemical reactions), generating forces that easily compete with thermal fluctuations that would still be present even if the molecular machinery was switched off. This means that single living cells are maintained out of equilibrium by chemical and mechanical processes, that these processes change during the cell cycle, that probes inserted into the cell may interrupt passive and active cell behavior, and that the influence of active forcing is spatially heterogeneous. For example, if an activating protein species binds at the bilipid membrane or in the cellular cortex to cause a local contraction, that deformation propagates. Similar mechanochemical processes are taking place throughout the cell. Chapters 5–8 highlight the challenges in characterizing material properties of cellular components in the presence of often unknown or uncharacterized active forces. Chapters 9 and 10 give further illustrations of other active materials ranging from bacterial suspensions to motor proteins.

With respect to biological materials, the first aim and challenge is to prescribe a series of tests and experiments to characterize the material of interest *in physiologically relevant conditions* (e.g., lung mucus at different disease states) or to observe the material of interest in biologically relevant conditions (e.g., yeast cells at specific phases of the cell cycle). Of course one has to have sufficient experience and data to compare these outcomes with other complex fluid species and with diverse samples of the fluid of interest from “normal” and “dysfunctional” sources. This phase could be described as comparative rheology or biology, where the measurements or observational data of a particular specimen are compared to known or possibly benchmark specimens of similar origin. If there is sufficient data, inferences can be made by performing various statistical tests to decide if a given specimen lies within certain percentiles or standard deviations from a population mean.

The second challenge is to build predictive model capabilities. The aim is to develop quantitatively accurate mathematical models that extrapolate beyond experimental controls and that can be coupled to physiological forces and in vivo geometry to predict complex fluid behavior and function. For instance, design and evaluation of drug or physical therapies for lung disease would be far more efficient with accurate, predictive models for airway mucus flow and particle diffusion within mucus layers. Cellular abnormalities likewise would be better understood and designer molecules for cell repair would be more efficient with accurate predictive models.

The first step, characterization, is the purview of rheology: the study of how materials deform or flow due to applied loads. This subject is reviewed in Chap. 1; subsequent chapters give detailed experimental, theoretical, and computational insights into biological fluid behavior when forces and strains are applied at macroscopic scales (macrorheology) and at microscopic scales (microrheology). The second step, modeling, relies upon fundamental conservation laws, physical, chemical, and mathematical principles to posit a predictive model (governing

equations, boundary conditions, and initial data) that when properly parameterized accurately reproduces experimental observations. An inverse problem can therefore be solved to infer all model parameters from wisely chosen experiments and data. Given the model and parameter fittings, accurate simulations of the model should be feasible and able to predict behavior under conditions more general than the characterization experiments. These goals are far from complete for almost all complex fluids in the human body, although progress has been made. For example, remarkable advances have been made in the modeling of tear films [5], the mathematical description of soft tissues like articular cartilage [6], and modeling blood flow discussed in Chap. 11.

To be honest at the outset, the novice to complex fluids is forewarned that the world of materials that are neither simple viscous fluids nor simple solids encompasses such diversity that there is no universal class of models to begin with. In fact, there are few complex fluids for which predictive models exist, have been validated, and used for biological or biomedical applications. We will mention some examples, and the other contributors to this volume provide more specific details. Significant research opportunities in this area lie in: the derivation of accurate, computationally feasible models for physiologically relevant complex fluids; characterization in terms of linear and nonlinear constitutive laws and parameter inference from experimental data; and predictive simulations, especially those that might support health assessments and therapeutic applications.

The term “fluid” in complex fluid is potentially misleading, since it conjures images of hydrodynamics to many physical scientists. However, the complexity of complex fluids lies in the intimate coupling between flow or deformation and the microstructure of the material. In complex fluids, the dynamics of the flow or deformation field is on equal footing with the dynamics of the microstructure. Indeed in rheological experiments, instruments are designed either (1) to control flow or deformation to learn how the microstructural stresses respond in approximately linear flows or deformations in the relevant geometry of the instrument; (2) to control stress and study the flow or deformational response. The classical text by John Ferry [7], or the more recent text by Chris Macosko [8], are excellent sources for rheological instruments and how to characterize materials based on experimental data. For predictive purposes, outside of such controlled experiments, one must have constitutive laws with material parameters that are inferred from rheological experiments and then simulated or analyzed to predict more general behavior. In this respect we refer the reader to Chaps. 1 and 6.

The term “soft matter” is more appropriate and indeed is gaining traction as a descriptive term that encompasses biological as well as synthetic materials that are neither simple viscous fluids nor simple solids. More traditional descriptors are viscoelastic or non-Newtonian. The complexity of soft matter is revealed by the memory exhibited in equilibrium and nonequilibrium responses of soft matter. This memory is evident macroscopically (press on your flesh, and it recovers on a time scale that doctors use to assess excess water retention), and with appropriate instrumentation, memory is evident down to the scales of the microstructure. A generic challenge in soft matter is to determine the time scales of memory and

their influence on their transport properties: how a sample deforms or flows under forcing, how the microstructure fluctuates and diffuses in thermal equilibrium, and how foreign particles of diverse size, shape, and surface chemistry diffuse within the material. Indeed, the field of passive microrheology (see reviews by Waigh [9], Squires and Mason [10], Chen et al. [11] and Crocker and Hoffman [12], and Chap. 3) aspires to measure the fluctuations of probe microscopic particles and exploit a generalized fluctuation-dissipation theorem (FDT) to infer the complex dissipative properties. The remarkable FDT states that the memory spectrum of the microstructure in the linear response regime is revealed from the colored noise of the fluctuations of probe particles. For biological materials, including the internal structure of single cells or lung mucus, there are unknown length scales and heterogeneity of the microstructure, so it is not sufficient to probe with one particle or at one location [13, 14]. This is why the techniques of microrheology have been developed and honed on benchmark complex fluids such as colloids or pure homogeneous solutions of naked DNA as explained in Chap. 5. The challenges in biological fluids are addressed in many other articles within this volume, as well as in the specific sections below on cells and mucus.

The integration of biology and medicine with mathematical modeling and computational simulations is moving the life sciences toward new frontiers where physiological and pathological information from living organisms can be quantitatively described *in silico* [15]. In this chapter, we review the properties of fluids and soft matter encountered in the human body. As new methods are applied to increasingly complex biological process, our understanding of the mechanistic role of these biological materials has grown. However, there is still much to be learned in order to apply and advance the available tools toward predictive medicine. Here, and in the subsequent chapters, some of the challenges faced in the mathematical modeling of biological fluids and soft matter are reviewed.

1.1 Biological Materials in the Human Body

Sciences like rheology [1, 3] and biomechanics [16] study biological materials to find relationships between forces and deformations or flows. In the human body, atoms and molecules are organized into cells, tissues, organs, and individual organisms. As a result, forces, deformations, and flows can originate within the individual organisms or around them and include a wide range of time and length scales.

Human biological materials include tissues, organs, blood, plasma, skin, DNA, RNA, proteins, cells, mucus, saliva, and other body fluids. Some of the common characteristics of these materials are [17]:

- They are composites, containing both inorganic and organic components.
- They are able to self-assemble.

- They are multifunctional, with the ability to change their characteristics tailored to a specific function.
- They are hierarchically organized at the atomistic, molecular, and larger scales.
- Many properties are length and time dependent and can vary significantly across various scales.

As an example, consider the flow of blood in the microvascular network. The rheological properties of blood are dependent on shear rate, shear history, and the dimensions and geometry of the system in which it is contained. The apparent viscosity of blood measured in tubes with diameters $\sim 200\ \mu\text{m}$ shows a precipitous decrease with decreasing diameter, reaching a minimum at diameters of $\sim 5\text{--}7\ \mu\text{m}$, corresponding to the diameter of capillary blood vessels [18]. That is, blood is a shear-thinning material, whose nonlinear response depends on the particulars of the flow properties in vivo and is highly specialized to meet the needs of the specific organ or tissue. Current advances on modeling blood flow as well as a comprehensive discussion of the constituents of blood are addressed in Chap. 11. There is even evidence that viscoelasticity of blood is relevant to the fluid-structure interactions in the highly dynamic conditions in heart chambers, coronary arteries, and valves [private discussions with Boyce Griffith, NYU and UNC].

1.1.1 Mathematical Modeling of Biological Materials

Above the atomic and molecular level, cells, tissues, organs, and organisms can be considered as continua and have traditionally been described by classical mechanics. In this way, descriptions of these systems are derived from fundamental physical laws like conservation of mass, moment, and energy, together with the respective constitutive equations for the material.

In their simplest form, constitutive equations relate forces and deformations, or more precisely, stresses and either strains or velocity fields. Depending on the experimental or physiological conditions the input can either be the stress or the strain (or velocity). For example, in active microrheology, the input is the strain rate if the particle is moved with a constant velocity, such as in optical tweezer experiments. Alternatively, the particle can be driven with a constant force, such as in magnetic bead rheology. Determining the stress-strain relationship is then crucial in the understanding of these materials through the formulation of constitutive equations.

The basis to solve these problems is the coupling of the conservation of mass and momentum equations for fluid density ρ and velocity field $\mathbf{u}$, with the appropriate constitutive equation for the stress, $\boldsymbol{\tau}$,

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0, \quad (2.1)$$

$$\rho \left(\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} \right) = -\nabla p + \nabla \cdot \boldsymbol{\tau} + \mathbf{F}. \quad (2.2)$$

Under isothermal conditions, simple liquids like water or honey behave as Newtonian viscous fluids. In this case, the stress is directly proportional to the rate of strain ($\dot{\gamma} = \nabla \mathbf{u} + (\nabla \mathbf{u})^T$) and the constant of proportionality is the viscosity (η), which measures resistance to flow:

$$\tau = \eta \dot{\gamma}. \quad (2.3)$$

For elastic solids, the simplest relation is that of an isotropic Hookean solid where the stress is directly proportional to the strain (γ , where $\partial \gamma / \partial t = \dot{\gamma}$) and the constant of proportionality is the modulus (G), which measures the stiffness of the material. The simplest elastic constitutive law is

$$\tau = G\gamma. \quad (2.4)$$

Most biological materials exhibit characteristics of both viscous fluids and elastic solids. The nature of the response (more viscous-like or more solid-like) depends on the magnitude of the imposed deformation or forces or on the time scale at which the input is being imposed. These types of fluids are known as viscoelastic. Chapter 1 provides a more in-depth introduction to modeling and constitutive laws.

The simplest viscoelastic constitutive laws are the linear viscoelastic models, where the relation between the stress and the strain is linear, with a combination of both viscous and elastic terms. For example, when a spring and a dashpot are combined in series, the force on both units is the same, while the total deformation is the sum of the individual deformations. With these conditions, one can easily find the constitutive equation for this toy mechanical model as

$$\tau + \frac{\eta}{G} \frac{d\tau}{dt} = \eta \dot{\gamma}. \quad (2.5)$$

Other linear mechanical models can be formulated by different configurations of dashpots and spring units. For a detailed review of these models, we refer the reader to the book by Tschoegl [19].

Besides differential models, as the one given by Eq. (2.5), constitutive equations can also be represented by integral models. For example, continuing with our spring-dashpot model, integration of Eq. (2.5) with respect to time gives

$$\tau(t) = \int_{-\infty}^t G \exp(-(t-t')/\lambda) \dot{\gamma}(t') dt', \quad (2.6)$$

where the stress is related to the strain history by a kernel, which in the case of Eq. (2.5) is $G(t) = G \exp(-t/\lambda)$, and $\lambda = \eta/G$ is the relaxation time.

As mentioned above, most biological materials are viscoelastic, but even more than that the relation between the stress and the strain is not linear for all conditions. Some simplifications can be applied to use linear viscoelastic equations to describe a material. For example in passive microrheology, the motion of imbedded

“Brownian” probes is followed to gain insight into the viscoelastic properties of the material. The fluctuations of the particle arise from fluctuations of the microstructure and any solvent, and a generalization of the FDT to generalized Langevin equations (GLEs) is exploited to infer both viscous and elastic moduli of the material. For a detailed description see Chap. 3.

Another approach is to apply small stresses or deformations to the sample, guaranteeing that the strain-stress relation is linear. One such experimental approach is the small amplitude oscillatory shear (SAOS) where a sinusoidal stress with small amplitude is applied to the sample, for example, using commercial rheometers [8]. In this setup, given a strain of the form

$$\gamma = \gamma_0 \sin(\omega t), \quad (2.7)$$

Equation (2.5) can be solved to show that the stress is given by

$$\tau(t) = G\gamma_0 \left[-\frac{\lambda \omega}{1 + (\lambda \omega)^2} e^{-t/\lambda} + \frac{(\lambda \omega)^2}{1 + (\lambda \omega)^2} \sin(\omega t) + \frac{\lambda \omega}{1 + (\lambda \omega)^2} \cos(\omega t) \right]. \quad (2.8)$$

At the steady state, $t \rightarrow \infty$, the stress becomes the sum of two functions: one proportional to $\sin(\omega t)$, i.e., the strain, and one proportional to $\cos(\omega t)$, i.e., the strain rate. Because of their relevance to elastic and viscous behavior, these functions are known as the elastic (or storage) modulus $G'(\omega)$ and the viscous (or loss) modulus $G''(\omega)$. For the so-called upper convected Maxwell (UCM) linear viscoelastic constitutive law, these functions are given by

$$G'(\omega) = G \frac{(\lambda \omega)^2}{1 + (\lambda \omega)^2}, \quad G''(\omega) = G \frac{\lambda \omega}{1 + (\lambda \omega)^2}. \quad (2.9)$$

In this way, if a material obeys $G' > G''$, it is said to be more elastic than viscous and if $G'' > G'$, the material is more viscous than elastic. For a detailed review of these types of responses and their extensions to nonlinear regimes, see Chap. 6.

Yet another set of experiments are known as creep measurements, where a constant stress is imposed and the resulting strain is measured. For a Hookean solid, the strain is directly proportional to the stress so that the resulting strain history is like the one shown by the purple circles in Fig. 2.1. For a viscous fluid, the stress is proportional to the time derivative of the strain, with the resulting strain described by a straight line as shown by the blue squares in Fig. 2.1. Viscoelastic materials exhibit both viscous and elastic behavior. For example, some experiments in cells [20] have shown that their behavior is that of a viscoelastic fluid shown in red triangles in Fig. 2.1. For a further discussion on rheological tests, we refer the reader to Chaps. 1 and 6.

The analysis of the material properties based on linear viscoelastic measurements have provided great insight into the behavior and function of these materials. However, as mentioned above under physiological conditions these materials are

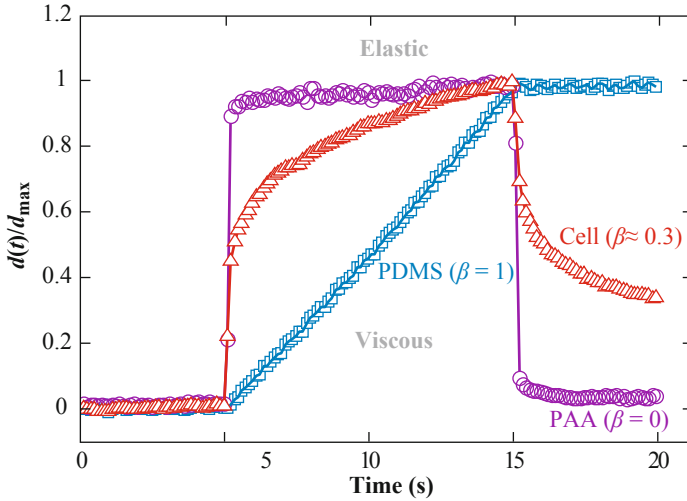


Fig. 2.1 Experimental creep response: the material is loaded at time $t = 5$ s with a constant force F and the displacement, $d(t)$, is measured. The figure shows typical responses of three types of materials: elastic material (purple curve) polyacrylamide-bis-acrylamide (PAA) hydrogel; viscous material (blue curve) polydimethylsiloxane (PDMS) silicone oil; and cellular material (red curve) F9 embryonic carcinoma cell. The creep function is defined as $J(t) = d(t)/F = j_0(t/t')^\beta$, where t' is a characteristic time scale of the experiment, the prefactor j_0 characterizes the softness or compliance of the material, and the power-law exponent β represents the type of material; $\beta = 0$ for elastic solids, $\beta = 1$ for viscous fluids, and $0 < \beta < 1$ for viscoelastic materials. Figure from [20]

often under nonlinear conditions. Consequently, experimental tests and models such as the ones we have discussed thus far fail to capture features relevant to the physiological functions of many biological materials.

