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# Viscoelasticity in Biological Systems: A Special Focus on Microbes

Supriya Bhat, Dong Jun, Biplab C. Paul and Tanya E. S Dahms

Additional information is available at the end of the chapter

## 1. Introduction

Over billions of years of evolution, living organisms have developed into complex biosystems, of which the basic unit is the cell. Cells have a complex molecular structure with a certain level of rigidity. Living cells, whether isolated or part of a larger collective, live under constant mechanical stress from their external environments. Cells have developed adaptive mechanisms to maintain homeostasis and viability, which interestingly follow the basic principles of classical mechanics.

Cell mechanical properties have myriad biological significance and so there has been significant interest in the past decade to measure the response of cells to external mechanical signals. Cellular mechanics and rheological properties (*e.g.* stress-strain relationships) are known to play a role in biological processes such as cell growth, stem cell differentiation, cell crawling, wound healing, protein regulation, cell malignancy and even apoptosis (programmed cell death) [1,2].

A living cell is a complex dynamic system, far from static, which constantly undergoes remodeling to adapt to varying environmental conditions. The mechanical changes in cells under normal conditions and in response to external signals are highly complex and extremely difficult to measure *in vitro*. The interplay of cellular constituents enables adaptation to changing demands of mechanical strength and stability. The field of rheological science deals with the mechanical behavior of biological materials and over the past decade several rheological methods have been developed to quantify the mechanical behavior of cells in response to external conditions and forces.

To understand cell mechanics we first need an appreciation of how cells operate in a mechanical context. Firstly, how do cells maintain their shape and flexibility to accommodate cellular requirements? Cell surface layers are strong, playing a crucial

mechanical role in maintaining cellular shape and resisting turgor pressure, yet at the same time flexible enough to allow cell growth and division. Multi-cellular eukaryotes are arranged into specialized structures of varied composition (*e.g.* tissues) which protect them from harsh environments. Single-celled prokaryotes or eukaryotes can also form sophisticated structures, such as biofilms or mycelia, but even as single cells they are able to bear mechanical stress and maintain integrity which is quite astonishing. Cells range from soft mammalian tissue cells to those with very firm plant cellulosic fibers, so understanding how different cellular components provide diverse mechanical properties is of great interest. Further, how do mechanical properties support locomotion? Active movements depend on cell type and ultimate function within tissues or the greater environment, and their associated mechanical properties change according to environmental demands. Another question relates to how external factors, such as temperature, pH, aridity and xenobiotics affect cell mechanical properties. Further, what is the role of cell mechanics in processes such as active and passive diffusion, adhesion, community formation and evolution of complex tissues in higher eukaryotes, and how do the mechanics of isolated components work in concert within dynamic live systems that are far from equilibrium?

Several of these questions remain unanswered, however early success studying the rheological properties of non-living materials has provided a strong foundation for studying biological systems. Over the past decade, several obstacles have been overcome and thus a new perspective of cellular mechanics is emerging [2].

## 1.1. Viscoelasticity in biology

If cellular metabolism could be frozen at any given moment, disrupting the production of metabolites and supra-molecular structures, the cell would simply become fluid-like with the associated mechanical properties. Living cells behave both as an elastic solid and as a viscous fluid, and so are considered viscoelastic. Such materials, including biological molecules and cells, cannot be fit using classical models of either elasticity or viscosity. Cellular viscoelasticity arises from the combination of high water content conflated with a polymerized structural matrix. On the one hand, the biopolymers which support cell shape provide strong enough mechanical properties to resist environmental pressures, but on the other hand their organization is highly dynamic and linked to metabolic conditions.

Cellular mechanical properties can be characterized using viscosity, elasticity and creep compliance. Herein we focus on viscoelasticity studies measuring either biological sample deformation induced by an external force or the force resulting from sample deformation. The cell cytosol, which contains the majority of cell fluid, can be treated as the coexistence of liquid and solid phases. The latter is composed of proteins, DNA, RNA and cytoskeleton filaments as well as organelles suspended in the viscous buffered saline, saturated with metabolites and proteins. The liquid component has a high compression modulus, meaning that without the structural components and macromolecules it would be very fluid (less viscous). In contrast, the solid phase has a lower compression modulus, exhibiting more elastic properties. In cells as a whole, the viscosity of the cytoplasm dominates the transport

and movement of subcellular macromolecules (such as DNA and proteins), elasticity controls the response of the cytoplasm to mechanical stresses at shorter timescales (seconds), organelles and cytoskeleton elements over long timescales (minutes), and the contractile mechanical regime governs responses at even longer time scales (minutes to hours).

The quantitative characterization of elastic material is the elastic modulus, described as the ratio of stress to strain, but the deformation (strain) of a fluid under an external stress changes as a function of time and is referred to as strain rate. Thus fluid viscosity is the ratio of stress to strain rate. The mechanical responses of biopolymers fall into a category between that of an elastic solid and a fluid, defined as viscoelasticity.

In this chapter we will describe the methods to measure viscoelasticity in biological systems and their significance. We generalize the mechanical properties of prokaryotic and eukaryotic cells since it has been argued that viscoelastic properties are universal across cell types and species, however the mechanistic basis of this phenomenon is not well understood [4]. This chapter is organized into four main sections: (i) a brief summary of various rheological methods used for cell micromanipulation and the novel application of atomic force microscopy to measure cell mechanical properties, (ii) the main mechanical load bearing components of cells and associated studies that have helped to establish a consensus understanding of cell mechanical properties, (iii) a survey of the various factors that affect cell viscoelastic properties, but which currently lack clear interpretation, and finally (iv) a future perspective on the significance of cellular viscoelasticity.

## 2. Methods of measuring cellular viscoelasticity

In microrheological studies, several experimental techniques and theoretical models are combined to accurately quantify cellular mechanics. Several methods that are routinely used for cell rheology are briefly reviewed in the following section. Micropipette aspiration, microplate rheometry, and optical stretching are used to probe whole cell mechanics, while probe- or particle-based techniques such as magnetic probes, optical tweezers and particle tracking cell rheology only measure local mechanical properties. The probes or particles within a structure are subjected to an external force and the subsequent response measured, usually by tracking their displacement, to characterize associated mechanical properties. Atomic force microscopy uses a probe, but attached to the end of a cantilever with well-defined rigidity able to the mechanics of whole cells and their surface layers. A schematic diagram of various devices is shown in Figure 1.

The mechanical properties measured by a probe or particle technique are highly dependent on the strength of, and relationship between the particle/probe and the structure being studied. This relationship is highly complex, varies with cell type and can lead to significant misjudgement of the stiffness. Probes can be modified with different molecules such as antibodies, peptides and cadherins to target antigens, integrins and cytoskeletal components. However these measurements do not account for interactions between the probes and cells which can lead to confounding results [5]. Cells have a heterogeneous

composition and their various compartments have diverse mechanical properties. If only one cell position is probed, as is common, the mechanical properties of the whole cell will not be well represented. Comprehensive methods have been developed for measuring the mechanical properties of mammalian cells and precise methods have not yet been adequately developed for prokaryotes. A summary of the advantages and disadvantages are listed in the Table 1.

