Chapter 1

Introduction
The Mechanical Environment of Cells and Tissues

Living organisms are made up of one or more cells, the smallest basic structural and functional unit of life. The cell is protected by a membrane which encloses the gel-like cytoplasm containing biomolecules that provide the essentials for the cell’s growth, self-replication and other functions [1]. In multi-cellular organisms, the cytoplasm contains various organelles such as the nucleus which contains genetic material needed to code the molecules synthesized by the cell, the mitochondria which generates most of the cell’s energy supply, the Golgi apparatus which packages the proteins and dispatches them to parts where they are needed, and the cytoskeleton which provides structural integrity to the cell, maintains cell shape, facilitates locomotion, helps anchor the cell to the substrate and neighboring cells, and speeds up the transport of materials within cells [1–3].

The function of cells is highly diverse, and cells carrying out a similar function often organize into an ensemble to form tissues. Myocytes—long tubular cells containing force-generating myosin proteins—assemble to form muscle tissue. Epithelial cells are bound together in sheets to form the epithelium that lines and supports most internal organs (Figure 1.1). Surrounding the cells is an interstitial space that holds the extracellular matrix (ECM), which acts as a substrate and glues together cells and organs. Besides this interstitial matrix, an extracellular basement membrane also separates the epithelium from underlying connective tissue, which connects the various types of tissues and organs in the body. More than just an inert scaffold, the ECM not only binds the cells together but also influences their survival, development, and overall behavior [1, 4], as well as determining tissue morphology and function [3, 5]. Thus, multi-cellular life would not be possible without the extracellular matrix [5].

Cell-matrix interactions are crucial for the overall healthy function of tissues. Living cells must not only remain connected to their neighbors and to the tissue substrate, but also move freely through it when necessary. For example, endothelial cells must stay attached to an ever deforming artery subjected to pulsatile shear forces from blood flow [3, 6, 7]. At the same time, these cells must be able to easily detach themselves from the surrounding matrix to allow large macrophages—blood cells that clean up debris and destroy pathogens and cancer cells—to pass through the arterial wall [8, 9], or to cover an injured region of the artery [10, 11]. Clearly, the mechanical properties of both cellular and extracellular materials are important in the underlying mechanical and biochemical processes involved in cell-matrix interactions.
**Figure 1.1**: Sketch showing hierarchy in the structural components of tissues, cells, and extracellular matrices. (a) Tissue section showing the epithelium of an organ, the basement membrane, and the endothelium of blood vessel, in relation to the connective tissue. Also shown are fibroblasts—cells that produce extracellular matrix (ECM) proteins such as collagen. (b) An epithelial cell showing organelles and cytoskeletal fibers. (c) Plasma membrane at the interface between the actin cortex and ECM.
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The mechanical environment of cells and tissues involves forces generated by cytoskeletal contractility, the modulation of these forces by the stiffness of the ECM, as well as external forces applied to the tissue. Cells react to changes in the mechanical environment of the ECM by influencing tissue development, cell differentiation or disease progression [12]. The forces exerted by cells onto the ECM and the feedback provided by the matrix stiffness are mechanical signals transduced into complex intracellular cues to regulate cellular functioning [13–15]. Understanding the material properties of cells and tissues as well as cell-matrix interactions are therefore equally important to take into account as biochemical signalling in designing biomimetic materials to engineer artificial tissues for regenerative medicine [12, 16–18] and to gain more insight into their role in diseases such as cancer and fibrosis [19–21].

1.1 Biopolymer Networks: Life’s Woven Fibers

The cytoskeleton is the principal determinant of cellular mechanical stability [22] and is an intricate network of three types of fibrous protein molecules known as biopolymers (Figure 1.2): F-actin, microtubules, and intermediate filaments, each polymerized within the cell from a specific protein composition. Actin filaments are double-stranded helical structures formed from globular actin and are about 7 to 9 nm thick and up to several micrometers long [1, 23, 24]. With cross-linking proteins, actin filaments organize into network assemblies. The most abundant protein in most eukaryotic cells, it comprises the actin cortex attached to the cell membrane to control cell shape [4, 25, 26] and binds...
with myosin to generate forces in muscle contraction [23, 27, 28]. Microtubules are tubular structures with inner and outer diameters of about 14 and 30 nm, respectively and are assembled from tubulin dimers [1, 29, 30]. They are dynamically involved in mitosis and cell migration [31–34]. Intermediate filaments (IFs) are assembled by tetrarmers of alpha-helical segments. Unlike actin and microtubules, IFs (~10 nm in diameter) have no polarity and do not play a direct role in cell motion and transport [23]. Comprising various types such as keratin which is an important structural element of skin cells [35], lamin which supports the cell nuclear membrane [36, 37], and vimentin which anchors the organelles in place [38, 39] and regulates cell adhesion [40, 41], IFs are mainly involved in providing mechanical strength to cells [4].