1.1.2 Nonlinear Viscoelasticity

Chapter 1 provides a detailed description of modeling hydrodynamics in viscoelastic materials. Here we highlight the main challenges involved in the formulation and modeling of nonlinear viscoelastic behavior; Chaps. 6 and 10 should also be consulted.

The main characteristic of viscoelastic materials is that the relationship between stress and strain depends upon their deformation history. In particular, their rheological properties are dictated by the evolution of the conformation of microstructures. One of the main challenges is how to develop methods to connect the configuration at the microscopic level to the dynamics involved at macroscopic length scales. However, this need to bridge disparate length and time scales presents numerous challenges. As an illustration, consider the range of length and time scales encountered in a typical polymeric system. Characteristic times can span from $O(10^{-13} \text{ s})$ for bond vibrations to seconds or minutes for the relaxation of chain orientation

to hours for glassy states and phase separation. Similarly, relevant length scales vary from angstroms for bond lengths to nanometers for average chain length to micrometers or larger for experimental and industrial processes.

Depending on the level of investigation, one can model these systems by using stochastic differential equations, Fokker–Planck-type equations or macroscopic constitutive relations that can be differential equations, integral equations, or a combination of these two. The choice of level of description depends on the objective, the questions one expects to answer, and the computational capabilities one has when it comes to solving the resulting coupled system of equations. For different macroscopic constitutive equations of viscoelastic materials, the reader is referred to Larson’s book [21]. For a stochastic modeling of the materials, we refer to Ottinger’s book [22] and for a review on multiscale methods to the article by Keunings [23]. In addition, Chap. 10 and the book by Owens and Phillips [24] discuss different numerical approaches used in the simulation of viscoelastic materials.

The kind of nonlinear behavior and how to tease out such behavior depends greatly on the type of system under study and the particular information one expects to gain with modeling. For the scope of this chapter, we will focus on two exemplary materials: mucus and cells. For other behaviors and fluids the reader is pointed to other chapters in this book.

Under *in vivo* conditions, these systems are often subjected to large deformations and/or stresses rendering the stress-strain relationship nonlinear and time dependent. The adequate formulation of constitutive equations for these materials is challenging. In addition, probing the validity of such equations requires testing of the materials in controlled conditions, e.g., using the protocols and instruments of rheology. Experiments involving biological fluids and soft matter can be difficult either because the sample cannot be isolated for testing, or the available volumes are too small or too “soft” for many devices, or because it is difficult to keep the specimen in normal living conditions (e.g., controlled temperature and humidity). Additional perspectives are given in Chaps. 3–6.

Other challenges faced in the modeling of biological fluids include:

- The role of geometry and confinement. The material may reside in a complex, nonstationary, 3D geometry, e.g., mucus in deformable lung airways or a cell within a living tissue or exposed to a foreign substrate. Numerical modeling of *in vivo* material behavior requires solvers that adapt to these dynamic, complex geometries.
- Since many biological processes are nonlinear, the constitutive relations that describe material behavior can include mathematical terms with complex functional forms, e.g., memory over a broad frequency spectrum.
- Material response depends on a large number of variables. For example, blood flow can be dramatically different depending on capillary diameter, compliance of surrounding tissue or capillary walls, temperature, pressure, stresses, and heart rate. For a detailed discussion see Chap. 11.

- Most biological materials are heterogeneous, consisting of substructures with markedly different properties. Modeling therefore must either explicitly resolve the heterogeneity (e.g., phase-field modeling of single cells or dynamics of molecular constituents of mucus) or posit homogenized models with effective material parameters that average over the microstructural heterogeneity.
- Biological materials are often anisotropic so that their behavior is directionally dependent. For example, the filamentous cortex in cells is aligned as opposed to randomly oriented.
- Some biological systems change their properties during a process in response to imposed stresses. In this way, there can be dramatic changes in the properties over both time and position. Modeling efforts must either model the substructures and their dynamics or capture these effects through coarse-grained parameters and their space-time variations.
- The development of accurate, meaningful boundary conditions is a major challenge, including fluid-structure conditions, adherence versus slip at interfaces, stress versus strain versus velocity boundary conditions, and their compatibility with models. All of these issues weigh heavily on the choice of numerical algorithms, and often potential boundary conditions need to be tested against experimental data to determine their validity.

2 Mucus in the Human Body

In the human body, mucus covers the luminal surface of the gastrointestinal (GI) [25], respiratory [26], and reproductive [27] tracts. Mucus also coats eyes, the epithelium of the nose, mouth, and salivary glands, as well as the peritoneal surface of intra-abdominal organs [28]. All mucus is not made equal; it is biochemically tuned for diverse barrier and flow transport properties depending on which organ it coats. For example, mucus acts as a lubricant, as a moisture barrier to prevent dehydration of underlying tissues, as a chemical barrier to prevent gastric acids from destroying tissues, and as a diffusional barrier to pathogens and airborne particulates. In many organs, mucus flows to clear its trapped contents, with the flow generation mechanisms as diverse as mucus itself, e.g., blinking eyelids, gravitational drainage in the reproductive tract, airway cilia, airdrag from breathing, coughing, or sneezing. Typical human mucus co-regulates diffusion of trapped pathogens and clearance of the trapped load. Since mucus is being swept away continuously, the particular organs or epithelial tissues are likewise continuously manufacturing a distribution of high-molecular-weight mucin molecules and controlling water content via ion-nucleotide feedback mechanisms [29, 30]. These molecules collectively endow mucus with its ability to recognize (in the sense of control over their diffusion) and discard (by clearance of the mucus layer) particles ranging from $O(10\text{ nm})$ antibodies to $O(100\text{ nm})$ viruses to $O(1) - O(10)$ micron bacteria and any number of environmental particulates.

Disease states are often associated with a breakdown in “normal” properties of the mucus barrier [31], from stomach ulcers to chronic obstructive pulmonary disease (COPD). While mucus is ubiquitous in the human body, and therefore fundamental to human health, the links between the molecular constituents of mucus, the mechanisms by which they are continuously replenished to maintain their critical functions, and indeed the key microstructural features and variations of mucus throughout the body that endow such diverse functional properties remain active areas of research. It is fair to say that mucus is now “hot,” with a resurgence of attention since the landmark paper on airway mucus by Knowles and Boucher [32].

Mucus functions as a viscoelastic, dynamic, semipermeable barrier that protects organs and epithelial tissue by selectively trapping and discarding pathogens, toxins, and other particulates [32]. At the same time, mucus layers allow the flux of water, gases, and nutrients that are transported through epithelial cells and distributed inside the body [28,33]. In performing these functions mucus is constantly secreted, shed, and digested, recycled, or discarded. The mechanical and chemical properties of mucus are critical to its functional specificity, as it coats every organ and surface not covered by skin or nails. These diffusive, flow, and lubricating properties vary not only across organs but among individuals, with age, and physiological and pathological conditions [28,32,33].

Given the fundamental role of mucus in human health, it is perhaps surprising how much is known yet how little is understood about its biophysical and rheological properties. However, the diversity of mucus across the body and across populations, coupled with the difficulty in procuring samples (extremely low-volume samples) and the sensitivity of mucus to handling, presents major challenges in the experimental and theoretical characterization of human mucus. It is only in the recent past that the heightened awareness of the role of mucus in human health has converged with new instrumentation and new theoretical advances, in particular the emergence of the field of microrheology, together with the historical shift of the physical and mathematical sciences toward the biological and biomedical sciences.

Next, we review the main composition of human mucus and how it affects its flow and diffusive rheology. We discuss one of the main mechanisms for mucus clearance, coordinated ciliary beating, and review modeling approaches. Finally, we discuss diffusion of particulates in mucus.

2.1 *Mucus Composition*

A typical “healthy” mucus sample consists of 90–95 % (by mass) of water, 2–5 % high-molecular-weight glycoproteins (mucins), 1–2 % lipids, 1 % salts, and 0.02 % of DNA and other molecules [34]. Despite its dominant water content, mucus readily exhibits both viscous and elastic behavior that may vary dramatically with frequency (or shear rate), with amplitude of forcing (imposed strain or stress), and with the length scale of forcing. These viscoelastic properties are biochemically regulated by the relative concentration of, and interplay between, the components listed above.

Some key studies point to a classification of mucus as a physical gel, distinct from an entangled polymeric material [35–37]. This gel quality derives primarily from mucins and other low-molecular-weight proteins that form a three-dimensional network or gel matrix [35]. Mucins are negatively charged, glycosylated proteins that are continuously synthesized and secreted to replenish the mucus layer. Mucins can be divided into secreted (gel-forming and non-gel-forming) and membrane-anchored. Gel-forming mucins are a complex group of high-molecular-weight, polymeric glycoproteins. The main gel-forming mucins present in human mucus are MUC2, MUC5AC, MUC5B, and MUC6 [38–42]. Mucins also contain cysteine-rich domains where no glycosylation is present. These “naked” domains have hydrophobic properties [38, 43, 44]. To avoid contact with water, the hydrophobic portions of the molecules form dynamic, physical mucin-cross-links. This “intertwining” of mucins with other musins and biomolecules presents in the mucus constitute the gel matrix. Among other things, the density of cross-links controls the characteristics of a gel network. In a 2–5 wt% mucus gel, each mucin molecule overlaps 10–100 other mucin [28, 45]. In addition, other types of intermolecular interactions of different characteristic time and length scales contribute to the formation and strength of the mucus gel network. In particular, electrostatic interactions [39], hydrophobic interactions [46], and calcium-mediated interactions [47] have a well-documented role. Figure 2.2 depicts a mucin network including hydrophobic interactions and strong disulfide bonds (s-s). For extended reviews on mucus gelation see [36, 45, 48–52].

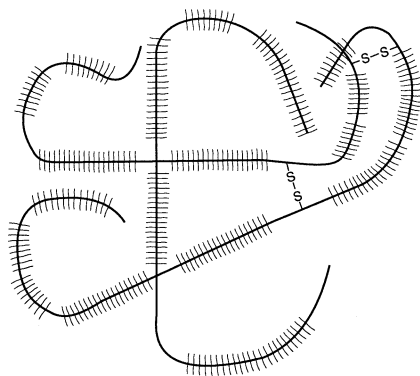


Fig. 2.2 Mucin molecules are made up of a peptide backbone (*solid curves*) with glycosylated regions (*perpendicular cross hairs*) and naked regions. These naked regions allow mucins to interact with other mucins and proteins through weak non-covalent bonds. These bonds together with strong disulfide bonds (s-s in the figure) result in the formation of a cross-linked network, and, at typical mucin concentrations, a high density of entanglements. Figure from [43]

2.2 *Mucus Viscoelasticity*

In general, all mucus secretions are viscoelastic; however, the absolute values of their viscous and elastic moduli and the stresses they are exposed to in vivo vary significantly depending on what organ, epithelial tissue, or cell culture the mucus is harvested from. Furthermore, the viscoelastic moduli from a single organ of a particular person evolve with environmental exposure (e.g., altitude), disease, and disease progression. To illustrate this, consider mucus in the stomach. Taylor and coworkers [53] showed that, in the stomach, two physically distinct mucus secretions are produced. One is a “shear-resistant” mucus gel that forms a protective mechanical barrier and the other is a “shear-compliant” secretion, which transforms into a viscous liquid when subjected to even low mechanical shear stress and acts as a lubricant facilitating the movement of solid matter through the gut during the digestive processes.

John Sheehan [54] led the effort to identify which mucin molecules were primarily distributed in sputum and airway mucus compared to the mucin distribution and concentrations in the periciliary liquid layer (PCL) of lung airways. It is remarkable to recognize that goblet and other cells in the epithelium manufacture all these molecules, and thus all are shed from the epithelial surface. Thus, they choose by physical and chemical affinities to reside in the PCL or mucus layers. Sheehan’s goal was to biochemically and biophysically characterize the functionalities of mucus and PCL layers in airways and to understand their evolution during aging, environmental conditions, and disease progression [34, 42, 44, 47, 48, 51, 54–56].

As is the case with other viscoelastic materials, the interplay between viscous and elastic properties directly affects the transport capabilities of mucus. However, other physical properties play an important role in the function of mucus. For instance, adhesiveness and wettability govern the properties of the interface between mucus and the epithelial surface [57]. Optimal conditions for the clearance and lubricant properties of mucus require that both wettability and adhesiveness are high enough to prevent flow of mucus under body forces (gravity) but low enough to mobilize mucus by ciliary beating and other air–liquid pumping mechanisms.

Although the mucin network primarily governs the rheological characteristics of mucus, other biochemical constituents such as proteins, proteoglycans, lipids, DNA and cellular debris affect the properties of mucus by modifying gel formation and strength. In the same manner, other macromolecular components of exogenous or pharmaceutical origin can influence the viscoelastic properties of mucus and as such alter its functional properties. Below we give several examples where changes in rheological properties of mucus are induced by factors other than the self-dynamics of the mucin network.

- Rheological measurements of human tears reveal shear-thinning viscoelastic properties. However, contrary to other types of mucus, the specific mucins and their concentration in tears are insufficient to produce the observed degree of non-Newtonian behavior. Gouveia and Tiffany [58] showed that if the lipids in tears are removed, the viscous response becomes Newtonian (shear-independent).

- Airway mucus is normally degraded by proteases [32]. Innes and coworkers [59] reported that an excess of plasma proteins present in acute asthma patients inhibits the degradation of mucins in a protease-dependent manner. This results in changes in the viscoelastic properties of mucus that reduces clearance, resulting in mucus plugs occluding the airway. Why clearance is reduced is an open question, relating back to the need for predictive models that evaluate clearance efficiency versus viscoelastic characterization and comprehensive experiments and theory to provide viscoelastic characterization.
- Dynamic light scattering and bulk rheology measurements reveal that gastric mucin solutions undergo a pH-dependent sol-gel transition from a viscoelastic solution at neutral pH (~ 7) to a soft viscoelastic gel in acidic conditions ($\text{pH} < 7$), with the transition occurring near a pH of 4 [45, 49].
- Several studies showed that the presence of salts in mucin solutions greatly affects their rheological properties [46, 49, 60]. In particular, the concentration of salts is correlated with decrease in mucin gel strength. This is consistent with other studies showing that inhalation of hypertonic saline increases mucociliary clearance (MCC) [61] and aids in mucus clearance for cystic fibrosis patients [62, 63].

We are still far from a synthesis of the biochemical basis that conveys mucus with its functional properties, still far from a viscoelastic classification of mucus specific to human organs or epithelial surfaces, still far from quantifying how mucus viscoelasticity varies during the life of a healthy human, and still far from quantifying how mucus viscoelasticity evolves with disease. However, the importance of the viscoelastic properties of mucus for many physiological functions is undeniable [38, 57–59, 64–68], nor can one deny the biomedical potential of tools that selectively modify mucus viscoelasticity [69–72]. Note that the awareness that mucus viscoelasticity is the controlling factor for barrier and clearance properties, and that efficiency of the diffusive barrier to specific foreign particles and of transport from specific clearance mechanisms, is relatively new in biology and medicine. This recognition can only be exploited effectively if the science and engineering tools are developed and implemented in physiologically relevant and clinically relevant conditions.