| Methods | Applications | Drawbacks | References |
|---|---|---|---|
| **Micropipette aspiration** | Measurements of non-linear deformations with high accuracy<br>Both soft and rigid cells can be used | Quantitative measurements rely heavily on theoretical models<br>Pipette geometry can limit measurements | [6,7] |
| **Microplate rheometer** | Several manipulations in the same instrument are possible<br>A large range of forces are measurable (1nN - 1μN)<br>Control of cellular pre-stress | No subcellular resolution | [8,9] |
| **Optical stretcher** | No physical contact required and non-destructive<br>Less time consuming with simple setup | Causes heating of cells<br>Extensive modeling is required to obtain force profiles | [10,11] |
| **Magnetic probes** | Large range of frequencies [0.01–1,000 Hz]<br>Parallel measurements of large number of cells possible<br>High timescale resolution | Can only probe microenvironment inside the cell | [12,13] |
| **Particle tracking microrheology** | Quantitative measurements of shear modulus possible<br>Can be used under physiological conditions | Used only for soft tissue cells | [14-16] |
| **Optical tweezers** | High timescale resolution<br>Force and position can be controlled more accurately | Local heating and prototoxicity can result<br>Can only be used at low forces in the linear regime | [17,18] |
| **Atomic force microscopy** | High spatial resolution<br>Measures large range of forces<br>More precise and easy to control cantilever position | Slow scan rate | [19, 20] |

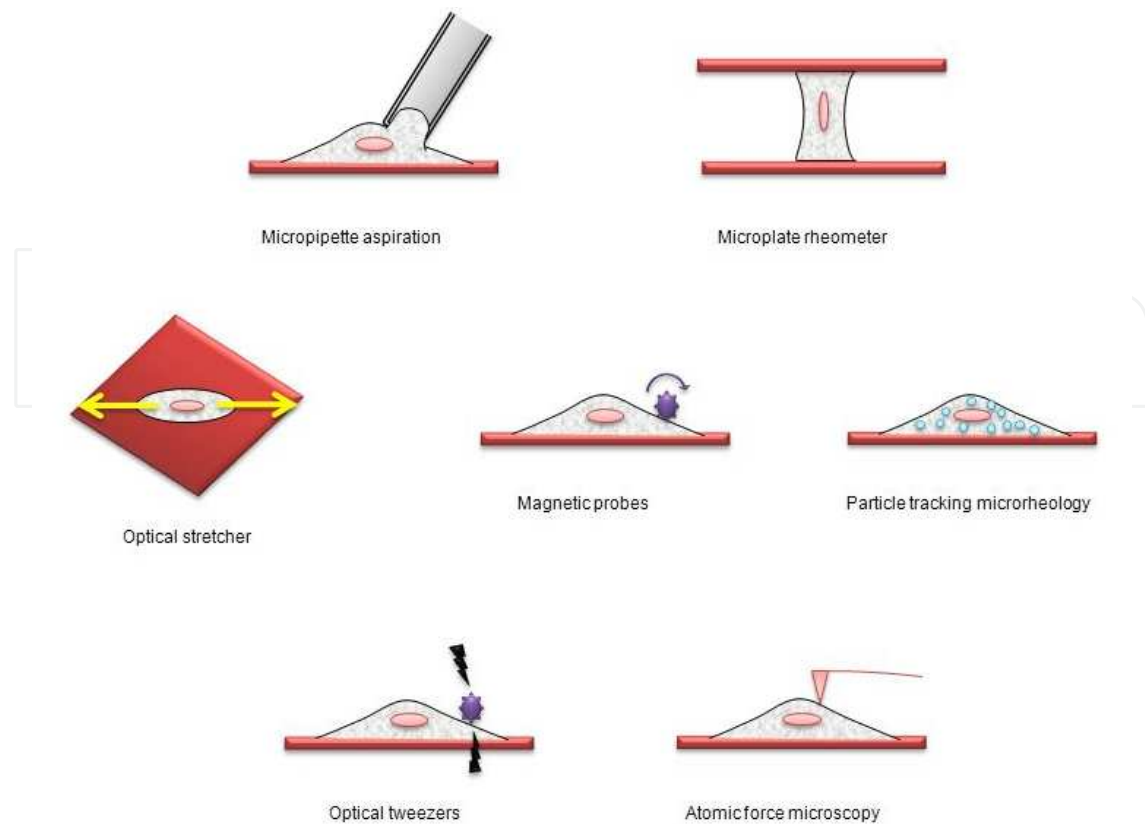**Table 1.** Comparison summary of different methods to measure cell rheology.

**Figure 1.** Schematics for the common methods used to measure cell rheology.

## 2.1. Micropipette aspiration

Micropipette aspiration is a widely used method and offers a versatile way of measuring the mechanical properties of living cells. A cell is aspired onto the end of a micropipette by a negative pressure gradient, and the aspiration length of the cell inside the micropipette is recorded as a function of time [21]. Cells experience large non-linear deformations in response to aspiration suction pressure (0.1 pN/$\mu$m$^2$), giving rise to forces (10 - 10$^4$ pN) that allow tracking of edges with high accuracy ($\pm$ 25 nm). This method can measure the elastic and viscous properties of very soft materials like red and white blood cells, and stiffer cells such as endothelial (skin) cells and chondrocytes (cartilage) [7]. Measurements are interpreted using basic continuum models to solve for elasticity and viscosity parameters.

In most cases, cells suspended in buffer or saline solution that are aspirated appear round, but shape and degree of deformation depends on cell type. Many mammalian cells are naturally spherical (*e.g.* white blood cells) or spontaneously adopt this shape when detached from a surface. Recall that cells can behave as liquids and/or solids [7], the response of which is similar until a hemispherical projection is formed in the pipette. Beyond that point, a further increase in the suction pressure causes constant cortical tension, creating a liquid-like cell able to flow completely into the pipette [22]. On the other hand, the surface of a solid cell will extend into the pipette to a new equilibrium position. Under constant pressure, the mechanical properties of the cell determine how far it is pulled inside the

micropipette. The measurement of suction pressure, location of the cell and movement of its edge in the micropipette can be used to calculate the viscous and elastic parameters [7].

There are several draw-backs associated with this method since quantitative measurement of cell stiffness relies heavily on theoretical models [6]. The most recent application of micropipette aspiration is to capture and hold cells for manipulation [7].

## 2.2. Microplate rheometry

Over the past decade there has been much progress in the study of rheological behaviour for single cells, including the development of several models which explain the mechanical properties of cytoskeletal networks. A microplate rheometer, invented by Thoumine and Ott in 1997 [8], consists of two parallel microplates that support cell adhesion and spreading. The plates can be made flexible and in some cases are coated with an adhesive material. A whole cell is stretched or compressed between the two plates while the flexible microplate is used as a nN-scale force sensor. Integration with an inverted microscope allows cells that are being manipulated to be directly observed. Several adaptations to the instrumentation allow measurement of creep function, area of expansion modulus, contraction forces of single cells, adhesive interactions and stress-strain responses. The latter can be used to measure viscoelasticity.

The force applied to the cell is directly proportional to the relative stiffness of the microplate, hence measurement of cell deformation offers valuable information on cell mechanical properties. Measureable forces range between 1 nN – 1 μN, and several manipulation modes are possible, including compression, traction, aspiration and adhesive rupture. Following their invention, microplate rheometers have been improved by a number of scientists. The behaviour of cells in response to compression, traction, aspiration or adhesion, for which elastic, viscous and contractile regimes can be distinguished based on time scale, can be used to calculate the viscoelastic modulus of living cells. The instrument has been modified for probing single layers of cells at once using the novel cell monolayer rheology (CMR) technique, making possible harmonic oscillation experiments and step shear or step stress experiments which reveal different viscoelastic regimes [9].