The extracellular matrix is a complex mixture of biopolymers and adhesion proteins interwoven into a hydrated gel of carbohydrate molecules, and provides a stable yet permanently reconstructing framework for cells and tissues to support their growth, morphology and regeneration [5]. Fibrillar proteins in the ECM include collagen that imparts structure and rigidity to the tissue and supports the resident cells, and elastin that provides elasticity to tissues [42, 43]. Adhesion proteins such as fibronectin connects cells to collagen fibers, and laminin assists cell adhesion [44, 45] by forming independent networks. In addition to networks of fibrous proteins, the ECM also contains various other components such as the hydrophilic proteoglycan aggregate shaped like bottle-brushes attached to a hyaluronan backbone, both involved in tissue hydration and providing resistance to compression via swelling forces [46–48]. All these components endow a wide range of material properties to diverse tissues: from soft gel-like vitreous humor of the eye, tension-resistant rope-like tendons, to rigid composites in bone [1, 19].

1.2 Elasticity of Biopolymers

In physiological processes involving mechanical cell-matrix interactions, fibrous biopolymers in cells and tissues experience a variety of deformations depending on their mechanical properties and the nature of the applied forces. Some forces act longitudinally along the fiber backbone causing it to stretch or compress, while others act perpendicularly which leads to bending or twisting deformations. These fibrous proteins are semiflexible: they possess enough bending rigidity to outcompete the natural tendency of a flexible synthetic polymer chain to crumple up into a random coil due to thermal fluctuations relative to a straight conformation [49, 50]. The semiflexible character of biopolymers and their assemblies give rise to unique physical properties including viscoelasticity [51–54], which has important implications on the mechanics of cells and tissues [55–58].
Figure 1.3: Schematic of a semiflexible fiber in a worm-like chain model. At any given point along the curvilinear coordinate $s$ that defines the fiber backbone, the local curvature is given by $d\hat{t}/ds$.

On the one hand, biopolymers must be strong enough to resist mechanical stresses and maintain the integrity of cell and tissue structure, while on the other hand they must be flexible to allow dynamic processes linked to metabolism, growth, and regeneration.

Regardless of the nature of the deformations, energy is required to deform biopolymers from their zero-temperature, unstressed conformation. The energy associated with the bending of stiff fibers is given by the worm-like chain (WLC) model [59]:

$$\mathcal{H}_{\text{bend}} = \kappa \int \left| \frac{\partial \hat{t}}{\partial s} \right|^2 ds, \quad (1.1)$$

and is determined by the flexural or bending rigidity $\kappa$, which has dimensions of energy times length. The integral runs along the fiber contour with respect to the curvilinear coordinate $s$ (Figure 1.3). The quadratic dependence on the local curvature $\left| \frac{\partial \hat{t}}{\partial s} \right|$ leads to a linear restoring force in terms of the bending displacement. For a homogeneous fiber, the bending rigidity can be expressed in terms of its material and geometric properties as $\kappa = EI$, where $E$ is Young’s modulus and $I$ is the cross-section area moment. Treating the fiber as a uniform cylinder of radius $r$, we have $I = \frac{\pi}{4} r^4$ and therefore $\kappa = \frac{\pi}{4} r^4 E$ [60]. In thermal equilibrium, an infinitely stiff fiber has zero curvature and therefore no bending displacements, i.e., it barely moves from a straight conformation. In contrast, an extremely flexible fiber can have a huge variety of conformations due to Brownian fluctuations as it exchanges energy with its environment. These fluctuations are characterized by a persistence length $\ell_p$, intuitively thought of as the typical length scale over which the fiber maintains a fixed orientation and is given by $\ell_p = \kappa/(k_B T)$ [22], where $k_B$ is Boltzmann’s constant and $T$ is the temperature of the surrounding heat bath. Table 1.1 summarizes the persistence lengths in relation to the diameter and contour lengths of biopolymers as well as their Young’s moduli.