2.2.1 Rheological Characterization

As discussed above, the rheology of mucus is determined by its composition and structure. However, rheological properties are dynamic rather than static measurements. In this sense, it is important to recognize that the rheology of mucus also depends on its interaction with dynamic forces like ciliary beating and airflow, eye blinking, gut contractions, etc. Thus, as with any other viscoelastic material, the rheological testing of mucus gels yields different results with different applied stresses or deformation frequencies.

- *Dynamic Moduli.* One way of classifying the rheological response of viscoelastic materials is through the frequency-dependent storage ($G'(\omega)$) and loss ($G''(\omega)$) moduli. In Fig. 2.3, the dynamic moduli of mucus from different sources have been plotted. The wide range of values observed in the figure arises from differences in measurement methods, functions and composition of the different types of mucus secretions, and samples obtained from normal and pathological individuals. Because of the dependence of mucus viscoelastic properties on the driving frequency, in the studies of the rheology of airway mucus, frequencies need to be chosen to mimic different transport mechanisms. Examples are the low-stress, high-frequency forces applied by beating cilia (~ 10 Hz or 62 Rad/s); the low-stress, low-frequency extrusion of secretions from glands (~ 1 , 2 or 6 Rad/s); or the high-velocity, high-stress forces imposed by cough or high-frequency ventilation or percussive therapies.

It is important to note that the viscosity of human respiratory mucus has often been given as 12–15 Pa·s with a relaxation time of about 40 s and elastic modulus of 1 Pa. These values are said to represent an optimal rheological profile for MCC [33]. However, it becomes apparent in Fig. 2.3 that such a classification of mucus viscoelastic properties in terms of a single value of the moduli fails to capture the complex spatial and temporal interactions of mucus with physiological forcing.

- *Spinnability.* Another rheological measurement used to determine mucus properties like adhesion and elasticity is spinnability. Spinnability is characterized by the mucus ability to be drawn into long threads under the effect of traction, which measures the cohesive forces that hold the mucus together. A typical measurement is performed with a given mucus volume (typically 30- μ L) stretched at some velocity (e.g., 10 mm/s). An electric signal conducted through the sample is interrupted at the point where the stretched thread is broken. This measured distance is reported as the spinnability of the sample. The spinnability of normal respiratory mucus ranges from 40 to 100 mm and becomes less for sputum [77, 78]. In a model study, a high spinnability was found to correlate inversely with cough clearance [79]. Table 2.1 shows several values of the spinnability of respiratory mucus.

2.2.2 Modeling Mucus Rheology

It is clear that, to faithfully characterize mucus in terms of predictive models, one must consider the dynamics of the gel network together with a wide range of interactions and conditions that affect the properties of mucus. One of the main challenges in the study of the rheological properties of mucus resides in the vast set of factors affecting such properties in vivo. Mucus harvested from human bronchial epithelial (HBE) cultures provides a model respiratory mucus free of inhaled infectious and inflammatory materials [56, 83, 84]. Here we show passive microrheology results for the linear dynamic moduli of HBE mucus and then use a canonical viscoelastic model, the UCM model, as a mode basis to fit the data. For a detailed review of microrheology methods see Chap. 3.

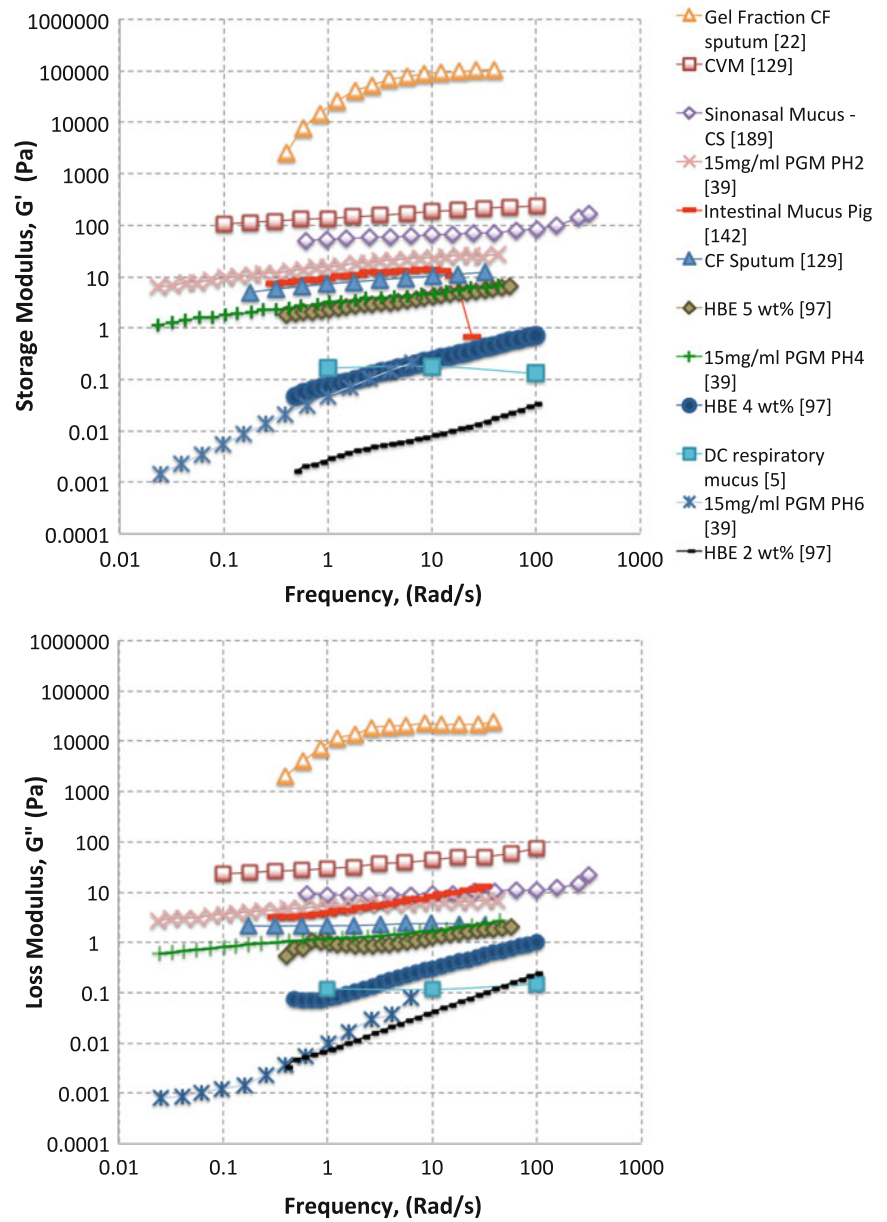
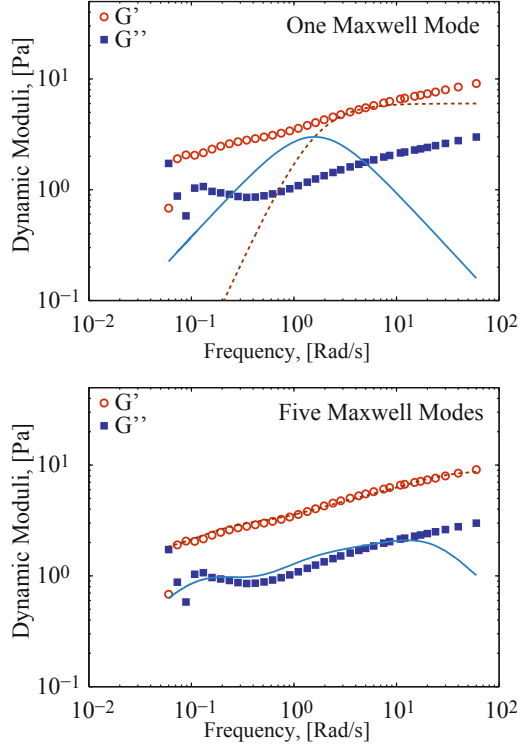


Fig. 2.3 Comparison of the viscoelastic properties of different types of mucus. Data points are a compilation of viscoelastic properties of mucus reported in the literature including: HBE mucus, [73], cystic fibrosis (CF) sputum [33], gel fraction of CF sputum [74], cervicovaginal mucus (CVM) [33], sinonasal mucus in patients with chronic sinusitis (CS) [68], pig gastrointestinal mucus (PGM) [49,75], and sputum obtained by direct collection (DC) [76]

Table 2.1 Values of spinnability for three different types of respiratory mucus

Source of mucus	Spinnability (mm)	Reference
Endotracheal tube technique	35.7 ± 17.5	[80]
CF sputum	12.6 ± 2.99	[81]
Patients with bronchiectasis	11.6 ± 0.4	[82]

Fig. 2.4 Fittings of 5 wt% HBE mucus to the UCM model with one relaxation time (*top*) and five relaxation times (*bottom*). Data courtesy of David Hill, Cystic fibrosis Pulmonary Research and Treatment Center, The University of North Carolina at Chapel Hill

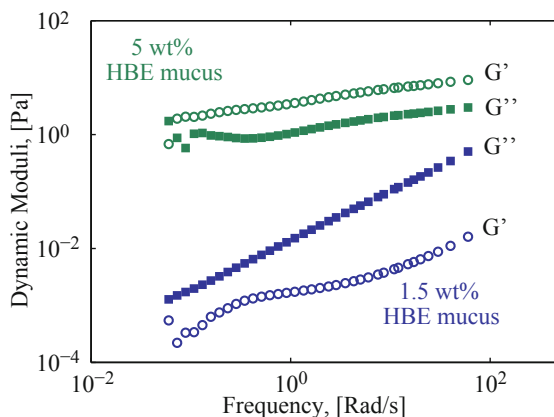
The moduli of a multimode UCM model, consisting of a linear superposition of UCM modes, are the summation of the moduli, given in Eq. (2.9):

$$G'(\omega) = \sum_i G_{0,i} \frac{(\lambda_i \omega)^2}{1 + (\lambda_i \omega)^2}, \quad G''(\omega) = \sum_i G_{0,i} \frac{\lambda_i \omega}{1 + (\lambda_i \omega)^2}. \quad (2.10)$$

Figure 2.4 shows fittings to a 5 wt% HBE culture sample. The presence of multiple relaxation modes describing the linear dynamic response of mucus is evident.

A viscoelastic material is said to behave like a liquid at a given frequency if it dissipates more energy than it stores, i.e., the loss modulus is greater than the storage modulus ($G'' > G'$) at that frequency. Similarly, the material behaves elastically (or gel-like) at a given frequency if $G'' < G'$. In the UCM model, the relaxation time, λ , marks a transition from liquid-like to elastic-like, as seen in the right top in Fig. 2.4 where the blue solid curve (G'') intersects the dashed red curve at $\lambda = 10 \text{ rad/s}$.

Fig. 2.5 Storage (G') and loss (G'') moduli for two concentrations of HBE mucus: 1.5 wt% (blue) and 5 wt% (green). When $G' < G''$ mucus dissipates more energy than it stores, behaving like a liquid. When $G' > G''$ the storage of energy is greater than the dissipation and mucus behaves like an elastic gel. Data adapted from [73]



Across physiological frequencies, e.g., from 0.1–100 Hz, HBE mucus has been shown to behave like a viscoelastic fluid ($G'' > G'$) at low mucin concentrations and like a viscoelastic gel ($G'' < G'$) at larger concentrations as shown in Fig. 2.5. The concentration at which the transition from a liquid to a gel occurs is called the gel point (GP). Recent studies show that the GP for HBE mucus is around a concentration of 4 wt% [73].

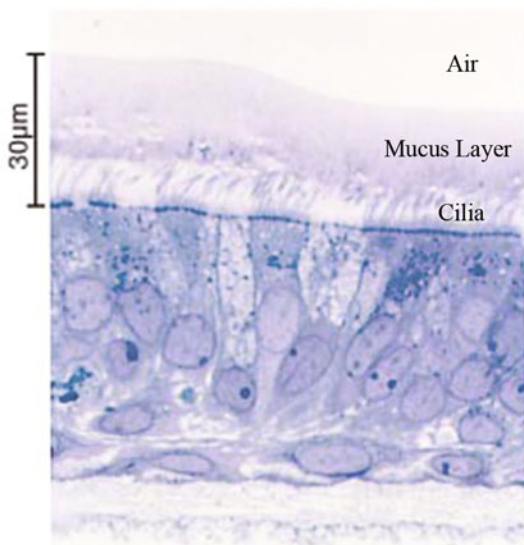
2.3 Respiratory Mucus Clearance

The airway epithelium is covered with a layer of fluid called the airway surface layer (ASL) composed of PCL and a mucus layer; see Fig. 2.6. In the human lungs, the mucus layer is believed to be $\sim 2\text{--}70\text{ }\mu\text{m}$ thick [85]. This thickness is determined by the balance between the rate of secretion and rate of degradation and shedding [28]. Mucus is produced at a resting rate of 0.5–1 ml of mucus per square centimeter of epithelial tissue surface over a 24-h period [43]. Studies of mucus transport velocity in vivo show that typical mucus transport rates in the trachea are 7–14 mm/min [86]. According to several in vivo studies, airway secretions loaded with organic and inorganic matter are typically cleared within 6 h [87]. This means that clearance of the entire tracheobronchial tree is mainly completed within 24 h [88]. In healthy individuals, the rate of mucus secretion is carefully balanced by mucus clearance.

2.3.1 Mucociliary Clearance

The human airway surface coating consists of an overlaying gel-like mucus layer and a lower PCL, which protects the epithelial surface from inhaled pathogens and particulates contained in mucus, as shown in Fig. 2.6. To prevent infection or

Fig. 2.6 Visualization of ASL surface setup. The cilia is in the lower PCL and it is in constant contact with the mucus gel layer. Figure adapted from [89]



inflammation, airway mucus must be cleared by a combination of coordinated cilia and airdrag; clearance requires a balance between the rheological properties of the mucus, PCL properties and volume, ciliary beat frequency and cilium length [32], and air–liquid transport [90, 91]. The PCL in healthy conditions is $\sim 7\mu\text{m}$ thick [92, 93]. The thickness of the luminal mucus layer varies throughout the respiratory tract, increasing from distal to proximal airways [93, 94].

The continuous ciliary beating propels mucus, in a proximal direction, up and out of the lung [28, 32, 43]. The coordinated beating of cilia can propel the mucus layer at reported speed of 1–10 mm/min [28, 95, 96]. During the effective stroke, the ciliary tips penetrate into the mucus layer, and, during the recovery stroke, they withdraw from this layer, as seen in Fig. 2.7 [97, 98]. Thus, most of the ciliary motions occur within the PCL, and only the tips of the cilia sweep against the mucus gel, thereby optimizing the propulsive force of the ciliary beating in MCC. When cilia penetrate mucus, they tend to bend backwards and if this bend is too great because of excessive mucus viscosity the system does not work effectively [99]. One can perform a back-of-the-envelope calculation to determine the dissipative losses per unit volume of the cilia beating in the PCL. These losses are proportional to $\eta_{PCL}(\omega L \delta_{PCL})^2$, where η_{PCL} is the viscosity and δ_{PCL} the thickness of the PCL, L is the length of the cilium, and ω the frequency of the beat. As an illustration, it has been shown that defects like cilium damage, reduced cilia beat frequency, and reduction of cilia length are factors that hinder MCC in smokers [100].