## 2.3. Optical cell stretcher

The optical stretcher is a non-destructive tool that can be used to quantify cell deformation. The optical stretcher was first developed by Guck et al. [23] based on the principle that a dielectric object, when placed between two opposed and non-focused laser beams, experiences a net force. The cell is suspended or "optically trapped" and is stable only if the total force is zero. Additive surface forces are capable of stretching an object, such as a cell, along the beam axes [11]. Both the exerted force and corresponding time-dependent deformations can be quantified. An optical stretcher allows the measurement of cell mechanics without physical contact, but the intense laser exposure tends to heat cells [24]. Recently, however, it has been demonstrated that heating does not affect cell cytoskeletal

structure, proliferation, motility, or viability [25]. Another potential limitation is the complexity of the force profiles, which require extensive theoretical modeling. Nonetheless, the optical stretcher has proven to be an effective way of measuring the contribution of cytoskeletal filaments to cell viscoelasticity since single suspended cells are probed as well-defined viscoelastic objects [25].

## 2.4. Optical tweezers

Optical tweezers can be used to capture tiny dielectric particles with a highly focused laser beam [17]. The movement of dielectric particles can be controlled with two main optical forces. The scattering force acts along the direction of beam propagation, while the electric field induces a dipole in the dielectric particles, producing an electric field gradient that pulls particles towards the focus. When the gradient force dominates, the dielectric particles can be confined in a stable three-dimensional optical trap. The trap is then moved to manipulate the bead, so the applied force and resultant particle displacement are interpreted in terms of mechanical response [26]. To obtain viscoelastic information, an oscillatory force is applied to the dielectric bead by oscillating the laser position with a movable external mirror. The resultant amplitude of the bead motion and the phase shift are interpreted in terms of viscoelastic response. The experimental data from optical tweezers can only be used to study the viscoelastic responses at low force in the linear regime [18]. Local heating and phototoxicity ("optication" as coined by Block) can result from the intense laser powers required to trap biological samples.

## 2.5. Magnetic probes

Another way to explore the viscoelasticity of cells involves manipulation of their movement through an externally applied magnetic field. The earliest application of this technique was pioneered by Freundlich and other researchers [12,27], in which magnetic particles were embedded in the material of interest (*e.g.* cells). The viscoelasticity can then be probed using magnetic tweezers or magnetic twisting. The ability to functionalize magnetic colloidal beads allows for their specific localization within the cell [28, 29].

The magnetic tweezer technique involves the manipulation of a super paramagnetic bead with an applied magnetic field generated by four pairs of soft ferromagnetic cores, each wound with a separate field coil arranged at special angles [30]. The movement of the bead is monitored by its induced magnetic dipole as it interacts with the field gradient of the strong magnet to which it is exposed. The corresponding displacement of the magnetic bead is used to measure cell properties. The movement of magnetic beads can also be controlled, albeit only in one direction, by a strong magnetic field gradient arising from electromagnets generated by axis-symmetrically arranged magnetic coils on a sharp iron tip [31]. Multiple pairs of electromagnetic tips are required for more complex movements of the magnetic bead, which for these experiments are smaller than the size of

the cell, and thus are limited to probing the viscoelastic response of a microenvironment rather than the whole cell.

Twisting magnetometry [32] and the more recently developed magnetic twisting cytometry [33] can also be used to measure the movement of magnetic beads, which usually consist of colloidal metal or polycrystalline iron oxide. The cell is deformed under a twisting magnetic field that is applied perpendicularly to the initial magnetic field once it has been turned off [34]. The change in the magnetic field direction causes reorientation of the magnetic bead towards the twisting field, and once both are turned off, the rate of magnetic bead rotation and the amount of recoil are measured to interpret local viscoelasticity.

## 2.6. Particle-tracking microrheology

In particle-tracking microrheology, fluorescent microbeads are injected into live cells and diffused randomly in their cytoplasm. These beads are so small (< 1μm) that their inertial forces are negligible and they move according to Brownian motion. The movement of the fluorescent beads can be observed by fluorescence microscopy, and route distance can be converted to bead displacement which is used to calculate frequency-dependent viscoelastic moduli and/or the creep compliance of the cytoplasm [14]. For particle-tracking microrheology of living cells, the applied deformation and resultant stress is not oscillatory and is used to probe the mechanical properties of adherent cells on planar substrates, showing strong elastic responses over short timescales but with dominant viscous responses over longer time periods [15].

Particle-tracking microrheology has been used to study the viscoelastic responses of live cells and their cytoplasm under pharmacological treatment, serum starvation and at the edge of tissue wounds, as well as the mechanical responses of their nuclei [35-37]. For these studies, target cells can be deeply embedded in a 3D matrix, a condition more similar to cells in their physiological environment and difficult to probe by other methods.

## 2.7. Atomic force microscopy

The advent of atomic force microscopy (AFM) provided a valuable tool to image cell surface structure at sub-nm resolution and to probe the global and local nano-mechanical properties of cells. Such a non-invasive method makes it possible to investigate live cells under physiological conditions. The key component of AFM is a sharp tip mounted on a cantilever (usually silicon or silicon nitride), which is raster-scanned over the sample surface by piezoelectric micropositioners (Figure 2). Lateral or vertical displacement of the cantilever is detected by a position sensitive photodiode, which signals the fast feedback loop to maintain a constant relationship (*e.g.* force or distance) between tip and sample and the computer which is used to generate an image of the sample surface. AFM can be operated in many different modes, including force spectroscopy (FS) which is used to probe the mechanical properties of the cell surface layer or whole cell [38].
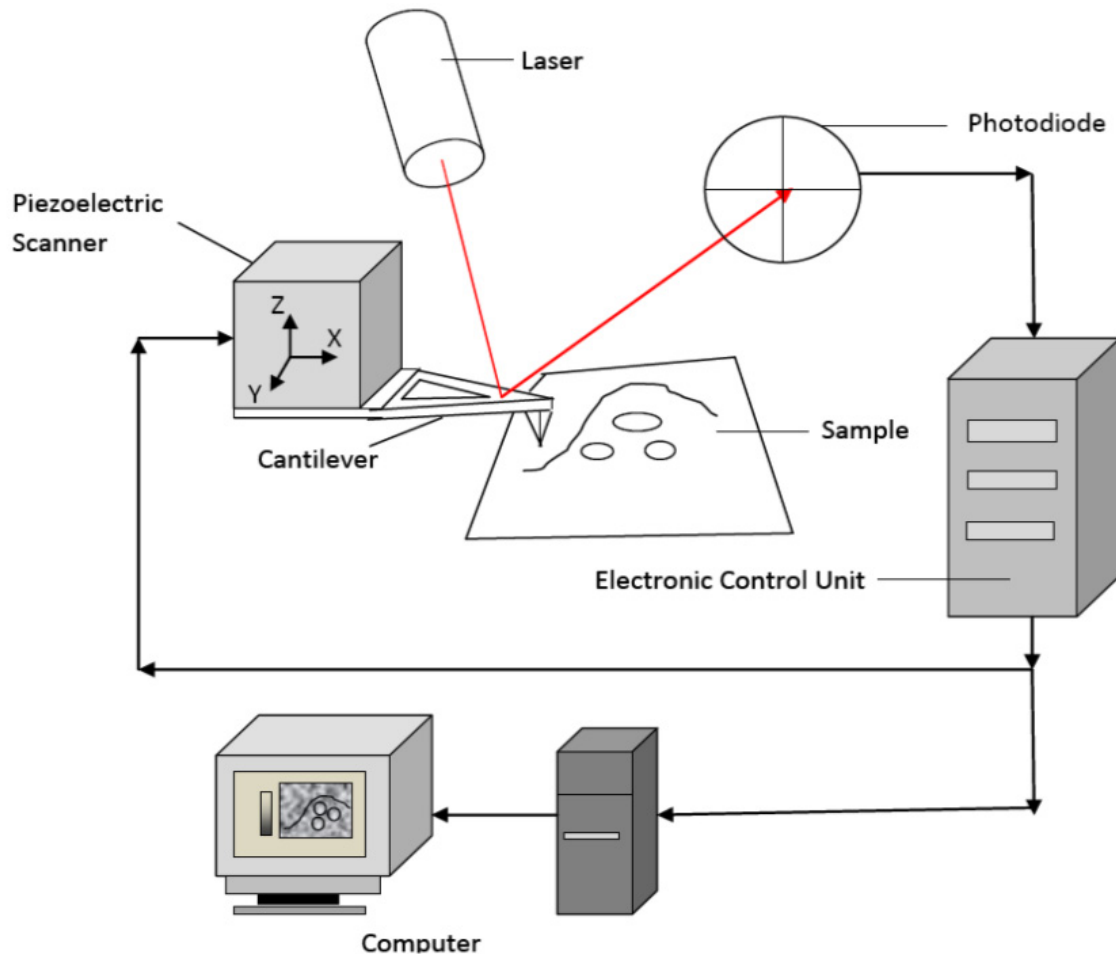
**Figure 2.** A schematic representation of the atomic force microscope. Printed with permission (Springer, USA).