A natural generalization of the WLC model addresses the response of the fiber to longitudinal forces along its backbone. The zero-temperature pure mechanical response can be expressed by a simple Hookean spring hamiltonian [50, 70–72]

$$\mathcal{H}_{\text{stretch}} = \frac{\mu}{2} \int \left( \frac{d\ell}{ds} \right)^2 ds, \quad (1.2)$$
where \( \mu \) is the stretch modulus and \( d\ell/ds \) is the relative local change in length of the fiber backbone. For a homogeneous cylindrical fiber, \( \mu = \pi r^2 E \) [60]. In the presence of thermal fluctuations, the transverse bending fluctuations give rise to additional longitudinal compliance. This added compliance exists because of the possibility of pulling out thermal fluctuations without stretching the fiber backbone, and has an associated modulus \( \mu_{\text{thermal}} = \frac{\kappa \ell}{\ell^3} \), where \( \ell \) is the length of a fiber segment [49]. The full stretching modulus of the fiber is obtained by effectively treating the mechanical and thermal components as springs connected in series [70].

Besides bending and stretching compliance, which for most biopolymers are dominated by the entropic response, a purely mechanical response that occurs under compression is Euler buckling [60]. With increasing compressive loads, a straight rod of length \( L \) can only withstand a maximum force \( F_c = \kappa (\pi/\lambda)^2 \) before collapsing, where \( \lambda = L \) is a characteristic bending wavelength. Under finite temperatures however, thermal fluctuations modifies this sharp force threshold into a smooth force-compression curve as it enhances compliance leading to reduced forces for the same degree of compression [50, 73]. The buckling threshold for an athermal fiber can also increase when it is surrounded by an elastic matrix, which has the effect of suppressing lateral deflections leading to shorter bending wavelengths \( \lambda < L \) [60, 74].

### 1.3 Network Mechanics and Nonlinear Effects

The mechanical properties of single fibers and their underlying interactions leads to collective mechanical properties of fibrous network assemblies. Indeed, semiflexible biopolymer networks possess unique mechanical properties that are in contrast to other soft and granular materials [75–79]. While these viscoelastic materials share common characteristics with conventional polymer gels and rubbers, they are significantly different in other aspects which cannot be accounted for by classic rubber elasticity theory [54, 80]. The unique properties of these gels which give rise to striking nonlinear effects are due to the

<table>
<thead>
<tr>
<th>Type</th>
<th>Approximate diameter</th>
<th>Persistence length</th>
<th>Contour length</th>
<th>Young’s modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-actin</td>
<td>7 nm</td>
<td>17 ( \mu )m</td>
<td>( \lesssim 20 ) ( \mu )m</td>
<td>2.6 GPa</td>
</tr>
<tr>
<td>Microtubule</td>
<td>25 nm</td>
<td>( \sim 1-5 ) mm</td>
<td>10s of ( \mu )m</td>
<td>~1.2 GPa</td>
</tr>
<tr>
<td>Intermediate filament</td>
<td>9 nm</td>
<td>0.2–1 ( \mu )m</td>
<td>2–10 ( \mu )m</td>
<td>9 MPa</td>
</tr>
<tr>
<td>Collagen-I</td>
<td>40–200 nm</td>
<td>( \sim 1 ) cm</td>
<td>10s of ( \mu )m</td>
<td>1–800MPa</td>
</tr>
</tbody>
</table>
combination of the semiflexible nature of the constituent fibers, the nature of the fiber interactions, as well as the underlying network geometry [62, 81–85].

1.3.1 Nonlinear stiffening

The rapid increase in the stiffness of biopolymer networks by up to two orders of magnitude in response to an applied deformation, stress, or motor activity [62, 75, 76, 86–89] is thought to mediate the transmission of mechanical signals across the plasma membrane through the cytoskeleton [90], as well as to prevent large deformations that may threaten tissue integrity [12, 76, 91, 92]. Nonlinear stiffening has been observed in gels of cytoskeletal and extracellular fibers [75, 76, 90, 93–97] and in soft human tissues [98], although its origins remain unclear. A number of mechanisms have been proposed to explain strain stiffening of fibrous networks, which include non-affine network rearrangements [72, 99–101], the inherent nonlinear response of the constituent fibers [49, 75, 76], and the stabilization effect of stress induced by the macroscopic deformation [88, 102, 103].

1.3.2 Negative normal stress

Most solid materials exhibit what is known as the Poynting effect, where the response is to expand in a direction normal to an externally applied shear stress. This effect explains why metal wires increase in length under torsional strain [104]. By contrast, gels of cross-linked biopolymer networks exhibit the opposite response to shear deformation. Such negative normal stress has been explained either in terms of the inherent asymmetry in the extension-compression response of thermal semiflexible polymers or non-affine deformations in athermal fiber networks [71, 78, 105, 106]. Moreover, the magnitude of these normal stresses is shown to become as large as the applied shear stress with increasing strain, which coincides with the onset of nonlinear stiffening [71, 72, 78, 105–107].