Phase shifts between beating cilia result in patches of adjacent cilia that coordinate in the propulsion of mucus [98, 102]. The mechanism of mucus transport by cilia is a biological illustration of active microrheology discussed in Chap. 3.

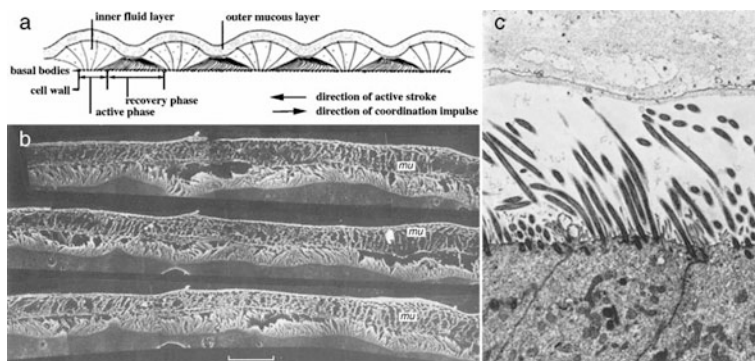


Fig. 2.7 Two layer hypothesis of airway secretions, showing the profile appearance of the cilia at successive stages of the beat cycle and their relationship to the overlying mucus (mu). Figure from [101]

Cilia-mucus interactions are related to swimmer-like propulsion in complex fluids, discussed in Chaps. 7 and 8, with a role reversal: the cilia aim to move the fluid past them rather than propel themselves. In order to produce a synchronous, wave-like movement of cilia (a so-called metachronal wave), it is widely believed that the PCL should have a low viscosity, which we argue against below. The characteristics of the synchronous metachronal wave are determined by the size and spacing of the cilia patches as well as the viscoelastic properties of the PCL. In addition, the thickness of the periciliary layer is critical for effective propulsion of mucus [98, 103]. The view that the PCL is a low-viscosity layer does not recognize that this layer is rich in many of the same mucin molecules that comprise mucus. A more recent view of the PCL has been proposed by our colleagues Button et al. [55]. The authors proposed a gel-on-brush model of the airway surface where the PCL is stabilized by osmotic effects and intermolecular repulsions. In addition, the macromolecules tethered to the cilia form a mesh that prevents large molecules and inhaled particles from penetrating the PCL. In general, if the periciliary layer is too shallow the cilia will not be able to perform a recovery stroke, and if it is too deep the cilia will not reach the mucus layer and the mucociliary action will be uncoupled [55]. Volume and composition of the PCL is mainly controlled by two mechanisms [104–106]: active ion transport and the continuous replenishment of loss of water by evaporation. For a more detailed discussion of the composition of the PCL we refer the reader to [55] and references therein. Finally, some reported characteristics of cilia are given in Table 2.2.

MCC relies not only on coordinated ciliary activity but also on specific rheological properties of mucus. It is widely believed that mucus viscoelasticity is optimized for clearance by coordinated cilia, yet as in any biological function, secondary mechanisms must exist such as tidal breathing and coughing. Indeed, in conditions such as the rare genetic disorder called primary ciliary dyskinesia or advanced lung disease, cilia are either asynchronous or unable to penetrate mucus, and air-liquid pumping is the primary mucus clearance mechanism [109]. Deep

Table 2.2 Reported properties of cilia

Quantity	Value
Cilium height ^a	5–8 μm
Cilium diameter ^a	0.15–0.3 μm
Frequency of beat ^a	10–20 Hz (60–120 Rad/s)
Wave length of metachronal wave ^a	20–40 μm
Cilia spacing ^a	0.3–0.4 μm
Density of cilia ^a	6–10 per μm^2
Number of cilia per cell ^a	200–400
Duration of effective stroke ^b	10 ms
Percentage effective stroke in beat cycle ^b	20 %
Duration of rest ^b	13 ms
Percentage rest in beat cycle ^b	26 %
Duration of recovery stroke ^b	27 ms
Percentage recovery stroke in beat cycle ^b	54 %
Speed of a tip cilium during effective stroke ^b	1 mm/s

References ^a[107], ^b[108]

in the lung, surfactants are prevalent whereas cilia density is very low and air is essentially stagnant; surfactant gradients play a significant role in clearing deep lung particles, a very slow mechanism relative to other mucus clearance modes. Under normal conditions, when the tip of a cilium engages the surface of the mucus layer, it sweeps with a shearing motion in the power stroke that is fast (recall cilia beat cycles are 10–15 Hz) and acts over micron scales. Silberberg argues that the elastic characteristic of the mucus gel dominates the efficiency of transport [26]. Hence, it is argued that cilia can only transport mucus if it has the proper viscoelasticity.

It turns out that frog palates are a good model system to evaluate MCC. Although the palate stops secreting mucus some time after excising the palate, the cilia continue to beat, allowing the placement of mucus samples or mucus simulants to be transported by cilia [110, 111]. With this setup, it has been shown that ciliary beating is unable to transport a variety of purely viscous materials. In addition, as the elasticity of mucus and mucus simulants is increased, there is a sharp increase in the clearance rate up to an optimal value, followed by a slow decrease in mucus transport [112]. Thus there is strong evidence of a mucus viscoelastic “sweet spot,” yet such results have yet to be reproduced with high-fidelity models. We point the reader to Chaps. 7 and 8 and the article by Teran, Fauci, and Shelley [113] for issues of optimal transport in complex fluids.

From the clinical and observational perspectives, there are numerous interpretations that have guided treatments for compromised mucus clearance. If respiratory mucus becomes too runny, i.e., more viscous than elastic, gravity may dominate ciliary transport, as observed when mucus drains from the nasal and sinus cavities. Often, gravitational drainage therapy is preceded by inhalation of hypertonic saline solutions to “thin” the airway mucus. On the other hand, if mucus becomes too

“hardened,” i.e., more elastic than viscous, it hinders transport by cilia for potentially many reasons. For instance, the storage modulus may be so high that mucus resists the stress that a beating cilium is capable of generating in the power stroke. Thus, cilia are unable to penetrate the mucus layer. Often a drug or physical therapy is capable of softening mucus temporarily to reinstate MCC. The viscoelasticity of mucus is apparently regulated to obtain the best compromise between the elasticity needed to prevent gravitational drainage (which leaves airway epithelia exposed to pathogens) and to provide efficient ciliary transport [26]. Healthy mucus is a gel with relatively low viscosity and elasticity that is easily transported by ciliary action, whereas pathological mucus has higher viscosity and elasticity and is less easily cleared [28, 73, 114]. Although, the viscosity of mucus results in energy loss, these losses are necessary for mucus to be displaced and either expectorated or swallowed [26, 32]. The elasticity of mucus is potentially important to minimize energy, with little energy loss from physiological forcing. As mucus becomes more viscous, there is a tendency for the ciliary beat frequency to decrease [43, 115] and the length and coordination of the metachronal wavelength become less efficient. We refer to several reviews related to these interpretations associated with efficiency of clearance and mucus viscoelasticity [95, 103, 116–119]. Again, without accurate modeling and simulations, it is virtually impossible to quantify all of these competing effects.

To explore the interplay between phasic forcing conditions and the viscoelastic properties of a complex fluid such as mucus, we consider the model problem of a viscoelastic material between two plates, with the upper plate stationary and the lower plate oscillating with frequency ω and maximum speed U_0 ,

$$u_x(y=0) = U_0 \cos(\omega t). \quad (2.11)$$

In this way, the movement of the lower plate captures, in a geometrically simplified sense, the effect of ciliary beating. We seek to assess the work consumed in phasic boundary forcing of a viscoelastic layer, and how elasticity contributes to the workload. Assuming “viscometric conditions” typical of rheometers, the velocity in the gap is given by [120]

$$u_x(y) = U_0 \left(1 - \frac{y}{H} \cos(\omega t) \right), \quad (2.12)$$

so that the shear rate is spatially uniform throughout the plate gap:

$$\dot{\gamma}_{xy}(y) = \frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x} = \frac{U_0}{H} \cos(\omega t). \quad (2.13)$$

Finally, the rate of work is given by the dissipation function [21]:

$$\boldsymbol{\tau} : \nabla \mathbf{u} = \tau_{xy} \dot{\gamma}_{xy}. \quad (2.14)$$

In one period, $2\pi/\omega$, of the oscillating plate, the net work done is

$$W = \int_0^{2\pi/\omega} \tau_{xy} \dot{\gamma}_{xy} dt. \quad (2.15)$$

For a Hookean elastic solid $\tau_{xy} = G\gamma_{xy}$, so $W = 0$, whereas for a simple viscous fluid, $\tau_{xy} = \eta \dot{\gamma}_{xy}$, and

$$W^* = \int_0^{2\pi\omega} \tau_{xy} \dot{\gamma}_{xy} dt = \eta \left(\frac{U_0}{H} \right)^2 \int_0^{2\pi/\omega} \cos^2(\omega t) dt = \eta \left(\frac{U_0}{H} \right)^2 \frac{\pi}{\omega}. \quad (2.16)$$

Thus the network is minimal for elastic materials and maximal for viscous materials.

For a *linear* viscoelastic material of Maxwell type, the scalar shear stress constitutive equation is

$$\tau_{xy} + \lambda (d\tau_{xy})/dt = \eta \dot{\gamma}_{xy} = \eta \frac{U_0}{H} \cos(\omega t), \quad (2.17)$$

which yields

$$\tau_{xy} = \eta \left(\frac{U_0}{H} \right)^2 \frac{1}{1 + (\omega\lambda)^2} \left[e^{-t/\lambda} + \cos(\omega t) + \omega\lambda \sin(\omega t) \right], \quad (2.18)$$

so that

$$W = \eta \left(\frac{U_0}{H} \right)^2 \frac{\pi}{\omega} \left(\frac{1}{1 + (\omega\lambda)^2} \right)^2 \left[\frac{\omega\lambda}{\pi} (e^{-2\pi/\omega\lambda} - 1) + 1 + (\omega\lambda)^2 \right]. \quad (2.19)$$

Figure 2.8, shows values of W normalized by the viscous work per cycle W^* , given in Eq. (2.16), and illustrates the compromise between elastic and viscous characteristics of the fluid. This simple calculation involves far too restrictive assumptions on geometry and constitutive modeling, and it assumes that work per cycle is a relevant metric for biology. The point of this illustration is to show that one can formulate any number of metrics, e.g., mass transport for asymmetric forcing conditions, but any quantitative analysis requires a constitutive model, so that a heavy premium is placed on the accuracy of constitutive modeling for lung mucus.

Since the mucin network controls viscoelasticity, the rheological properties of mucus can be modified, potentially dramatically, by overproduction of mucins (common in COPD), by the presence of other molecules that disrupt the gel (such as inhaled or ingested drugs or environmental toxins), or by dehydration of the mucus layer (common in cystic fibrosis (CF)). It follows that the balance between secretion and clearance must be maintained so that mucus viscoelasticity remains within efficient ranges for the mechanism(s) that mucus performs in different parts of the human body. The evolution from healthy to pathological mucus occurs by multiple processes such as abnormal secretion of salts and water, increased

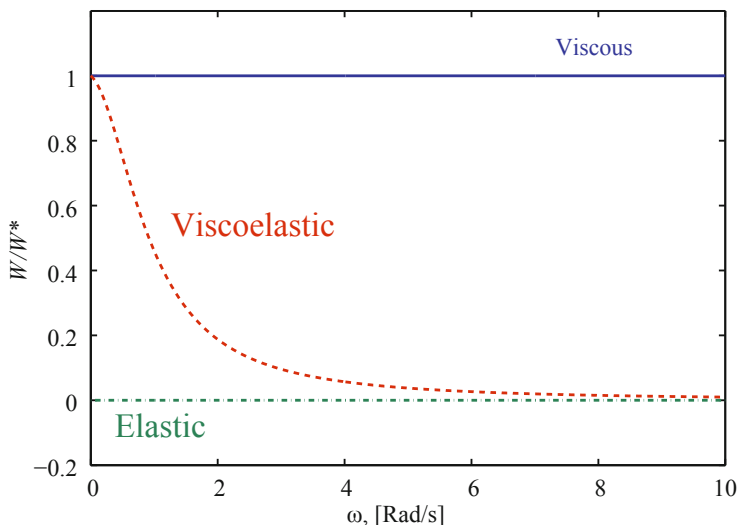


Fig. 2.8 Normalized rate of work for viscous, elastic, and viscoelastic materials as a function of driving frequency in a parallel plate shear cell under viscometric flow assumptions. Normalization is with respect to the viscous work per cycle W^* , given in Eq. (2.16)

production of mucins, infiltration of mucus with inflammatory cells, and heightened bronchia-vascular permeability [121]. Some inflammatory conditions induce mucin overproduction and hypersecretion. In cystic fibrosis (CF) and other chronic inflammatory airways diseases [57, 122–125], other large polymers predominate in the airway secretions; these biological macromolecules together with bacteria and other cell components are prevalent in the larger airways of the respiratory tract, constituting *sputum* that is recovered in the clinic. Breakdown in mucus clearance leads to a cascade of deleterious effects, including clogging of airways and safe harbor for infectious microbes [121]; see Fig. 2.9.

On the other hand, if the mucus mesh becomes more dilute or porous or chemically unresponsive so that bacteria and viruses diffuse more freely and rapidly, epithelial cells and tissue are under-protected and once again risk of infection increases [126, 127]. Similar conditions arise in the viscoelasticity of cervical mucus (CVM). For instance, in women with bacterial vaginosis, the viscosity of the CVM is lower than in those with normal flora, which may be responsible for the increased risk of infection by HIV and other sexually transmitted pathogens, as well as other adverse gynecological conditions [126]. An understanding of the diffusive barrier properties of mucus in the human body and the development of predictive models based on experimental rheological data are major areas of open research, with public health implications.