AFM offers the further advantage of being able to correlate sample topography with mechanical properties across the sample surface using indentation forces as small as 10 pN. With force spectroscopy (single point) or force mapping (multiple points), the tip approaches the sample, indents the sample and then retracts at each point, generating a force versus distance curve at a specific point on the cell surface (Figure 3). Cantilever deflection as a function of distance of the tip from the cell surface is initially represented by photodiode voltage as function of piezo displacement. This voltage is then converted to cantilever deflection and finally a force or indentation distance. The extent to which the sample is deformed depends on its viscoelastic properties.

Cantilever deflection can be converted to force using Hook's law:

$$F = k \times d \tag{1}$$

where k is the cantilever spring constant, and d is cantilever deflection.

Force spectroscopy and mapping are used to quantify the mechanical behavior of the cell with the help of theoretical models. The indentation of the biological sample can be determined by subtracting the difference between cantilever deflection on hard surfaces and

on soft biological surfaces. Based on the Hertz model, Sneddon [40] developed a theory describing the relationship between loading force and indentation. Most commercially available AFM tips are either conical or parabolic, and hence these two types of AFM tips are considered during modeling. The relationship between loading force and indentation are given by following equations [41]:

$$F_{parabolic} = \frac{4E\sqrt{R}}{3(1-v^2)}\delta^{3/2} \tag{2}$$

$$F_{con} = \frac{2E\tan\alpha}{\pi(1-v^2)}\delta^2 \tag{3}$$

where R is radius of curvature for a parabolic AFM tip, $\alpha$ is the half opening angle of conical tip, $\delta$ is the indentation of the cell as a result of loading force F, 'E' is the Young's modulus of the sample, which describes the magnitude of elasticity and $v$ is Poisson ratio, which is assumed to be 0.5 for soft biological materials. The Young's modulus of microbial cells is determined from the non-linear portion of the force indentation curve with equations 2 or 3 [41].
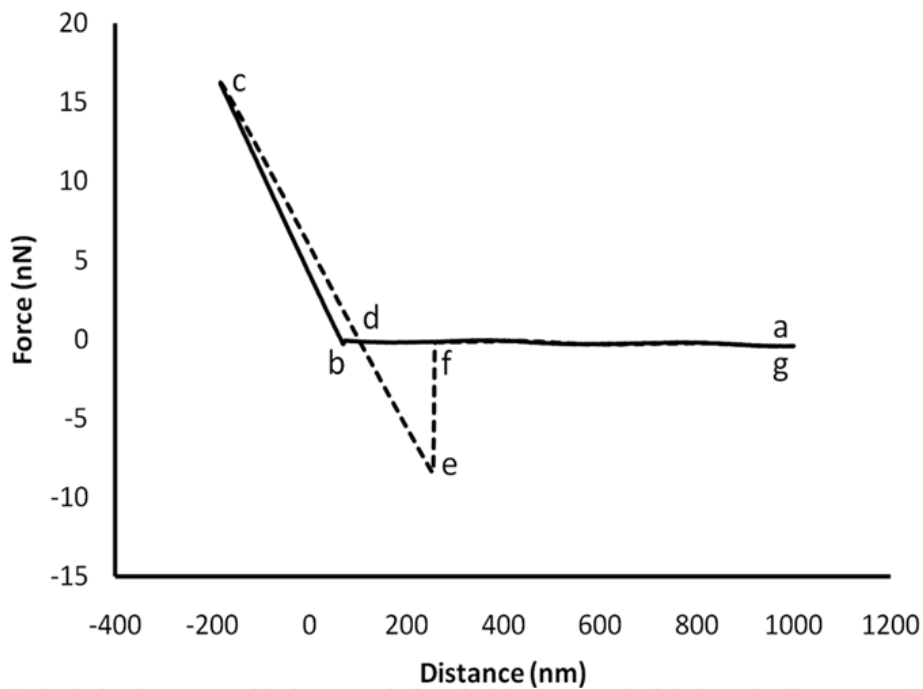


**Figure 3.** A representative force-distance curve taken on the surface of an *Aspergillus nidulans* cell wall. Solid and dashed lines represent approach and retract cycles respectively. Point b indicates jump into contact of the AFM tip to the sample. Section b-c represents the force required to indent the sample a given distance, and is used to measure cantilever deflection and to calculate sample indentation [39].

The spring constant of a fungal cell wall can be determined using the following equation:

$$k_w = \frac{K_c m}{1-m} \tag{4}$$

where $k_w$ is the spring constant of the hyphal cell wall, also called relative rigidity, $K_c$ is the spring constant of the cantilever and m is the slope of the approach curve, corrected for that of a hard surface. This equation can also be used to determine the spring constant of

cylindrical bacterial cells [42]. Models developed by Zhao et al. [20] can be used directly to calculate the Young's modulus of fungal hyphal walls. Fungal cell wall elasticity depends not only on the spring constant, but also hyphal radius (R), and cell wall thickness (h):

$$E = 0.8 \left(\frac{k_w}{h}\right)\left(\frac{R}{h}\right)^{1.5} \tag{5}$$

Since the slope of the approach portion of the force curve provides information on sample stiffness, the spring constant determined from the equation 4 can also be used to determine the elastic modulus of round-shaped fungal spores using the following equation [43]:

$$k_w = 2E\left(\frac{A}{\pi}\right)^{1/2} \tag{6}$$

where E is the elastic modulus of the spore and A is the contact area between the AFM tip and sample. The contact area between an AFM tip and spore sample can be determined from the following equation [44]:

$$A=\pi\left(2R\delta_p - \delta_p^{\,2}\right) \tag{7}$$

where $\delta_P$ is the indentation below the circle of contact calculated the from following equation:

$$\delta_P = \left(\frac{\delta_t - \delta_r}{2}\right) \tag{8}$$

where $\delta_t$ is the maximum indentation and $\delta_r$ is the residual depth of indentation. These values are determined experimentally from the force versus distance curve.