1.4 Experimental and Theoretical Approaches

The recent research progress in studying the mechanical properties and elastic response of biopolymer networks has been due to the advances in both experiments and theoretical/computational modeling. In this section, we describe a common experimental
1.4 Experimental and Theoretical Approaches

Figure 1.4: Schematic of a rotating plate rheometer in oscillatory mode. The sample undergoes an oscillatory shear deformation $\gamma$ at a frequency $\omega$, and responds with an oscillatory shear stress $\sigma$. An axial stress $\tau$ can also exist normal to the shear boundaries.

technique—rheology, which appears in parts of this thesis where comparison with simulations and theoretical predictions are made. An overview of theoretical models closely related with the approach used throughout this thesis is also presented.

1.4.1 Rheology experiments

The method of macroscopic rheometry uses a rotating plate rheometer (Figure 1.4) and has been used to measure the viscoelastic properties of gels from a wide variety of reconstituted biopolymer networks to study nonlinear stiffening and normal stresses [63, 66, 78, 88, 103, 108–110]. There are two commonly used modes for which one can perform measurements using a rheometer: creep mode for measuring the creep compliance, i.e., the tendency of a material to move and deform slowly when subjected to mechanical stress, or oscillatory mode for measuring the dynamic storage moduli to characterize viscoelasticity. In the oscillatory mode, one could directly measure the macroscopic response of the sample by subjecting it to an oscillatory shear deformation $\gamma$ and relating it to the corresponding shear stress $\sigma$. If the sample is purely elastic, the shear stress under small deformations is linear: $\sigma = G\gamma$, where $G$ is the shear modulus. For viscoelastic materials, the modulus $G(\omega) = G'(\omega) + iG''(\omega)$ is dependent on the frequency of oscillations, and is composed of storage $G'$ and loss modulus $G''$ components. For biopolymer gels, the storage modulus dominates low-frequency measurements. For higher frequencies, both moduli scale as a power-law with $\omega$ [54]. Throughout this thesis, we focus only on the elastic component as captured by the storage modulus.

The nonlinear elasticity of a sample is measured in terms of the differential or tangent modulus $K = \frac{d\sigma}{d\gamma}$. There are two commonly used protocols in measuring the nonlinear response, and are described in more detail in chapter 3. In the strain-controlled protocol, a time-dependent strain ramp $\gamma(t)$ is imposed at a fixed rate and the resulting stress $\sigma(t)$ is measured. The stress-strain data set is then interpolated before calculating the local derivative to determine $K$. In a stress-controlled protocol, the sample is subjected to a
constant stress $\sigma$, and superimposed on it is a small oscillatory stress $\delta \sigma(t) = \delta \sigma e^{i\omega t}$. By monitoring the resulting oscillatory strain $\delta \gamma(t) = \delta \gamma e^{i\omega t}$, the differential modulus $K = \frac{\delta \sigma}{\delta \gamma}$ can be determined.

1.4.2 Theoretical models

There is a wide range of theoretical modeling approaches used to study the elasticity of biopolymer networks. Here and throughout this thesis, we limit ourselves to athermal models of isotropic fibrous networks with permanent freely-hinged noncompliant crosslinkers. These kinds of models have been used to understand certain specific aspects of linear or nonlinear elasticity, often combined with direct quantitative comparison with rheology experiments on reconstituted networks.

The off-lattice two-dimensional Mikado networks of straight rods [70, 71, 100, 101] or with built-in undulations [72] highlight the importance of non-affine network rearrangements and fiber alignment, which coincide with other signatures of nonlinearity such as strain stiffening and negative normal stress. Another off-lattice model makes use of a Monte Carlo scheme to generate more realistic thermalized networks in 3D [111, 112], which also emphasizes the role of non-affine displacements in the nonlinear stiffening of networks with either homogeneous fibers or heterogeneous fiber composites.

Networks generated from lattice-based structures [113–117], which can also be combined with a mean-field approach [102, 113, 118, 119] have also gained a wide interest because of their computational efficiency, the flexibility in the choice of spatial dimensionality, as well as the relative ease with which one can generate increasingly larger system sizes. However, most of the work done using lattice-based networks have focused on linear elasticity, in contrast to random networks.