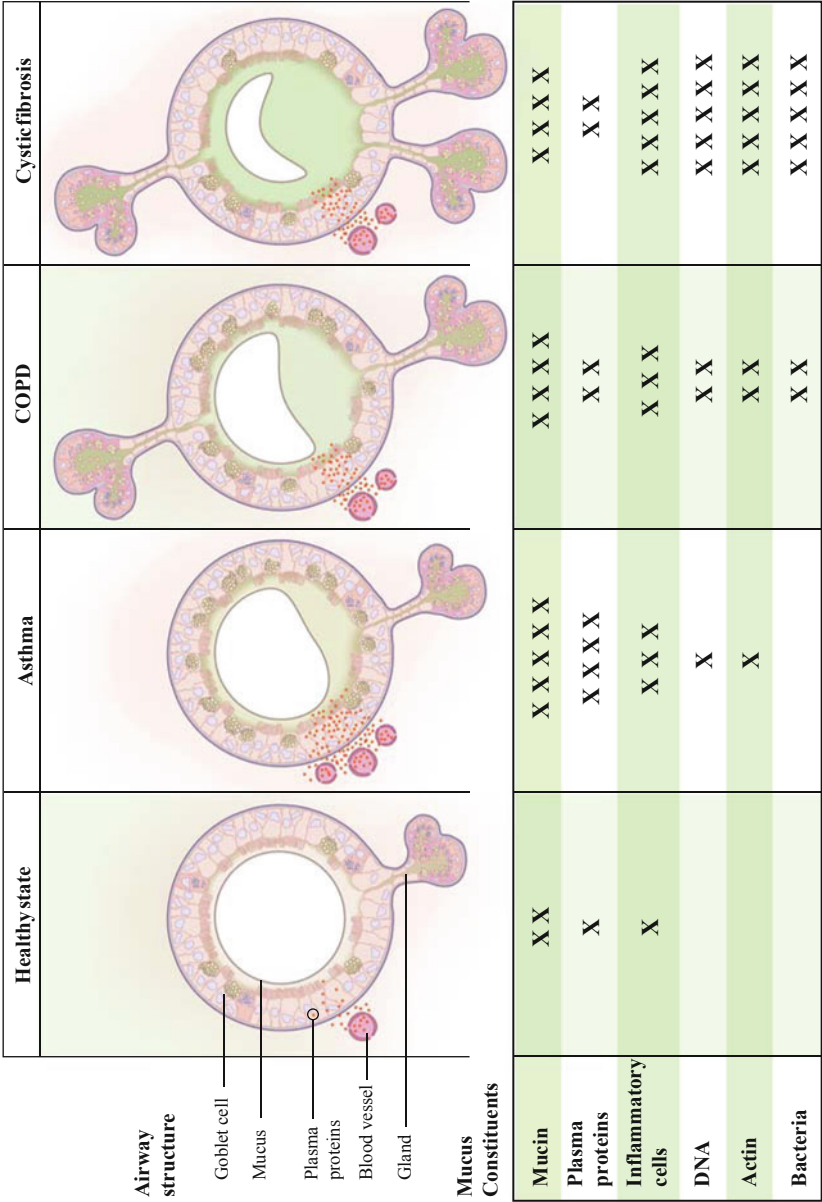


Fig. 2.9 Contributions of mucosa disease to abnormal mucus in healthy state, asthma, COPD, and CF. Figure from: [121]

2.3.2 Modeling Mucociliary Clearance (MCC)

Mathematical models of mucociliary transport incorporate the effects of a large number of cilia beating in a coordinated manner with the two-layer (mucus and PCL) airway surface liquid (ASL). Low Reynolds numbers characterize the fluid mechanics of the ASL, since small velocities (microns per second), length scales (microns), and high viscosities (low-frequency shear viscosity of typical lung mucus is 2–3 orders of magnitude greater than water [33]) are prevalent. The reader is forewarned that low Reynolds number is a simple viscous fluid “guiding parameter” and only a small part of the story in viscoelastic fluid mechanics. The sentence just above makes perfect sense in viscous fluids, yet it does not say anything about the elasticity of the medium, the frequency-dependence of viscous and elastic moduli, nonlinearity of the microstructure during transport, etc. Theoretical models, to the extent that they encode the relevant viscoelastic properties of mucus, can provide information on the transport induced by an imposed cilia beat pattern or stresses from air drag. However, several length scales are involved: molecular length scales, relative to the biochemical structure of mucus; length scales associated with the cilium tip ($\sim 1\ \mu\text{m}$); lengths associated with the cilium length, cell size, and ciliary wavelength; length scales associated with the length and diameter of an airway (5–10 mm). Of particular importance with regard to cilia-mucus interaction is the scale of the cilium tip with respect to the entangled network of molecules constituting the mucus, since at these scales the cilium tip is comparable to the molecular characteristics of the mucin network [28, 107, 128].

Flow models, to be useful, should enable the prediction of MCC and, more importantly, suggest means for modifying the system. King et al. [129] proposed a planar two-layer fluid model to study the transport of mucus in the respiratory tract due to cilia beating and air motion. While their model was based on certain restrictive assumptions, such as zero mean PCL transport, they predicted that clearance increases as the elastic modulus decreases in agreement with experimental observations.

In contrast to the well-described axial transport of mucus along airway surfaces via ciliary action, theoretical analyses predict that the PCL is nearly stationary. However, experimental studies have concluded that the entire PCL is transported at approximately the same rate as mucus, 39.2 ± 4.7 and $39.8 \pm 4.2\ \mu\text{m/s}$, respectively [117]. Removing the mucus layer reduced PCL transport by $> 80\%$, to $4.8 \pm 0.6\ \mu\text{m/sec}$, a value close to that predicted from theoretical analyses of the ciliary beat cycle; hence, the rapid movement of PCL is dependent upon the transport of mucus [117]. In addition, movement of the PCL has proven important for mixing effects [117]. These seminal studies, and a revisitation of the conclusions drawn from tracer markers in the PCL and mucus layers, were the focus of initial conversations by Forest and the applied math group at UNC with faculty in the cystic Fibrosis Center at UNC and Richard Superfine from Physics at UNC. That was a decade ago, with interactions leading to the Virtual Lung Project at UNC.

Several theoretical mucociliary transport models are discussed by Sleight et al. [93, 130]. We summarize some of them next and point the reader to Chaps. 7 and 8 for a related discussion of locomotion through complex fluids.

- *Envelope model* [131]. This model assumes that cilia are densely packed, so that the fluid effectively experiences an oscillating material “sheet”.
- *Sublayer model* [132]. Initially proposed by Blake, in this model individual cilium is modeled as a flexing cylindrical body anchored at the cell surface. The action of a large number of cilia is modeled by a continuous distribution of force per unit volume within the cilia sublayer.
- *Discrete cilia model* [133]. The cilia are represented by a distribution on of Stokeslets with appropriate mirror images to satisfy the no-slip velocity boundary condition at the cell surface. The flow in the cilia sublayer is then determined by summing the individual cilium velocity fields, in the infinite plane approximation.
- *Traction layer model* [134, 135]. This model is the continuous version of the sublayer model. The action of a large number of cilia is modeled by a continuous distribution of force per unit volume within the cilia sublayer.

2.3.3 Modeling Mucus Transport in the Human Respiratory Tract

In healthy subjects, the layer of mucus lining the respiratory tract epithelia is only $\sim 5\text{--}10\mu\text{m}$ thick and rests on an $\sim 7\mu\text{m}$ sol phase. The diameter of an adult human trachea is $\sim 1\text{ cm}$, while the diameters for subsequent branches are successively smaller [136]. The manipulations of physical therapists are known to be clinically efficient, but outcomes are mostly empirical since the biophysical mechanisms involved in these manipulations are not well understood. Mauroy and coworkers [137] developed a model of mucus clearance in idealized rigid human bronchial trees to study the interaction between tree geometry, mucus physical properties, and amplitude of flow rate in the tree. Their results showed that airflow rate and viscoelastic properties of mucus determine the maximal possible mucus thickness in each branch of the tree, resulting in a specific distribution of mucus thickness and properties along the tree. In general, most models of mucus transport in the respiratory tract include the following assumptions [105]:

- Mucus layer thickness is assumed constant within single airway bifurcations.
- Transport of the mucus layer has a constant net velocity.
- Mucus production rates in the terminal bronchioles are considered equal.
- Thickness of the mucus layer lining the wall of a given airway tube is negligibly small with respect to the airway diameter.

Recent work by Sorin Mitran [138, 139] attempts to bridge cilia geometry, cilia force generation, cilia density, a viscous PCL layer, a viscoelastic mucus layer, and an air phase toward a predictive tool for mucus hydrodynamics. Similar modeling and simulation tools are under development by (and partially with) our colleagues, including Ricardo Cortez, Lisa Fauci, Anita Layton, and Karin Leiderman.

2.3.4 Cough Clearance

A second mechanism for the expulsion of mucus from the airways is cough clearance, which becomes a primary clearance mechanism when MCC fails. The hydration of mucus dramatically affects its viscous and elastic properties, which in turn determines how effectively it is cleared by ciliary action and cough [121]. This may help explain why lung diseases caused by impaired ciliary function are less severe than those caused by dehydration, which impedes both clearance mechanisms [121].

King and coworkers [140] examined the relationship between mucus rheology, depth of the mucus layer, and clearance by simulated cough. Cough clearance and adhesion were explored in experiments on mucus transport. They found that high elasticity of the mucus, $G' > G''$, impedes clearance. These findings show an opposite relationship to that seen in ciliary clearance, suggesting that healthy mucus may exhibit intermediate levels of elasticity because it must be capable of responding to both forms of clearance, i.e., mucociliary and cough. It follows that medications that decrease viscosity, such as mucolytics, may benefit ciliary clearance but hamper cough clearance, while medications that decrease the adhesion of secretions to the epithelial surface are likely to improve airflow-dependent clearance [141].

Camassa et al. [90] studied, experimentally and theoretically, flows where an annular viscous liquid film lining the wall of a tube is forced upwards against gravity by turbulent airflow up the core of the tube. This core-annular flow configurations mimics mucus clearance in the trachea and was pursued to reproduce and extend seminal experiments by Kim et al. [91, 142, 143]. The authors derived a longwave, fully nonlinear asymptotic model to interpret experimental observations and data against model simulations. Traveling wave solutions of their model predict a transition between different mass transport regimes. Past a certain threshold that can be identified with surface tension of the liquid-air interface, sufficiently large-amplitude waves begin to trap boluses of fluid, which propagate upward disconnected from the wetting layer similar to vortex rings sliding between the viscous film and air. This theoretical result is then confirmed by a second set of experiments that show ring waves of annular fluid propagating over the underlying creeping flow. By tuning the parameters of the experiments, the strength of this phenomenon can be adjusted in a way that is predicted qualitatively by the model. Recent results based on a different turbulent air drag closure have brought the theory and experiments much closer to quantitative accuracy [Camassa et al. 2013, preprint]. The extension of these experiments and models to viscoelastic fluids that mimic mucus is in progress. Furthermore, the modeling platform of Mitran [144] is proposed to explore fully resolved air-liquid pumping simulations. The goal of these studies is to assess the efficiency of turbulent air drag in transporting mucus layers in airways. The relative efficiency of air-liquid pumping versus coordinated cilia or chest cavity compressive pumping is unknown.

2.4 *Diffusion in Mucus*

The following discussion can be more profitably read after Chap. 3, since passive microrheology is based on diffusion of microbead probes in soft matter. The deposition and clearance of particulates in mucus layers have been under investigation for decades [145]. Generation of suitable therapies that effectively deposit particles in the mucus layer has long been thought to be the only relevant factor for successful drug absorption. However, efficient deposition of particles, although still important, is not always a sufficient condition for successful drug delivery. Particle diffusion through the mucus barrier, just like the flow of mucus, is not a simple process where particles obey simple diffusion laws. New experimental tracking methods have provided a wealth of data on particles with diameters down to tens of nm up to microns, where the particles not only range in size (hydrodynamic radius) but also in their surface interactions with the mucin gel network. Because of this heightened awareness, there is a compelling need to understand and control what happens after particles have landed in the mucus layer. We refer to our recent articles and references therein [73, 146–148].

In the lungs, objects trapped in the mucus gel are transported at rates up to 5–10 mm/min by beating cilia and are delivered to the GI tract for inactivation and digestion. The luminal gel layer of respiratory mucus is replaced as rapidly as every 10–20 min, resulting in extremely efficient clearance of inhaled particles. Trapping and rapid clearance is crucial to protect the airway epithelium from the onslaught of pathogens and environmental toxins we breathe everyday. However, the “mucociliary escalator” also serves as a major barrier to the delivery of therapeutic nanoparticles. Strategies to address this barrier and more efficiently deliver therapeutic nanoparticles to the lungs include mucoadhesive particles, mucus-penetration particles, and mucolytics [145]. The success of these strategies lies in the experimental and theoretical understanding of the diffusive properties of mucus and the underlying interactions between the deposited particulates and the mucus gel matrix.

The study of self-diffusion of nm and micron-scale particles in mucus is important to determine the movement of toxic agents within the layer, as well as for drug delivery and gene delivery therapies [96]. This study is twofold. On one hand, there is the need to understand to what extent particles, characterized by a given surface charge and hydrophobic or hydrophilic properties, are able to diffuse through the network structure. On the other hand, one needs to understand how the network hydrophobic and hydrophilic regions, negatively charged biopolymers [96], and topological constraints arising from the pores present in the network affect such diffusion. That is, if one controls the particle diameter and modifies surface chemistry, then physics of adhesion or repulsion of nearby polymer chains can likewise dramatically alter the diffusive paths. If one varies the particle diameter with controlled surface chemistry, then the observed diffusive scaling varies significantly. This is accentuated if the particle diameter is comparable to the pore/mesh scales in the polymeric network [149, 150].

The various mucin molecules and other proteins in mucus form a heterogeneous, three-dimensional network with a potentially fractal length-scale distribution. The incorporation of the complex mucus microstructure into estimates of particle diffusion in mucus has been modeled in various ways. One approach is to consider the microstructure as a physical obstruction to particle diffusion. This effect is then modeled as a reduced self-diffusion coefficient of the particles. In addition to reduced particle mobility (i.e., lower diffusion coefficients than in the pure saltwater solvent), these obstructive effects can result in a broad distribution of effective particle diffusivities within the gel matrix; clearly one particle may not sample the same length scales of the mucus network as another. It is an intriguing open problem to explore how these effective diffusivities (again, assuming particles obey simple diffusion but with reduced diffusivity relative to the solvent) reflect the heterogeneity of the mucus microstructure. One can, for example, use the mean diffusivity of multiple particle paths with a range of particle diameters to infer a mesh distribution of pore sizes. Carrying this out in a reasonably rigorous manner remains an open problem, yet it is highly relevant to understand the diffusion of diverse species in mucus, including viruses, bacteria, airborne particulates, and many other inhaled substances.

Studies of passage times through mucus layers are riddled with complications that arise because the particles not only have to overcome the gel matrix barrier but also it is not completely known how different particles will interact with mucus under pathological conditions. For instance, Sanders and coworkers [96] performed a study of passage times in CF and COPD sputum. They observed that a low percentage of nanospheres, with a diameter of 270 nm, moved through a 220- μ m-thick CF sputum layer after 150 min. Whereas larger nanospheres (560 nm) were almost completely blocked by the sputum, smaller nanospheres (124 nm) were retarded only by a factor of 1.3 as compared with buffer. In addition, they found that sputum from a patient with COPD retarded the transport of nanospheres to the same extent as CF sputum [96]. Interestingly, the authors found that nanospheres diffused significantly more easily through the more viscoelastic sputum samples. These findings are in contrast to studies that showed that mucolytic agents, which decrease the viscoelasticity of biogels, enhance the transport of drugs and colloidal drug carriers [151,152]. Sanders and coworkers argued that this increase in the mean diffusion of the nanospheres in the more viscoelastic medium is due to the increased heterogeneity in the network. This claim is supported by studies with synthetic gels. Mallam et al. [153] observed that increasing the concentration of junctions in these gels changes the network structure from a homogeneous microporous matrix into a more heterogeneous macroporous network. These studies point to the importance of determining the mesh length-scale spectrum and identifying degrees of heterogeneity in the sample. Below we summarize several research efforts to determine mesh sizes in mucus samples. Characterization of heterogeneity is more challenging and relies on the formulation of mathematical models and tools capable of faithfully reproducing the observed diffusive behavior of particles in mucus. Several steps have been taken in this direction and we summarize them at the end of this section.

If we are able to screen two related effects: the binding-unbinding kinetics of the various diameter particles to the mucus mesh and the repulsion vs. attraction of the mesh to the particle surface chemistry, *then, in principle*, single particle microrheology should reveal sufficient information about the mesh length-scale distribution of a given mucus sample. One should note, however, that the inference of mesh length scales is not immediate. The particle increment process (the measurable data) is a result of all lengthscale fluctuations of the mucus network, not simply those associated with the particle diameter. Therefore, the challenge is to somehow learn how different length-scale particles behave across the entire relaxation spectrum of the mucus gel and then to be able to learn the mucus network structure from the cumulative results of particle paths across a range of particle diameters. This inverse problem is far from solved; each particle diameter reflects a different sampling of the colored noise spectrum from the mucus microstructure.