In the above section we have outlined how to quantify the elastic behavior of microbial cells, and most of the available literature describes bacteria elastic properties with Young's modulus. However, biological samples are not purely elastic but viscoelastic. Therefore, the microbial cell can be modeled as a combination of both properties.
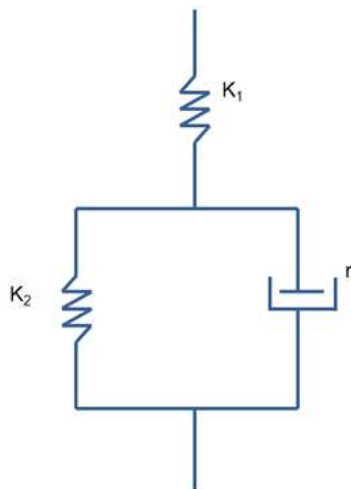


**Figure 4.** A schematic representation of standard solid model used to determine viscoelastic parameters. Adapted from [19] with permission.
$k_1$ is the instantaneous elastic response, $k_2$ is the delayed elastic response as a function of creep and $\eta$ is the viscocity.

Vadillo-Rodrigue et al. (2009) [19] explained the viscoelastic properties of bacterial cell walls using a standard solid model which describes both an instantaneous and a delayed elastic deformation. Based on this model they have derived the following equation that describes the experimentally obtained creep response data:

$$Z(t) = \frac{F_0}{K_1} + \frac{F_0}{K_2}\left[1 - \exp\left(-t\frac{K_2}{\eta_2}\right)\right] \tag{9}$$

where, $Z(t)$ is the position of the z piezoelectric transducer as a function of time $t$, $K_1$ is the spring constant that represents initial deformation, $K_2$ is the spring constant after creep response, and $\eta_2$ is viscosity.

The contribution of elastic and viscous components can be determined from the force-time curve taken at the center of cells when applying a constant force, $F_0$, for at least a 10 second period. Cantilever deflection is determined and using equation 1 is converted to force and then to an indentation-time curve, which is also called creep response. The indention of the cell over time at a constant force can be theoretically determined from equation 4 and fitted to the indentation-time curve shown in Figure 5. The experimentally determined data fit very well with the theoretical data obtained from the model. Microbial cells in particular exhibit two types of responses when a force is exerted on their surface. The first is the instantaneous linear relationship of the force versus distance curve, attributed to whole cell turgor pressure, while the non-linear region is thought to correspond to the response of the cell envelope.
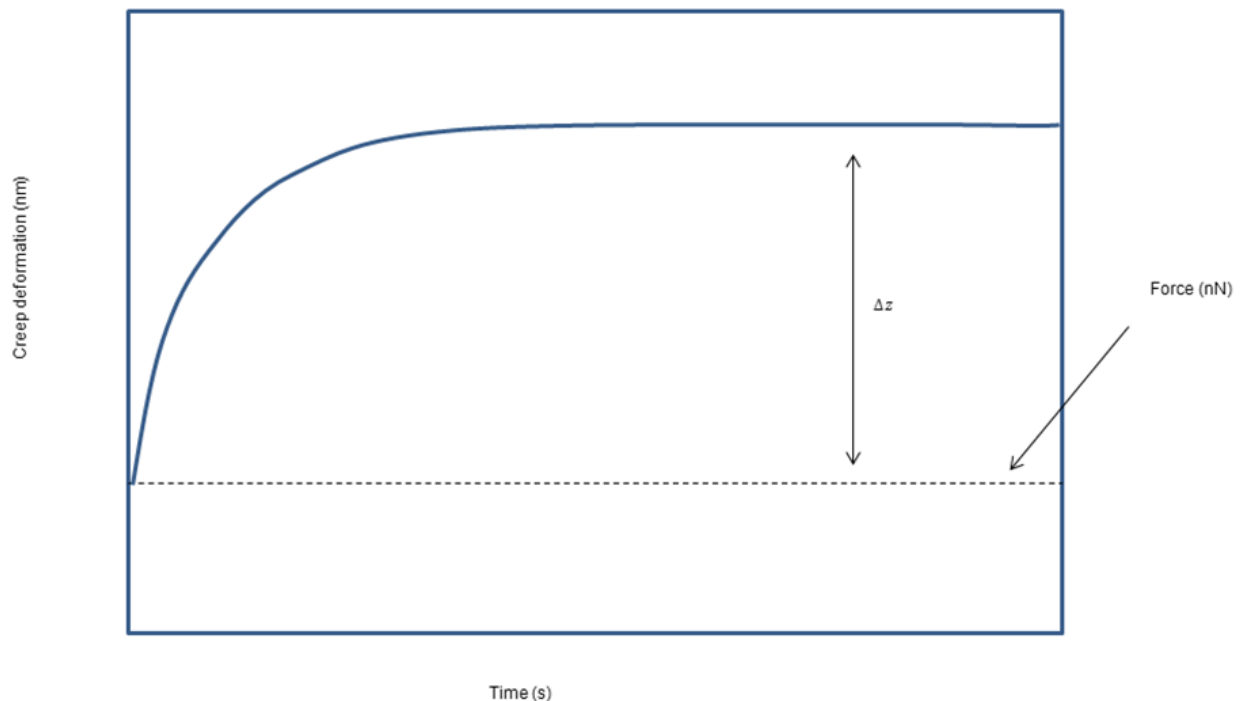


**Figure 5.** A typical creep deformation of an *Escherichia coli* cell at a constant force as a function of time. Adapted from [45] with permission.

AFM has been employed to measure the elasticity of a wide variety of cells ranging from bacteria, fungi, cancer cells, stem cells, osteoblasts, fibroblasts, leukocytes, cardiocytes developing embryos, cells at different cell cycle stages, and those treated with drugs. A broad spectrum of new measurements is possible by exploiting and manipulating the interaction between tip and sample in a quantitative way. Elasticity is most often measured with conical AFM tips. Spherical tips give rise to elasticity measurements 2-3 times that of conical tips, likely based on the large contact surface area. In comparison with other methods, AFM is more advantageous based on its ability to image the sample surface at high resolution while measuring an indentation map of the sample. The combination of imaging and force spectroscopy provides information about how cell surface structure affects elasticity and viscoelasticity. However, measurements depend on tip shape, which cannot be determined during sample scanning. Despite some limitations, AFM applications are rapidly developing. New instrumental designs and modification of the associated theoretical models will ensure an effective way to measure the elasticity and viscoelasticity for a wide variety of biological samples.

## 3. Viscoelastic cellular components and super structures

Although several methods have been developed to quantify cellular responses to deformation during locomotion, adhesion and mitosis, reliable tools are not available to quantify the distribution of mechanical forces between the various sub-cellular components [26]. Biological cells range in size between 1-100 μm and are comprised of constituents that provide mechanical strength, such as the cell envelope composed of multiple complex and distinct structures, cell walls composed primarily of polysaccharides interspersed with proteins, the cell membrane composed of phospholipid bilayers and membrane proteins, complex cell organelles of different sizes and shapes made of a variety of macromolecules, the cytoskeleton composed of microtubule networks, actin and intermediate filaments, other proteins and macromolecules such as DNA and RNA. The structure and function of each of these constituents may vary depending upon cell type. For instance, fungi are encased in cell walls, whereas bacteria have more elaborate cell envelopes with a peptidoglycan (polysaccharides cross-linked with peptides) layer and one or more cell membranes. Human cells, generally by virtue of being part of more elaborate structures, have only a cell membrane. It is not well understood how cells and their associated components sense mechanical forces or deformation, and convert such signals into biological responses [46].

The small size of prokaryotes, in comparison with larger eukaryotic cells, was a considerable obstacle in the development of methods for directly measuring their mechanical properties [47], solved largely by FS methods now routinely used. Cellular mechanical strength mainly relies on the outermost layers, such as the cell wall, envelope, or membrane, in addition to internal structural components such as the cytoskeleton. Extracellular components, such as those used to help form elaborate community structures (*e.g.* biofilms) also contribute to viscoelasticity and mechanical strength. There has been a major focus on the viscoelastic properties imbued to the cell by its cytoskeleton, which has been highly conserved

throughout evolution and influences not only internal cell dynamics but overall function. Nonetheless, external cellular components also play a significant role in mechanics despite their limited study.