Whether lattice or off-lattice models are used, the usual approach in simulating rheology is to impose an affine deformation and subsequently minimize the total elastic energy using a numerical function minimization scheme, with the goal of finding the nearest local minimum in the energy landscape. This approach assumes no interaction between network and solvent, i.e., that there is no viscous component $G''$, and therefore emulates a zero-frequency rheology measurement to obtain $G'$. Furthermore, steric interactions between fibers are also disregarded since typical network volume fractions in biopolymer gels are too low ($\lesssim 0.1\%$) in contrast to synthetic polymers [120, 121]. The low volume fraction consequently leads to a low probability of fibers crossing each other under typical strain ranges covered in rheology experiments.
1.5 Outline of the Thesis

Chapter 2: Modeling athermal sub-isostatic fiber networks

This chapter explores the full range of elastic regimes in lattice-based network models and the results are compared with those obtained from a random network. It begins with a detailed description of these network models, which allow the independent control of fiber rigidity and cross-link connectivity. The network consists of elements with linear response to fiber bending and fiber stretching deformations. A unified description of elasticity is presented that makes it possible to have a direct comparison of simulation results across different network models as well as a quantitative comparison with experimental measurements. Interesting implications are presented at the end to set the background for the succeeding chapters.

Chapter 3: The role of normal stress in collagen network mechanics

The chapter presents striking experimental observations on reconstituted collagen networks: the stiffness becomes independent of protein concentration in the nonlinear elastic regime over a range of concentrations and applied shear stress, in strong contrast to other biopolymer networks. A minimal model is presented using disordered networks of linear fiber elements, and that it accounts for the nonlinear mechanics of collagen. The model highlights the importance of local network geometry in determining the onset of nonlinearity, such that a given network architecture can thereby account for the concentration independence in the nonlinear regime. In addition, the local network architecture controls the susceptibility of the network stiffness to the applied stress. The chapter concludes with the important role of normal stresses in determining the nonlinear shear elastic response of collagen networks. The experimental results shown in this chapter that confirm the model predictions are from close collaborations with S. Münster et al from the Weitz group in Harvard and the Fabry group in Erlangen, and with K. Jansen from the Koenderink group at AMOLF.

Chapter 4: Mechanically-controlled criticality in fiber networks

In this chapter, the transition in elasticity regimes of disordered fiber networks under simple shear strain and isotropic expansion is explored. Well below the isostatic threshold, these networks exhibit a line of second order transitions that depend on network connectivity. Critical behavior along this line is demonstrated by investigating the characteristic signatures of criticality observed in continuous phase transitions. The
critical exponents are different from mean-field predictions, yet are surprisingly insensitive to the dimensionality and the type of network architecture. The nature of the applied deformation however, affects the dependence of the critical exponents on local connectivity. With rheology experiments on collagen networks performed by K. Jansen from the Koenderink at AMOLF, this chapter further demonstrates that the nonlinear mechanics of collagen networks can be quantitatively captured by the predictions of a strain-controlled rigidity transition.

Chapter 5: The mechanics of floppy rope networks under stress

The chapter addresses the stabilizing effect of stress on a floppy sub-isostatic rope network under shear deformation. Without stress, rope networks are only stable above a finite shear strain. Above this threshold, the network stiffness can be described as a continuous rigidity transition, with a critical exponent that depends on network connectivity. Below the critical strain, rope networks can be stabilized by various effects such as finite fiber bending resistance. By applying a uniform extensional stress to an otherwise unstable rope network, its contribution as a stabilizing field is isolated from other possible fiber interactions. The associated linear shear modulus scales as a power law with the applied stress, with an exponent that depends on the network connectivity. Moreover, these networks are infinitely susceptible to stress. A stiffening relation summarizes these findings at the end that reasonably captures the mechanics of rope networks stabilized by stress.

Chapter 6: Decoupling of shear and Young’s moduli in extracellular networks

The final chapter presents rheological experiments that provide a mechanical characterization of collagen and fibrin networks under multiaxial deformations that mimic typical strains under physiological conditions. Such characterization is directly relevant to the theoretical models that form the focus of this thesis. The chapter presents results from experiments performed by A. van Oosten at the Janmey group in Pennsylvania and simulations by M. Vahabi at the MacKintosh group in Amsterdam. The axial strain response of these systems are found to be highly asymmetric: they exhibit strong shear hardening when extended and weak shear softening when compressed. These features are captured by our stiffening mechanism and minimal model of disordered networks with linear fiber elements. Furthermore, the Young’s moduli are decoupled from the shear moduli, demonstrating that biopolymer networks violate continuum elasticity theory.