2.4.1 Modeling Diffusion in Mucus

A standard practice in the microrheology and drug delivery literature is to report mean-squared displacement (MSD) of particle position data on a log-log plot. MSD is calculated as

$$\langle \Delta \mathbf{r}^2(\tau) \rangle = \left\langle [\mathbf{r}(t + \tau) - \mathbf{r}(t)]^2 \right\rangle, \quad (2.20)$$

where $\mathbf{r}(t)$ is the position of the particle at time t , τ is the lag time between the two positions taken by the particle used to calculate the displacement $\Delta \mathbf{r}$, and the average $\langle \dots \rangle$ designates a time average over t and/or an ensemble-average over several trajectories.

Reported values of MSD in mucus [73, 75, 154–156] show a sub-diffusive MSD scaling over an intermediate dynamic range,

$$\langle \Delta r^2(\tau) \rangle = D \tau^\alpha, \quad (2.21)$$

where the power-law exponent is $0 < \alpha < 1$ and often a transition to linear scaling ($\alpha = 1$) over sufficiently long lag times. The prefactor D is the effective diffusion coefficient with units $\mu\text{m}^2/\text{s}^\alpha$.

For direct modeling of tracer particles in a viscoelastic medium, a sufficiently robust family of stochastic processes is needed that reflects these fundamental MSD signatures of transient sub-diffusion. With a robust family of stochastic processes in hand, then one can build inference methods to fit model parameters to experimental data and give a means to characterize diffusive properties of a given particle in a viscoelastic medium [146, 148]. Below we summarize two models that have been successfully used to describe diffusion of particles in mucus, together with simple Brownian motion.

- **Brownian Motion**

The velocity of a particle driven by Brownian motion is described by the Langevin equation

$$m \frac{d^2 \mathbf{x}}{dt^2} = -\zeta \frac{d\mathbf{x}}{dt} + \mathbf{F}(t), \quad (2.22)$$

where $\mathbf{x}$ is the position of a Brownian particle with mass m and drag coefficient ζ . The force $\mathbf{F}$ comes from random fluctuations and is assumed to be white noise, i.e.,

$$\begin{aligned} \langle \mathbf{F}(t) \rangle &= 0 \\ \langle \mathbf{F}(t) \mathbf{F}(s) \rangle &= 2\zeta k_B T \delta(t-s), \end{aligned}$$

here k_B is the Boltzman constant and T the absolute temperature. In the zero mass limit (inertialess), Brownian motion is described by the equation

$$\zeta d\mathbf{x} = \mathbf{F}(t) dt. \quad (2.23)$$

The MSD of a particle with a diffusion coefficient $D = k_B T / (6\pi\eta a)$ and undergoing Brownian motion is

$$\langle \Delta r^2(\tau) \rangle = D\tau.$$

- **Fractional Brownian Motion**

Fractional Brownian motion [157] is a self-similar Gaussian process with stationary increments and a uniform MSD scaling behavior,

$$\langle \Delta r^2(\tau) \rangle = D_{\text{fBm}} \tau^\alpha, \quad (2.24)$$

where D_{fBm} is the generalized diffusion coefficient with dimensions L^2/t^α . The autocorrelation function for fBm is likewise known and given by

$$E[B_\alpha(t)B_\alpha(s)] = \frac{1}{2}(|t|^\alpha + |s|^\alpha - |t-s|^\alpha). \quad (2.25)$$

Furthermore, long-range correlations are given by

$$\langle \xi_\alpha(0)\xi_\alpha(t) \rangle \approx \alpha(\alpha-1)t^{\alpha-2}, \quad (2.26)$$

where ξ_α is the fractional Gaussian noise, so that the Langevin equation describing simple fBm is

$$\zeta d\mathbf{x} = \xi_\alpha(t) dt. \quad (2.27)$$

From Eq. (2.26) is easy to see that uncorrelated, regular Brownian motion corresponds to $\alpha = 1$. For $0 < \alpha < 1$ the prefactor is negative and the increments are negatively correlated, rendering the associated process sub-diffusive. Conversely, when $\alpha > 1$ the motion is persistent (positively correlated), resulting in superdiffusion in which successive steps tend to follow in the same direction. fBm has been used to model a variety of processes including diffusion of biopolymers inside cells [158], monomer diffusion in a polymer chain [159], bacteria chromosomal loci [160], polymer translocation [161], diffusion in crowded fluids [162], and diffusion of one micron particles in HBE mucus [73].

Hill and coworkers [73] found for different mucus concentrations and over the experimental time scales (1 min) that single particle and ensemble MSD data were remarkably well approximated by a uniform power law and therefore consistent with a scaling of the form (2.24). Furthermore, the power law and prefactor were well described by the following functions of wt% of solids in the mucus samples:

$$\alpha \approx -0.17 \text{ wt\%} + 1.1, \quad \text{for } 1.5 \leq \text{wt\%} \leq 5, \quad (2.28)$$

$$D_{\text{fBm}} \approx 1.6 \exp(-1.5 \text{ wt\%}), \quad \text{for } 1.5 \leq \text{wt\%} \leq 5, \quad (2.29)$$

where the units of D_{fBm} are $\mu\text{m}^2/s^\alpha$. These excellent fits to fBm need to be validated for longer time scales in order to apply the fBm models to predictions of passage time distributions of particles through mucus barrier layers. It is an open problem of intense study in our research group to predict passage time distributions and their scaling with thickness of the mucus layer.

- **Generalized Langevin Equations**

Inspection of Eqs. (2.23) and (2.27) shows a general form of Langevin equations describing the diffusion of particles. In fact, if $\mathbf{F}(t)$ in Eq. (2.22) is not white noise, the motion of the particle has been described by a GLE of the form

$$m \frac{d^2 \mathbf{x}}{dt^2} = - \int_0^t \frac{d\mathbf{x}(s)}{ds} K(t-s) ds + \mathbf{F}(t), \quad (2.30)$$

and the FDT connects the memory kernel function, $K(t)$, with the random fluctuation force by

$$\langle \mathbf{F}(t) \mathbf{F}(s) \rangle = k_B T K(|t-s|).$$

In the Laplace transform space, the one-dimensional GLE becomes

$$m z^2 \tilde{X}(z) = -\tilde{K}(z) z \tilde{X} + \tilde{F}. \quad (2.31)$$

This is similar to the simple Langevin equation for viscous diffusion, except with a frequency-dependent drag coefficient given by the transform of the memory

kernel. In the viscous limit, $\tilde{K}$ reduces to the constant Stokes drag coefficient: $6\pi\eta a$, where η is the fluid viscosity and a is the particle radius.

Fricks et al. [146] explored GLEs with memory functions consisting of an arbitrary sum of exponentials (a Prony series) and showed that the GLE can be transformed to a large vector Langevin equation. This transformation allows the application of well-known tools for inference from noisy time-series data (e.g., maximum likelihood estimators and the Kalman filter) to GLEs and for numerical generation of paths for given Prony series kernels. These tools were implemented to illustrate the transient anomalous diffusive statistics for the two classic, solvable models of polymer chain dynamics, namely Rouse and Zimm models. These models exhibit transient sub-diffusive behavior with intermediate time scale MSD scaling of $t^{1/2}$ and $t^{2/3}$, respectively, which are reproduced accurately in [146]. However, the inference of parameters in the memory kernel was restricted to a small number of memory time scales, on the order of less than 10, whereas mucus and similar biological fluids have a broad spectrum with decades of memory time scales.

McKinley et al. [148] made an important advance for GLEs and their application to transient anomalous sub-diffusion, showing how to prescribe an arbitrarily specified sub-diffusive power-law scaling in MSD on intermediate time scales (between the shortest and longest time scales of memory in the fluid). To do so, they generalized the Rouse and Zimm models, showing (and proving) how the scaling in the memory spectrum can be dictated so that the MSD scales with a tunable power law between 0 and 1. Shortly afterward, Amitai et al. [163] published a related result on tunable MSD exponents. McKinley et al. [148] further showed that the zero mass limit of the GLE, for an arbitrary memory kernel, is given by a sum of exponentials and is exactly solvable, giving an explicit particle path formula [148],

$$X(t) = CB(t) + \sum_{k=1}^N C_k Z_k(t), \quad (2.32)$$

where $X(t)$ is the particle position at time t , $B(t)$ is a standard Brownian motion, and the second term in the right-hand side is a sum of Ornstein–Uhlenbeck (OU) processes. In this sum, each term represents one color of noise in the process and the full-colored noise spectrum is related by explicit polynomial interpolation to the kernel memory spectrum. Each Z_k satisfies the stochastic differential equation:

$$dZ_k(t) = -\lambda_k Z_k + dB_k(t). \quad (2.33)$$

These results now pose a three-parameter family of GLE memory kernels, essentially dictated by the shortest and longest time scale of memory and the intermediate power law exponent of MSD, with which to fit to experimental particle time series data. These kernels are candidates along with fractional

Brownian motion for best fit to particle paths in mucus and other biological fluids. The determination of which models fit the data more accurately is an open challenge currently being explored in collaboration with S. McKinley, J. Mellnik, N. Pillai, and M. Lysy [164].

2.4.2 Mesh Size Distribution

Since the distribution of mesh spacing (and attractive versus repulsive interactions) is determinant in the diffusive properties of particulates through mucus, several studies have focused on determining the mesh size of different types of mucus based on an obstruction scaling model. We note that these studies must assume a model for the mucus mesh, and all inferences are based on those model assumptions. A more fundamental approach than the one summarized next is a very important project.

2.4.3 Obstruction Scaling Model

The obstruction scaling model [165, 166] assumes that the reduction in diffusivity is due to the particle encountering polymer chain obstacles. However, this approach assumes an effective diffusivity (i.e., Brownian motion), even though it is now evident that particles above a few hundred nm have sublinear scaling of MSD with time. Said differently, if one fixes a time scale of observation of paths, then it is possible to associate a viscous diffusivity of the sample fluid for that fixed time scale. The downside of this approach is that one will get different results for the effective diffusivity for every time scale. Nonetheless, this modeling approach is a common standard so it is worth understanding before proposing alternatives.

The model assumes that the effective radius of the mesh spacing is greater than the hydrodynamic radius of the diffusing particle and there is no interaction between the solute and the polymer. From this model, the ratio of diffusion in a gel and diffusion in pure water is given by

$$\frac{D_g}{D_w} = \exp \left[-\frac{\pi}{4} \left(\frac{r_s + r_f}{r_g + r_f} \right)^2 \right], \quad (2.34)$$

where D_g is the diffusion coefficient of the particle in the polymer gel, D_w is the diffusion coefficient in water, r_s is the particle radius, r_f is the gel fiber radius, and r_g is the effective radius of the pore. For an extended discussion about these models of diffusion through mucus, we refer the reader to Cu and Saltzman [167]. Some reported values of mucus mesh sizes inferred from these assumptions are shown in Table 2.3.

Note that the results from these studies imply that the mucus gel network has pores that are larger than the diameter of many known viruses [168]. It is clear that mucus employs methods other than obstruction to prevent viruses from infecting

Table 2.3 Mesh size in mucus samples

Mucus type	Mesh size (nm)
Cervicovaginal mucus (CVM) ^a	340 ± 70
Cystic fibrosis sputum ^b	145 ± 50
Chronic rhinosinusitis mucus ^c	150 ± 50
CVM treated with nonionic surfactant N9 ^d	130 ± 50

References: ^a[168], ^b[150], ^c[156], ^d[169]

mucosal surfaces, indicating that mucus is not just a steric barrier to deposited particulates. Namely, mucus is also an effective “adhesive” that can immobilize particles by hydrophobic and electrostatic interactions and hydrogen bonding [145]. These binding affinities suggest at the very least a generalization of the obstruction scaling model to include waiting times for particle binding and unbinding, which leads to sub-diffusive scaling [170]. Note, it is precisely these binding affinities that surface treatments of synthetic particles are designed to screen, in order for passive microrheology to faithfully reflect the innate fluctuations of the material. In particular, antibodies found in mucosal secretions have been reported to immobilize viruses and bacteria [171–175]. While one region of an antibody is capable of forming low affinity bonds with mucus, the other region can specifically link to the surface of pathogens. Thus, even viruses that are smaller than the average mucus mesh spacing and that do not bind to mucin molecules can be trapped with the help of antibodies [28]. Our research group has teamed with Sam Lai in the School of Pharmacy at UNC to study the intricate kinetic and diffusive interactions between antibodies, viruses, and mucus gels [176, 177]. However, to circumvent the mucus barrier, some viruses have evolved to contain hydrophilic coatings that enhance their mobility through the barrier by minimizing interactions with the components of the mucus gel network [173].

One also has to keep in mind that the mucus gel is not a rigid structure. As discussed above, the orientation and spacing between the components of the matrix gel is maintained by a series of interactions between the various macromolecules and small molecules in the solvent. Mucus network fluctuations can be strongly affected by changes in the small molecules in the solvent, e.g., significant changes in GI mucus versus PH has been observed by several research groups [46, 49, 178–181]. Then the use of average mesh values to describe the gel network is a crude way to estimate mean passage time. Our focus has been on more accurate methods of estimating passage time distributions for particles in mucus versus particle diameter, which can vary dramatically with disease conditions [73]. To do so, it is necessary to first identify models to describe diffusion through mucus. Our group has spent a significant effort on modeling of the primitive particle time series afforded by the advanced microscopy and particle tracking tools. As noted earlier, we have focused on parametric methods [73, 146–148], based on assumed stochastic processes and models from which we infer parameters of the model from the experimental particle time series data, and on nonparametric methods [73], based on statistical analyses of the time series data without any assumptions on

an underlying model for the particle paths. Continued advances are needed in the understanding of how particle size and surface chemistry affect the passage times through mucus layers. These studies also provide insights into the length-scale distribution of the mucus network, although it is an open and intriguing problem to infer details of the mucus network structure from particle fluctuations. Clearly, attractive and repulsive particle-mucus microstructure kinetics need to be filtered in order to isolate fluctuations arising purely from mucus microstructure. This goal is the reason why two-particle microrheology was developed, for a discussion see Chap. 3.

3 Modeling Structure and Dynamics Within a Single Cell: The Mitotic Yeast Spindle

A predictive simulation of the structure and dynamics within an individual living cell remains a fundamental modeling and computational challenge. Cells are highly complex structures and there are multitudes of organizational charts for a single cell, e.g., search for “eukaryotic cell component chart.” Most research labs in cell biology focus on specific aspects of cells and cellular processes. We will restrict our discussion to mitosis in yeast. Yeast are a model system for eukaryotic cells, which are distinguished by a membrane-bound nucleus and nuclear chromosomes packaged into chromatin fibers. The lab of Kerry Bloom at UNC explores the intricate behavior of these structures during different phases of the yeast cycle, and our work in yeast mitosis has been with Kerry Bloom and his students, specifically Andrew Stephens and Jolien (Verdaasdonk) Tyler.