## 3.1. Cell envelopes, walls and membranes

The bacterial (prokaryotic) envelope is a structurally remarkable cell component that defines a cell from its external environment and serves a protective function. The envelope also helps maintain cell shape and mechanical integrity and is responsible for important biological functions, such as the selective transfer of material in and out of the cell, and necessary changes accommodating cell growth and division. The envelope has a multilayer geometry which withstands positive turgor pressure exerted by the cytoplasmic fluid from the cell interior, and so protects the cell from osmotic swelling and burst. Thus quantifying the cell envelope mechanical properties of prokaryotes that contribute to their mechanical strength is of interest.

The mechanical properties of the cell envelope relate to its structural composition, comprised of distinct layers made up of polysaccharides, lipids and proteins (Figure 6). Structural differences in the two different types of prokaryotic cell envelopes led to the classification of two distinct groups of bacteria namely, Gram-positive and Gram-negative. The former is named for the Gram stain retained by the thick peptidoglycan layer outside the cytoplasmic membrane (Figure 6A), while the latter having a relatively thin peptidoglycan layer sandwiched between inner cytoplasmic and outer membranes (Figure 6B) does not retain Gram stain. Many researchers have studied the flexibility of the bacterial cell envelope [48-50], for which the majority of its viscoelastic nature is attributed to the peptidoglycan layer.

Cell stiffness, required to maintain bacterial shape, is dependent on the cell envelope. When the peptidoglycan layer is removed from rod-shaped whole cells by chemical treatment, soft, highly deformable and osmotically sensitive spherical cells (spheroplasts) are the result, indicating that the elastic response of cells is largely dominated by the peptidoglycan matrix [51,52]. In isolation, peptidoglycan is very flexible, exhibiting purely elastic properties with a modulus of 25 MPa [53]. However, bacterial cell envelopes show a time dependent response to externally applied forces, meaning that their overall mechanical properties are more accurately described as viscoelastic [45].

Cell envelope composition makes a major contribution to viscoelasticity. The cell envelope of the Gram-positive bacterium *Bacillus subtilis* is significantly stiffer than the Gram-negative *E. coli*, attributed to the difference in their peptidoglycan layer thickness. FS measurements of local viscoelasticity for live bacterial cells show that the time required for *B. subtilis* to reach asymptotic creep deformation is higher than that of the Gram-negative *E. coli* and that covalent crosslinking increases cell envelope stiffness. The same study also showed a difference in the deformability of wild type *E. coli* (*lpp+*) and its Lpp mutant *lpp-* (Lpp a major peptidoglycan-associated lipoprotein and one of the most abundant outer membrane proteins in *E. coli* cells). Thus lipopolysaccharides, peptidoglycan thickness, the bound form of the peptidoglycan–lipoprotein complex and stabilizing cations all play an important role in maintaining

viscoelasticity [19]. A micromanipulation study using optical fiber probes to test bursting strength also showed *E. coli* to have a weaker envelope than the Gram-positive *Staphylococcus epidermis*, more susceptible to mechanical stress [47]. Researchers thus attribute the elastic component of the cell envelope to the peptidoglycan layer and the viscous component to the liquid phase of the membranes [19,45]. The viscoelasticity of the bacterial cell envelope has also been shown to depend on its degree of hydration [19] and is thought to play an important role during cell division. During cell division, polymerization–depolymerization reactions in the FtsZ assembly cause softening and fluidization, reducing viscoelasticity and reflecting the more dynamic and active motion of individual FtsZ filaments in the lipid membrane [54].
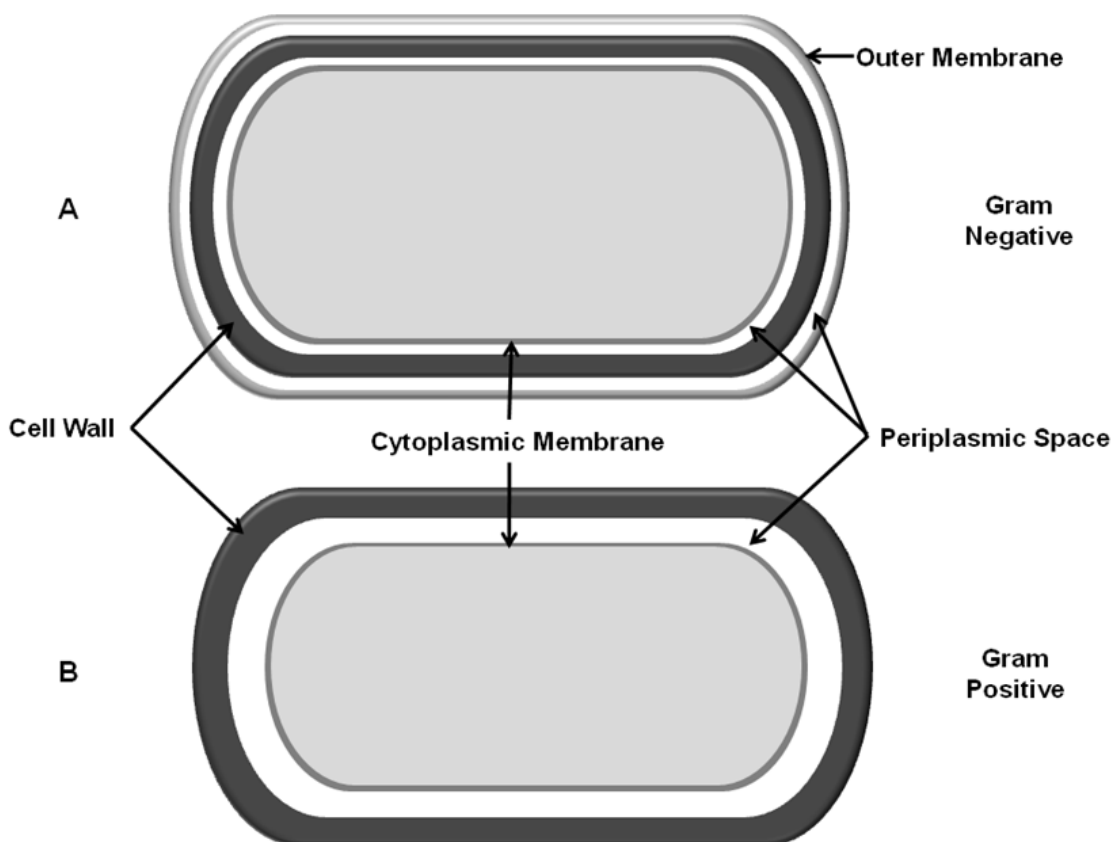


**Figure 6.** Simple models of (A) Gram-positive and (B) Gram-negative bacteria.

Earlier work on several fungi showed that the viscoelasticity of cell wall components allow for growth, cell division and spore germination [55-57]. The mechanical strength of fungal cells is largely attributed to their rigid but flexible cell walls which contain four major structural components: β-(1,3)-glucan, β-(1,6)-glucan, chitin (N-acetylglucosamine) and glycoproteins. The β-[1, 3]-glucan and β-[1,6]-glucan are more fibrous components whereas glycoproteins form a gel-like matrix, thought to impart viscoelasticity to the fungal cell wall. The mature cell wall has covalent cross-links formed between the chitin and glucan residues to provide the wall with sufficient strength and viscoelasticity. A study of the fungi *A. nidulans* showed that the immature hyphal wall region, having less well ordered wall components, and those devoid the cell wall component β-galactofuranose have lower viscoelasticity than wild type mature regions [58, 59] and this was conjectured to relate to reduced cross-linking. Determining the

mechanical properties for cell walls of live fungal hyphae is challenging [60] and so there have been few studies measuring their mechanical properties.