3.1 Modeling Mitosis in Yeast Cells

In cell biology, yeast provide model systems for the study of the cell cycle and regulatory mechanisms. Yeast are readily available, and while less complex than animal cells, the cell cycle in yeast is remarkably similar. In general, the cell cycle encompasses a series of events leading to the division and duplication of the cell. Within these processes, mitosis and its regulation play a key role. *Mitosis is the stage of the cell cycle where the cell focuses its energy toward a single goal: chromosome segregation.* Models of cell mitosis based on experimental evidence serve as in vitro labs, where different theories and mechanisms, not accessible experimentally, can be tested. Modeling of cell mitosis can be performed at different levels. On one hand, one can use generalized logical network models where the self-regulating cell division system is modeled as an intricate molecular network [182–185]. In addition to the different molecular players, the network includes a series of checkpoints that place cell division under external control and ensure that every single step

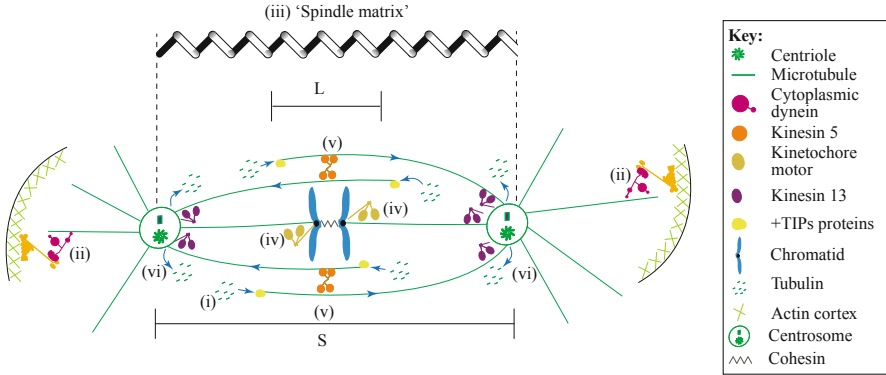


Fig. 2.10 Schematic of the mitotic spindle. Figure from [185]

has been completed before the next one begins. For example, sister chromatids are not separated until chromosomes are correctly aligned during the metaphase phase. Another approach consists on describing the mechanical and physical interaction of the main components of the cell via force balances [185–191]. The approach described next falls within this category. In this model the regulation of cell division results from a force balance applied to the mitotic spindle.

The mitotic spindle ensures the equal distribution of chromosomes during cell division. In the mitotic spindle, sister chromatids are bi-oriented and bound via the kinetochore to microtubules emanating from opposite spindle pole bodies; see Fig. 2.10. The kinetochore is a specialized protein/DNA structure built on centromere DNA that binds to the plus-end of dynamically growing and shortening kinetochore microtubules (kMTs). In yeast, each of the sixteen chromosomes is tethered to the spindle via a single kMT [192, 193]. Other microtubules extend inward from the spindle pole bodies and are not attached to replicated sister chromatids. These microtubules are known as interpolar microtubules. Interpolar microtubules (ipMT), from opposite spindle poles, overlap and are cross-linked by microtubule motor proteins and microtubule-associated proteins, represented in red in Fig. 2.10. These proteins (when double bound) exert outward forces as they slide pulling the ipMTs apart [194, 195]. In addition, the replicated chromosomes form the sister chromatids (blue in Fig. 2.10) and exert inward forces through their connection to the kMTs. Two proteins play a fundamental role in the structure of the sister chromatids: cohesin and condensin [196–200]. These complexes, together with DNA, constitute the “chromatin spring” [201, 202]. The balance of microtubule-based extensional force and a chromatin spring contractile force is necessary to produce a steady-state spindle length and tension at the kinetochore that satisfies the spindle checkpoint [203]. The spindle checkpoint is a control mechanism that ensures that sister chromatids are attached and aligned before the two poles separate to form the two daughter cells.

The microtubules, microtubule-based motor proteins, and kinetochore components of the segregation apparatus have been explored with biophysical techniques, leading to a detailed understanding of their function [204–208]. However, the inherent complexity of the cell division process has made it challenging to understand the underlying mechanisms, even for a single phase such as metaphase. Mathematical and computational models are necessary to integrate experimental results with biochemical and biophysical cellular components, with the goal to understand the mechanochemical principles of the cell division process. From such a basic framework, one can then begin to understand cellular dysfunction and then apply that understanding in beneficial ways. Different models have been formulated to study individual components of the metaphase spindle. For instance, mathematical models of the spindle aim to account for the distribution and dynamics of spindle microtubules [188, 189, 209–212]. One class of models consists of stochastic equations describing kMT plus-end dynamics [188, 211, 213, 214]. These models include spatial gradients in dynamic instability across the spindle, as well as tension-mediated regulation of kMT plus-end dynamics [188]. Another stochastic model that includes kinetochore attachment and detachment was formulated by Gay and coworkers [189]. Although microtubule dynamics were not explicitly modeled, this model incorporated a spatial gradient in kMT detachment rate that is analogous to the spatial gradients of Gardner and colleagues [188]. Overall, with appropriate tuning of the spatial gradients, these models were able to recapitulate experimentally observed features of microtubule plus-end dynamics and kinetochore separation. However, none of the models explicitly consider the physical properties of the chromatin spring (for a review see the article by Mogilner and Craig [206]) which is one focus of the Bloom lab.

Some models have coupled the microtubules and motor dynamics to the chromatin spring. For a review, the reader is referred to Mogilner et al. [185]. What these models have in common is that they assume the chromatin behaves as a Hookean spring, i.e., a linear force-extension relation. The chromatin spring is presumed to be derived via cohesion between sister chromatids [189], cohesin and condensin-based chromatin loops [202, 215], or an entropic worm-like chain [216]. However, Stephens et al. [191, 202] developed a series of models based on experimental observations in budding yeast cells and showed that the dynamics of the chromatin spring are not explained by a simple Hookean spring assumption. Using spindle length, chromatin dynamics, and stretching of individual and multiple chromosomes in the spindle, Stephens and coworkers probed the physical nature of the spring by comparison of measurable data with a predictive mathematical model, described next.

3.1.1 Force Balance Within the Budding Yeast Mitotic Spindle

The components contributing to force balance in the mitotic spindle are depicted in Fig. 2.11. We consider three main force-generating processes: (i) an extensional force arising from double-bound motors walking directionally along ipMTs, F_{ip} ;

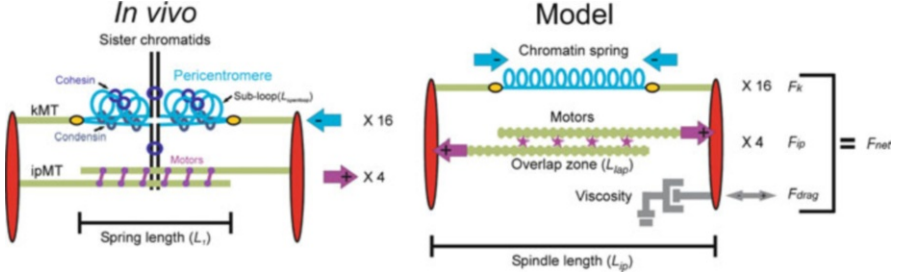


Fig. 2.11 Representation of the mitotic spindle in vivo and modeling assumptions. Figure adapted from [191]

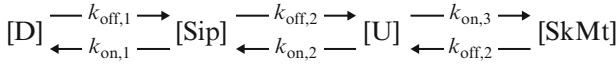


Fig. 2.12 Schematic representation of the motors population balance. Values of the “off” and “on” rates are discussed in the text and in Table 2.4. Figure from [191]

(ii) an opposing contractile (restoring) force generated by the chromatin spring, F_k ; and (iii) a viscous drag force, F_{drag} , that accounts for the cumulative viscous drag on the spindle. Holding one spindle pole fixed and summing all forces acting along the primary spindle axis, the net force on the spindle pole is

$$\sum F_{on\ spindle\ pole} = F_{ip} + F_k + F_{drag} = F_{net}. \quad (2.35)$$

The basis for a stable spindle length in the model is that a quasi-steady state is reached between inward and outward forces, with fluctuations about a mean spindle length arising from microtubule-based motor activity. Each force contribution is calculated as follows.

1. Motor Force, F_{ip}

The outward force (F_{ip}) arises from the sliding of antiparallel ipMTs due to plus-end-directed motors bound in the overlap region (denoted double-bound motors, $D(t)$). The forces exerted by each motor, F_m , are additive, so F_{ip} is proportional to the total number of double-bound motors:

$$F_{ip} = D(t) \cdot F_m. \quad (2.36)$$

Four types of motors are considered in the model: S_{ip} for motors single bound to ipMTs, S_{kMT} for motors single bound to the kMTs, U for unbound or free motors, and D for double-bound motors in the ipMT overlap zone. The total number of motors $D + S_{ip} + U + S_{kMT}$ is conserved. The population dynamics is summarized in Fig. 2.12.

Table 2.4 Transition rates of motors; see Fig. 2.12

Description	Dependence	Value	Notes
$k_{\text{on},1}$: attachment rate of single-bound motors to ipMTs	Constant	0.13 s^{-1}	(a)
$k_{\text{on},2}$: attachment rate of unbound motors to ipMTs	$L_{\text{ip}}, L_{\text{kMT}}^{\text{left, right}}$	Eq. (2.37)	(b)
$k_{\text{on},3}$: attachment rate of unbound motors to kMTs	$L_{\text{ip}}, L_{\text{kMT}}^{\text{left, right}}$	Eq. (2.38)	(b)
$k_{\text{off},1}$: detachment rate of double-bound motors	Constant	0.3 s^{-1}	(c)
$k_{\text{off},2}$: detachment rate of single-bound motors	Constant	0.3 s^{-1}	(c)

- (a) The dynamics of attachment of single-bound motors in ipMTs to become double-bound motors is assumed to follow a binomial process,

$$S_{\text{ip}} \rightarrow D \sim B(S_{\text{lap}}, 0.12),$$

where S_{lap} is the number of motors in the overlap region, L_{lap} , of the ipMTs,

$$S_{\text{lap}} = S_{\text{ip}} \cdot \frac{2L_{\text{lap}}}{L_{\text{ip}} + L_{\text{lap}}}.$$

The probability of success is constant and equal to 12 %. Since the relation between the probability of attachment, p_{on} , and the rate of attachment, $k_{\text{on},1}$, is

$$p_{\text{on}} = 1 - e^{k_{\text{on},1}},$$

it follows that $k_{\text{on},1} \approx 0.13$.

- (b) The rate of attachment of free motors to ipMTs and kMTs is assumed to be proportional to the tubulin concentration (constant) and the percentage of the total length that is available for attachment:

$$k_{\text{on},2} = [\text{tubulin}] \cdot \frac{L_{\text{ip}} + L_{\text{lap}}}{L_{\text{ip}} + L_{\text{lap}} + L_{\text{kMT}}^{\text{left}} + L_{\text{kMT}}^{\text{right}}}, \quad (2.37)$$

$$k_{\text{on},3} = [\text{tubulin}] \cdot \frac{L_{\text{kMT}}^{\text{right or left}}}{L_{\text{ip}} + L_{\text{lap}} + L_{\text{kMT}}^{\text{left}} + L_{\text{kMT}}^{\text{right}}}. \quad (2.38)$$

- (c) The rate of detachment of bound motors is assumed constant:

$$k_{\text{off},1} = k_{\text{off},2} = 0.3 \text{ s}^{-1}.$$

2. Drag Force, F_{drag}

At the low Reynolds numbers inside the cell, F_{drag} is proportional to the velocity of the spindle length (denoted L_{ip}) given by the Stokes drag law [217],

$$F_{\text{drag}} = -C_{\text{drag}} V_{\text{ip}}, \quad (2.39)$$

where $V_{\text{ip}} = dL_{\text{ip}}/dt$.

3. Spring force, F_k

The length of the chromatin spring L_{sp} is total spindle length L_{ip} minus the length of each kinetochore microtubule $(L_{kMT}^{left}, L_{kMT}^{right})$, where L_{kMT}^{left} and L_{kMT}^{right} are the length of the left and right kMT, respectively. Thus the chromatin length is $L_{sp} = L_{ip} - L_{kMT}^{left} - L_{kMT}^{right} - L_{rest}$, where L_{rest} is the rest length of the spring in the absence of force. To determine L_{sp} , the length of the left and right kMTs need to be defined. We follow the model of Gardner et al. [188] as explained next.

3.1.2 Kinetochore Microtubule Length Dynamics

The kMTs grow and shrink stochastically through polymerization and depolymerization, but the process is biased by the state of the kMTs, i.e., their length relative to a threshold length and the tension in the kMTs. The dynamics of this process was studied by Gardner et al. [188], giving the following relationships for the probabilities of rescue (growth), p_r , and catastrophe (shortening), p_c ,

$$p_r = |0.21 - 9.5F_k|, \quad (2.40)$$

$$p_c = \left| 0.38 - 0.65(L_{kMT} - 0.75)^2 \right|. \quad (2.41)$$

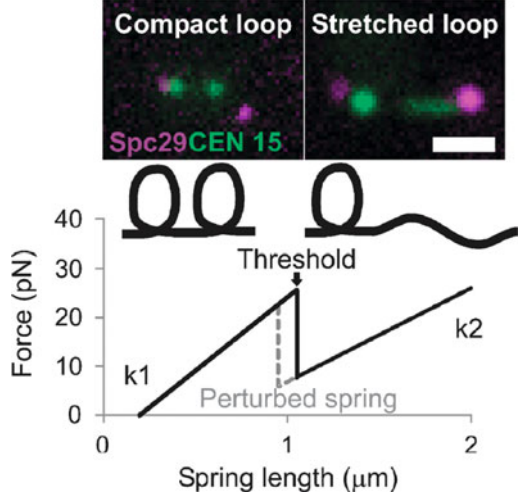
To find if at a given time step a kinetochore microtubule is growing or shortening, the following procedure is implemented. Two random numbers, r_c and r_r , are drawn from a uniform distribution. These numbers are used to compute two logical values, a_1 and a_2 , as

$$a_1 = \begin{cases} 0 & \text{if } p_c < r_c \\ 1 & \text{if } p_c > r_c \end{cases} \quad a_2 = \begin{cases} 0 & \text{if } p_r < r_r \\ 1 & \text{if } p_r > r_r \end{cases}$$

Changes in kMT length are then determined by the following rules:

a_1	a_2	Process
0	0	Do nothing
0	1	Rescue (rate 17 nm/s)
1	0	Catastrophe (rate 25 nm/s)
1	1	Same as previous time step

Fig. 2.13 Experimental evidence and graphic representation of piecewise continuous spring law. In the experimental data (top) the spindle pole bodies (Spc29) are labeled *red*, while the *green spots* correspond to labeled DNA arrays that are 1.8 kb from the centromere (CEN 15). Figure adapted from [191]



3.1.3 Functional Form of the Spring Force

The model assumes a *nonlinear spring force* that posits a threshold value for the spring extension. Spring lengths above the threshold value result in a decreased spring constant and an increase in rest length as depicted in Fig. 2.13.

The spring piecewise continuous spring force is given by

$$F_{k,i} = \begin{cases} -k_{\text{sp}} (L_{\text{sp}} - L_{r1}), & L_{\text{sp}} < X_{\text{thres}}, \\ -k_{\text{sp}} \left(\frac{L_1}{L_1 + x_{\text{loop}}} \right) (L_{\text{sp}} - L_{r2}), & L_{\text{sp}} \geq X_{\text{thres}}, \end{cases} \quad (2.42)$$

where x_{loop} and L_1 are constants and the “switching” between states is assumed instantaneous.

In addition, the 16 springs are arranged in parallel and linked to their two nearest neighbors. The links are assumed soft linear springs with spring constant $k_{\text{cross-link}} \ll k_{\text{sp}}$. Then the force law in each link is given by

$$\begin{aligned} F_{\text{cross-link}}|_{n+1} &= -k_{\text{cross-link}} [(L_{\text{sp}} - L_{\text{sp}}|_{n+1}) \cos \theta], \\ F_{\text{cross-link}}|_{n-1} &= -k_{\text{cross-link}} [(L_{\text{sp}}|_{n-1} - L_{\text{sp}}) \cos \theta], \end{aligned}$$

where θ is the angle between a cross-link and a spring, and we assumed that adjacent springs are close enough to each other so that $\theta \approx 0$ and $\cos \theta \approx 1$. The force exerted by the spring is then

$$\begin{aligned} F_{k,i}^{\text{cross-link}} &= F_{k,i} + F_{\text{cross-link}}|_{i+1} + F_{\text{cross-link}}|_{i-1}, \\ F_{k,i}^{\text{cross-link}} &= F_{k,i} - k_{\text{cross-link}} (L_{\text{sp}}|_{i-1} - L_{\text{sp}}|_{i+1}), \end{aligned}$$

where $F_{k,i}$ is calculated using Eq. (2.42). The total spring force is

$$F_k = \sum_{i=1}^{16} F_{k,i}. \quad (2.43)$$

4. Force-Velocity Relationship.

To calculate the force balance, Eq. (2.35), in a physically meaningful way we impose a linear force-velocity relationship defined by two parameters: a maximum (stall) force, $F_M = 6$ pN, and a maximum speed, $V_{\max} = 50$ nm/s. The net force (sum of forces) felt by double-bound motors on ipMTs is $F_{\text{net}} = F_{\text{ip}} + F_k + F_{\text{drag}}$, which is then distributed evenly across double-bound motors and gives an average force per motor:

$$\begin{aligned} \frac{F_{\text{net}}}{D} &= \frac{F_{\text{ip}} + F_k + F_{\text{drag}}}{D} = \frac{F_M D + F_k + F_{\text{drag}}}{D}, \\ F_{\text{per motor}} &= F_M + \frac{F_k + F_{\text{drag}}}{D}. \end{aligned}$$

The interpolar microtubule (therefore spindle) velocity can be determined as

$$V_{\text{ip}}(t) = \frac{F_{\text{net}}}{D} \cdot \frac{V_{\max}}{F_M} = V_{\max} \left(1 + \frac{F_k(t) + F_{\text{drag}}(t)}{F_M D(t)} \right). \quad (2.44)$$

From Eq. (2.44) we see that:

- If $F_k + F_{\text{drag}} = 0$, then $V_{\text{ip}} = V_{\max}$. In other words, if the forces from the chromatin spring and fluid drag cancel, the spindle will move at the maximum speed of one motor.
- If the net force on the ipMTs is zero, $F_{\text{net}} = 0$, then $V_{\text{ip}} = 0$ and the spindle is stationary. In this quasi-equilibrium condition, each motor is at or near stall force (6 pN) and this force arises from the spring and drag forces acting on the motors.
- The average force acting on a motor can be found as $F_{\text{per motor}} = F_M(1 - V_{\text{ip}}/V_{\max})$.

5. Numerical Integration

Solving Eq. (2.44) for the spindle velocity gives

$$V_{\text{ip}}(t) = \left(\frac{U_{\max}}{F_M D(t) + U_{\max} C_{\text{drag}}} \right) \cdot (F_M D(t) + F_k(t)),$$

so that

$$\begin{aligned} V_{\text{ip}}(t) &= \left(\frac{U_{\max}}{F_M D(t) + U_{\max} C_{\text{drag}}} \right) \times \\ &\quad \left(F_M D(t) - k_{\text{sp}} \left(L_{\text{ip}}(t) - L_{\text{kMT}}^{\text{left}}(t) - L_{\text{kMT}}^{\text{right}}(t) - L_{\text{rest}} \right) \right). \end{aligned}$$

To integrate this equation in time and find $L_{ip}(t + \Delta t)$, we perform a predictor-corrector scheme. The spindle length in the predictor step, $L_{ip}(t^*)$, is calculated as

$$L_{ip}(t^*) = L_{ip}(t) + \Delta t V_{ip}(t).$$

After this predictor step, we use $L_{ip}(t^*)$ to find $D(t^*)$ and $L_{kMT}^{\text{left,right}}(t^*)$ and perform a corrector step

$$L_{ip}(t + \Delta t) = L_{ip}(t) + \frac{\Delta t}{2} [V_{ip}(t) + V_{ip}(t^*)],$$

where

$$V_{ip}(t^*) = \left(\frac{U_{\max}}{F_M D(t^*) + U_{\max} C_{\text{drag}}} \right) \times \left(F_M D(t^*) - k_{\text{sp}} \left(L_{ip}(t^*) - L_{kMT}^{\text{left}}(t^*) - L_{kMT}^{\text{right}}(t^*) - L_{\text{rest}} \right) \right).$$

Solutions of the model have been shown to correctly recapitulate the observed experimental behavior and give insight into the dynamics of the chromatin network; for a detailed discussion see [191, 218].

Finally, we point out several aspects of the modeling amenable to further investigation. In this sense, the open research questions regarding this model include:

- *How microtubules attach to the chromosomes.* It is well known that as the kMTs grow and shorten, they “probe” space until they capture the chromosomes in a process rightly called search-and-capture [206, 219, 220]. The dynamic instability leading to growth/shrinkage of kMTs is included in the model; however the attachment/detachment dynamics are not included, i.e., the model assumes that the chromosomes are attached to the kMTs at all times.
- *Three-dimensional resolution.* The model assumes one-dimensional force balances. A generalization to a full 3-D geometry is a current research project.
- *Spring force law.* The piecewise continuous function used in the model correctly recapitulates the experimental observations. However, it assumes a single “unfolding” event and an *instantaneous* transition. New laws can be incorporated into the model based on a formal description of the transition states of the chromatin loops. For example, dynamics like those used to describe folding and unfolding of proteins [221] are being explored.
- *Astral microtubules.* In yeast cells, during metaphase there are approximately 2–3 astral microtubules extending from the spindle pole into the cytoplasm (see Fig. 2.10) versus the ~ 40 spindle microtubules. The astral microtubules are

critical for spindle orientation and are acted upon by cytoplasmic dynein [222]. While the spindle force balance proposed in the model guarantees that the sister chromatids are “centered” with respect to the spindle poles, the position of the chromatids with respect to the cell nucleus is governed by the astral microtubules. This coupling is another ongoing project.

- *Nuclear membrane forces.* The nuclear membrane has a heterogeneous morphology. As interactions of the spindle with the nucleus wall are introduced in the model, nuclear shape needs to be considered.
- *Inward motor forces.* An important component of the spindle machine is the minus-end motor Kar3 [194, 223, 224]. Kar3 is a nonessential gene that nonetheless contributes to the fidelity of chromosome segregation in mitosis. Kar3 is found in metaphase along the ipMTs as well as kMTs and microtubule plus-ends. The simplest model is that Kar3 opposes the outward motors Cin8 and Kip1. This more detailed resolution of motor activity is another research project.
- *Kinetochore forces.* Kinetochore forces are implicit as the mechanism that translates the spatial catastrophe gradient and tension-dependent rescue from the chromatin spring to kinetochore microtubule plus-ends. The rupture force to dissociate the kinetochore from a microtubule is approximately 9pN [225]. Note that this is comparable to the force of a single motor protein (6pN) so that its effects on the model might not be negligible.
- Variation among the chromatin springs is not incorporated in the model including: histone exchange in the pericentric chromatin [226], the likelihood that spring constants for different chromosomes are not identical nor are the switching thresholds, and variation in chromatin protein number (e.g., cohesin, condensin). These sources account for in vivo noise but are not believed to alter the overall trends or behavior of the model.

We end this discussion with a quote from Mogilner et al. [185] “A complete understanding of complex mitotic processes will inevitably require multidisciplinary efforts, of which modeling will undoubtedly be a major part. Three aspects of modeling will be crucial for success. First, the iterative character of the model-experiment loop will allow models to be adapted and improved. Although the initial models proposed will probably not survive experimental scrutiny, the development of first, even relatively crude, models is essential for the emergence of second generation models. Second, modeling will become more comprehensive and powerful through the combination of mathematical and computational approaches. Also, detailed mechanistic models will have to be combined with informatics-type models to deal with incomplete and sometimes noisy data of high-throughput studies. Third, simplistic models might have to become very detailed [...] Which way mitotic models will turn out is unclear, but the great challenge is to build adequate models of mitosis without making the models as complex as the mitotic spindle itself.” Our philosophy is aligned with theirs!

4 Modeling Cell Motility

In the interests of space, we only briefly highlight the modeling approach of our group (Q. Wang, X. Yang, J. Zhao at U. So. Carolina, A. Chen, T. Wessler at UNC) toward the fundamental phenomenon of living cell motility. There are many reviews easily found in a search, such as [227, 228], that can lead a modeling approach. Our group has teamed with Ken Jacobson, Maryna Kapustina, Denis Tsyngkov, and Tim Elston to explore an intriguing cell oscillation phenotype and to search via modeling for the mechanochemical mechanisms to explain this remarkable oscillatory phenomenon. For details of our approach, we refer to our book chapter [229]; for details of the oscillatory cell phenotype, we refer to [230], and we recommend in particular the “biosights” video podcast for their paper. Our modeling approach assumes a phase-field formulation of the cell, based on a relatively coarse organizational structure that ignores molecular-scale complexity. This level of description is chosen because we want to understand the minimum mechanical and chemical species and processes necessary to reproduce the experimental observations. For an excellent overview of cell mechanics refer the reader to the recent article by Hoffman and Crocker [231].

In our approach, the cell is modeled as a composite of substructures (see Fig. 2.14): a lipid bilayer membrane, a thin cortex (cytoskeleton) that is the primary structural component, a nucleus, and a cytoplasm that fills the remainder of the cell’s interior volume. Each substructure (phase) is governed by specific material properties and constitutive relations. In the phase field formalism, the boundary between adjacent phases is diffuse, modeled with a thin transition layer, and an energy functional prescribes the mass, momentum, and energy exchange across the transition layer. The cytoplasm contains various protein filaments, other organelles, and aqueous cytosol [232] and should properly be modeled as a viscoelastic fluid phase. Many laboratories focus specifically on the viscoelastic properties of the cytoplasm and in particular on fluid flow and transport within; we posit a

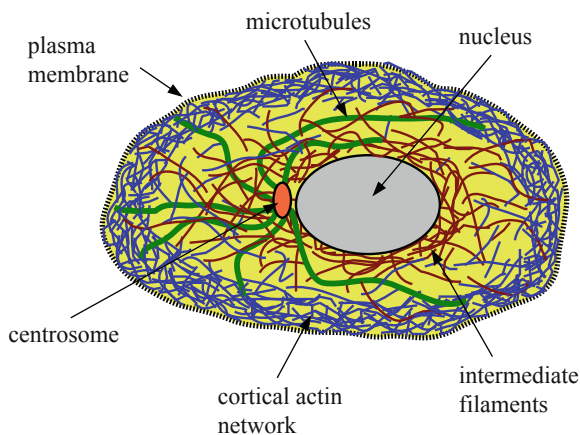


Fig. 2.14 Schematic of cell components. Figure from [232]

homogeneous viscoelastic fluid phase in our level of modeling, averaging overall the molecular complexity. The cortical layer not only provides the cell with mechanical integrity, but also provides a pathway for chemical activation and momentum transfer to the rest of the cell. The cytoskeleton is a network of protein filaments that spans the cell body and it is continuously remodeling. This constant remodeling makes the cytoskeleton highly adaptable, allowing the cell to change shape, to move, and to divide or merge. In our modeling, we adopt the description of the cortical layer as an active nematic gel [233].

Active polar nematic gel models have emerged as a new and popular topic in soft matter and complex fluids [233–235]. In an active material system, energy is continuously supplied by internal as well as external sources to drive the movement of the material system. The driving force behind these cell motility studies was the urge to understand the interaction between molecular motors and cytoskeletal filaments in cell motion and self-propelled motion of certain living cells. In a living cell, cross-linking proteins bind two or more self-assembled filaments (e.g., F-actin or F-actin and microtubules) to form a dynamical gel, in which motor proteins bind to filaments and hydrolyze nucleotide ATP. This process coupled to a corresponding conformational change of the binding protein turns stored energy into mechanical work, thereby leading to relative motion between bound filaments. In active nematic gel models, these molecular processes are upscaled to activation terms in the velocity field and extra stresses in the momentum balance.

Self-propelled gliding motion of certain bacterial species is another example of such an active material system, where molecular motors drive the cellular motion in a matrix of another material [233–245]. Both continuum mechanical models and kinetic theories have been proposed for active complex fluid systems [235, 240, 245, 246]. The mathematical framework incorporates the source of “active forcing” into an otherwise passive material system. The models are based on free energy considerations, both equilibrium and nonequilibrium, where one can keep track of dissipative and conservative principles, and the challenge for biological fidelity is to construct relevant energy potentials and chemical-mechanical activation functions. These potentials require detailed viscous and elastic properties of the fundamental cell components or phases, for which experimental techniques are now advanced enough to make progress. The energy formulation is likewise compatible with mathematical modeling, numerical algorithms, and simulation tools that have been developed for the hydrodynamics of multiphase complex fluids in evolving spatial domains. The simultaneous modeling of reaction and diffusion of biochemical species is self-consistent with the energetic formulation. These advances lay the groundwork for our approach.

Given the collective advances in membrane and cytoskeletal modeling, cell-substrate coupling, and biochemical kinetics, it is now feasible to develop a coarse-grained, whole cell model for migration on substrates or suspended in a saltwater solvent. This global cell-substrate model will enable us to investigate cell motility, dynamics of signaling proteins, cytoskeleton-substrate coupling, and contact cue guidance of motile cells. The model predictions will provide qualitative comparisons with cell experiments in the first proof-of-principle stage and potentially guide future experiments on detailed mechanisms associated with

motility. As properties of each substructure become more quantified, the model will be able to make predictions to guide cell motility experiments. Given the complex nature of cell migration on topographically designed substrates, one should adopt a theoretical and computational platform that is capable of reproducing a variety of dynamical modalities.

Among the competing mathematical models for multiphase soft matter phenomena, a field phase approach is sufficiently versatile to handle the complexity of this challenge and to incorporate additional biological complexity. This is, admittedly, a top-down approach and is not to be expected to resolve detailed molecular or even supramolecular structure of the lipid bilayer, cortex, cytoplasm, and nucleus. These four phases are coarse-grained into constitutive relations and material parameters, with laws for interphase mass, momentum and energy exchange, and activation (what makes the cell alive) provided by a set of chemical species. These small molecule proteins responsible for activation are the focus of molecular biologists. There are many to choose from, and we begin with a handful of species that diffuse within the four phases with phase-specific diffusion coefficients that allows the model to enforce membrane-bound or cortex-bound constraints, for example. The concentrations of these kinetic species, their reactions with one another, and their activation energy within each phase are hypothesized and tested with simulations. Our current simulations are focused on the oscillatory phenotype. We posit one protein species that binds to filaments in the cortex, modeled as a contractile activation proportional to protein concentration; another protein species triggers unbinding by the kinetics of reaction and diffusion among the protein species.

The test of this modeling approach rests on whether we are able to establish fundamental mechanisms of cell motility that follow from a minimal set of coupled mechanochemical processes and structures within single cells, together with cues from their environment. We refer again to our review article [229] where we put the mechanical phases, chemical processes, and external environment into one formulation. The success of this framework remains to be determined. In particular, there are significant challenges to full three-dimensional simulations of these models, requiring the tuning of all material phases and chemical species. A current focus of the Jacobson lab and our modeling effort calls into question the geometry of cells suspended in a solvent. Essentially all modeling efforts assume a spherical equilibrium cell morphology. However, Jacobson and Kapustina have convincing evidence that the equilibrium morphology is highly folded if viewed from two-dimensional focal planes and indeed severely blistered if viewed as a three-dimensional structure. This evidence implies in our phase field formulation that cells have far more surface area available than that required to enclose the cell volume with a sphere. We are currently exploring excess surface area versus volume constraints, which are natural within the phase-field formulation. We likewise are exploring the current leading constitutive characterizations and material properties of cell membranes, cortex, cytoplasm, and nucleus, as well as the current leading candidates for chemical activation species and their mechanical activation rules within the cortex.

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