Cell wall mechanical properties of the budding yeast *Saccharomyces cerevisiae* were determined through compression in a micromanipulator. Individual cells were compressed between two parallel surfaces and elastic parameters extracted from the force-deformation data [52]. A FS study of *Termitomyces clypeatus* showed an increase in cell wall rigidity and elasticity until the organism reached the stationary phase, followed by a decrease in these parameters at the onset of the death phase. The adsorption of chromium was also found to significantly affect the surface nano-mechanical properties of *T. clypeatus* [61].

Spores, another morphological form of fungi, appear in several studies describing their surface morphology [56,62,63] and adhesive properties [3,64] however, little information is available regarding their mechanical properties. Changes in the cell wall mechanical properties are a key factor in the emergence of the germ tube [55,56]. Both rodlet-covered and rodlet-free spores of *A. nidulans* were subjected to nano-indentation measurements by FS in air, showing that the rodlet layer is significantly softer than the underlying portion of the cell wall [43].

The nano-mechanical properties of whole human cells has been a topic of great interest for the past few decades and the viscoelastic properties of epithelial cells [4,65], stem cells [66], red cells [67,68] and cancer cells [69,70] are well-studied but beyond the scope of this chapter. The microbial cytoskeleton is less well studied, so the next section highlights significant data from isolated cytoskeletal components and those in the context of human cells.

## 3.2. Cell cytoskeleton

The filamentous network inside eukaryotic cells is a major contributor to the 3D morphology of a cell, acting as a scaffold to support the cell interior (Figure 7). This cross-linked biopolymer network has a role in cell mechanics, resisting deformation in response to external mechanical stresses. Besides being the determining factor for cellular shape, the cytoskeleton is involved in cell division, cell movement, adhesion and locomotion. Cellular viscoelastic responses can be largely dependent on the cytoskeleton, composed of three major groups of elements: microtubules, intermediate filaments and microfilaments. With the tremendous progress in biophysics, the structures of each cytoskeletal components are now well understood even at the molecular level, but we are just beginning to determine their contribution to cell mechanics.

### 3.2.1. Microtubules

Microtubules are the largest of the filamentous structures making up the cytoskeleton, for which the basic building block is tubulin heterodimers made up of $\alpha$ and $\beta$ subunits. Structural analysis shows that the $\alpha$ and $\beta$ tubulins alternately line up to form proto-filaments, which are further laterally arranged into a small lattice and closed to form a 25 nm wide cylindrical structure [71]. The $\alpha$ subunit forms the end of the microtubule localized

to the centrosome, close to the center of the cell, and the microtubule grows out to peripheral regions of the cell ending with a β tubulin. The alternating α and β tubulins render the microtubule polar and its orientation guides the unidirectional movement of microtubule motor proteins from the kinesin and dynein families [72,73]. Microtubule aggregation is dependent on temperature and the relative amount of tubulin-GTP dimers [74]. The inherent dynamic instability caused by the hydrolysis of tubulin-GTP dimers puts microtubules in a state of continuous and rapid assembly and disassembly, depending on cell cycle phase. Given the limited generation of cellular microtubules, they are expected to play a minor role in cell mechanical responses and a major role in cell shape. Nevertheless, they do indirectly influence the mechanical properties of cells by regulating the actin network through myosin-II. In neural cells, axons, dendrites and microtubules play a more direct role in cell mechanics, because they form tight bundles in which microtubule-associated proteins (MAPs) bind and stabilize parallel arrayed microtubular filaments [75-77].

Experiments carried out on gels and cross-linked networks made of microtubules demonstrate that they exhibit the strongest bending stiffness among all the cytoskeletal components. Therefore, elasticity dominates over viscosity when microtubules are deformed under an external force. Under a low external load, microtubule network stiffness exhibits a linear elastic response as a function of microtubulin unit concentration [78], which closely relates to microtubule polymerization rate and the final microtubule length of tubulin dimers formed [79,80]. Fast growing microtubules are more likely to form short filaments, based on defects in the microtubule lattice that further influence the tubulin dimer bonds and make the microtubules more flexible. Microtubule viscoelasticity also depends on the intermolecular interactions between tubulin dimers, but their lateral and longitudinal interactions along the microtubule do not equally contribute to the total mechanical response. Both the shear and circumferential moduli of the longitudinal bonds in microtubule protofilaments are several orders of magnitude higher than those of lateral bonds. Since microtubules exhibit a mechanical response with enthalpic elasticity arising from the bending and stretching of microtubule filaments, factors influencing the tubulin spacings play a critical role in determining microtubule viscoelasticity [78]. Among these factors, the microtubule-associated proteins are an important contributor as they bind to the microtubule surface through electrostatic interactions. In the presence of MAPs, the spacing of adjacent microtubules is doubled creating a cushion against compression. There is also evidence to show that the elastic modulus increases with the addition of crosslinkers to the microtubule networks [81]. Factors affecting microtubule stiffness, such as taxol and GTP analogs, also influence their mechanical properties by stabilizing and preventing their depolymerization [82]. Therefore, microtubule viscoelasticity is a function of both subunit concentration and crosslinking.

### 3.2.2. Intermediate filaments

Intermediate filaments are the non-polarized cylindrical fibrils of the cytoskeleton named for their size with a diameter of around 10 nm, intermediate to microtubules and

microfilaments [83,84]. Intermediate filaments are constructed by a group of related proteins, which have been divided into five subgroups according to their sequence identity. All of the proteins associated with type I, II, III, and IV immediate filaments are localized to the cytoplasm, while those of type V (*e.g.* lamins) localize to the cell nucleus and form a network underneath the nuclear membrane. Similar to microtubules, intermediate filaments are composed of protein dimers, but in this case elongated ones.

Intermediate filaments have been discovered in diverse cell types, with more than 60 associated genes coded in humans [85,86]. They are found to be highly extensible compared to filamentous actin (F-actin) and microtubules, which are able to retain enormous strains while sustaining the intact filament structure [87,88]. Their unique extensibility implies their it could play a special role in cell mechanics, which would set it apart from other cytoskeletal elements. Since most genes associated with intermediate filaments are responsible for coding keratins, keratin-based intermediate filaments are considered an important player in cellular mechanics [89,90], regulating viscoelastic properties and the motility of cancer cells. The architecture of the keratin filament is regulated by phosporylation, accounting for the viscoelastic responses of carcinoma cells during large deformations, and the actin network is also regulated by phosphorylation under the regulation of kinases [91-93], phosphatases and other regulatory proteins [89,94].

The mechanical properties of intermediate filament networks have been studied by applying classical models to homogeneous and isotropical samples, which link the elastic shear modulus to the mean mesh size of the cytoskeleton [95]. This means that the viscoelastic response of the network is dependent upon its subcellular organization, filament composition, and overall protein concentration. The nonlinear relationship between the mesh size of these networks and the elastic shear modulus [96] underscores whole cell mechanical properties, elasticity and viscoelasticity of the network, which can be significantly changed by small perturbations [97]. In the non-polar network, the intermediate filaments branch in an attempt to adapt to the cellular demands of the micro-environment [98].

The crosslinking interactions between proteins making up intermediate filaments are postulated to be vital for cell mechanics, inspiring the study of the associated protein tail. Mutation of the desmin proteins at the filament tail causes slight changes in network flexibility, but does not have a significant effect on the mesh size or shear modulus [99]. The crosslinking interaction may be expected to rely on electrostatic interactions between filament proteins, and by extension the salt concentration in their local micro-environment. Indeed, an increase in cations enhances the stiffness of the intermediate filament network. In the case of small external stresses, the elasticity of the network shifts from the linear to nonlinear state as a function of stress magnitude. On the other hand, under greater stress the network has a modified nonlinear elasticity [100]. Intermediate filament networks with a greater number of bundles have higher persistence lengths and flexural stiffness, in contrast to those with a lower number of bundles, demonstrating the importance of bundle number and thus protein type on cell elasticity.
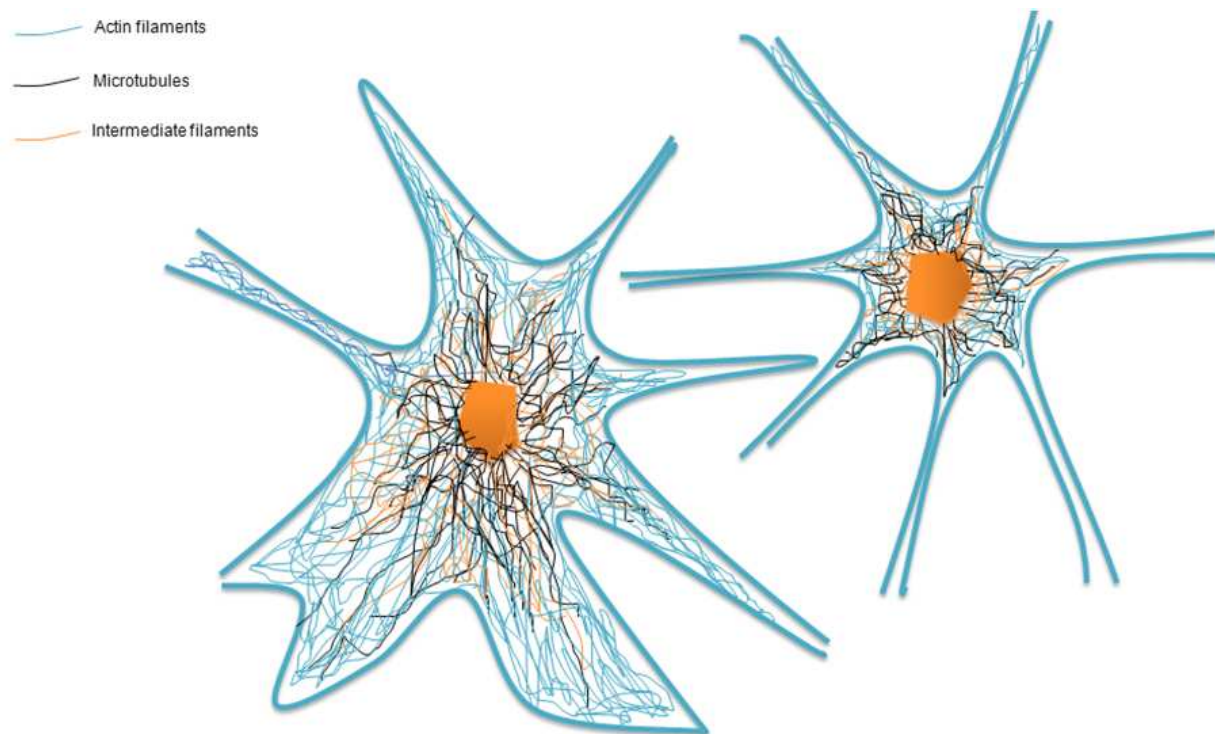
**Figure 7.** Schematic of a cytoskeleton as a dynamic 3-dimensional scaffold made up of actin filaments, intermediate filaments and microtubules.

### 3.2.3. Actin filaments

Actin filaments are the thinnest ($\approx$ 8nm) and most plentiful among all the components of the eukaryotic cytoskeleton [101]. Under physiological conditions, actin filaments grow by polymerization of the ATP-bound actin monomers, addition of monomers to the end of the severed filament fragments, or by branching out of the existing filaments. Actin filaments are polar fibers, with helically arranged actin monomers all pointed in the same direction, which can form a cortex underneath the cytoplasmic membrane to support cell movement. More than 100 proteins promote the formation of a single actin filament, filament cross-linking, networks and bundle formation. Actin filaments are continuously assembled and disassembled in response to cell signaling.

F-actin exhibits viscoelasticity as a semi-flexible polymer with linear and nonlinear responses to external forces [102,103]. To quantitatively evaluate the contribution of F-actins to cytoskeleton mechanics, such networks have been reconstituted *in vitro* with purified protein. The reconstituted F-actin forms a gel-like network in which the mesh size is dependent on the F-actin concentration [104]. Under a low applied force, semiflexible networks only composed of F-actins deform in a manner proportional to the force load, but under a larger force which affects F-actin filament contour length, the deformation can be described as strain-stiffening [95]. The mechanical properties of F-

actin filaments are affected by thermal fluctuations, which cause transverse bending, decreasing its end to end distance [105]. When an F-actin filament is under strain, fluctuations in the F-actin filament cause straightening with an associated consumption of energy. Thus, F-actin filaments exhibit an entropic elastic response. The magnitude of F-actin deformation reflects the reduction in the number of fluctuations in the actin filament [106].

Since the elastic response of F-actin filaments is length dependent, the concentration of actin and crosslinking proteins are closely related to the magnitude of the filament elastic modulus. The characterization of F-actin filament mechanical properties is also convoluted with actin concentration, the type and concentration of crosslinking protein, and the magnitude of the applied force. If the fluctuations are deconvoluted, the inherent elastic modulus of the filament dominates its mechanical response [106]. Crosslinking proteins covalently link individual subunits, allowing the actin filament to form a bundled structure with a larger diameter. Therefore, F-actin networks in combination with cross-linking proteins could behave differently from networks formed by only F-actins, the mechanical properties of which are more akin to the properties of cells. Once the actin filament becomes stiffer with the addition of crosslinkers, its elasticity dominates, and contributions from thermal fluctuations play a smaller role in the total mechanical response [106]. The entropic elasticity is reduced as actin and cross linker concentrations increase. In this case, the deformation of the actin filament depends on the bending and stretching of the filament itself [106]. The rigidly cross-linked actin filament has a more linear response and tends to break under small strains [107,108], and such filaments can sustain much larger stresses compared to pure F-actin networks, providing a reasonable explanation for their ability to resist a broad range of external stresses or internal tension.

## 3.3. Biofilms contribute to viscoelasticity

Biofilms have serious implications in industry, medicine and environmental systems making the study of their physical properties imperative to shed light on their growth mechanisms and adhesion properties, key factors in biofilm control. A biofilm is a surface-associated three dimensional consortia of microbial communities, surrounded by a matrix of protective biopolymers, macromolecular debris, sediment and precipitate, making it a highly complex mechanical, compliant, and viscoelastic structure. Biofilms can also be highly heterogeneous consisting of mixed populations of bacteria, fungi, protozoa [112] with interspersed pores and channels. Biofilms exhibit enormous resistance to external stress factors, with exopolysaccharides (EPS) contributing to their overall mechanical stability and enabling them to withstand external forces. Hydrodynamic forces can have a strong influence on biofilm formation, structure and thickness, EPS production, mass and metabolic activities [109-111]. Their dynamic structure ultimately affects how we view, model and study their mechanical behaviour. A schematic presentation of biofilm dynamic behavior is depicted in Figure 8.
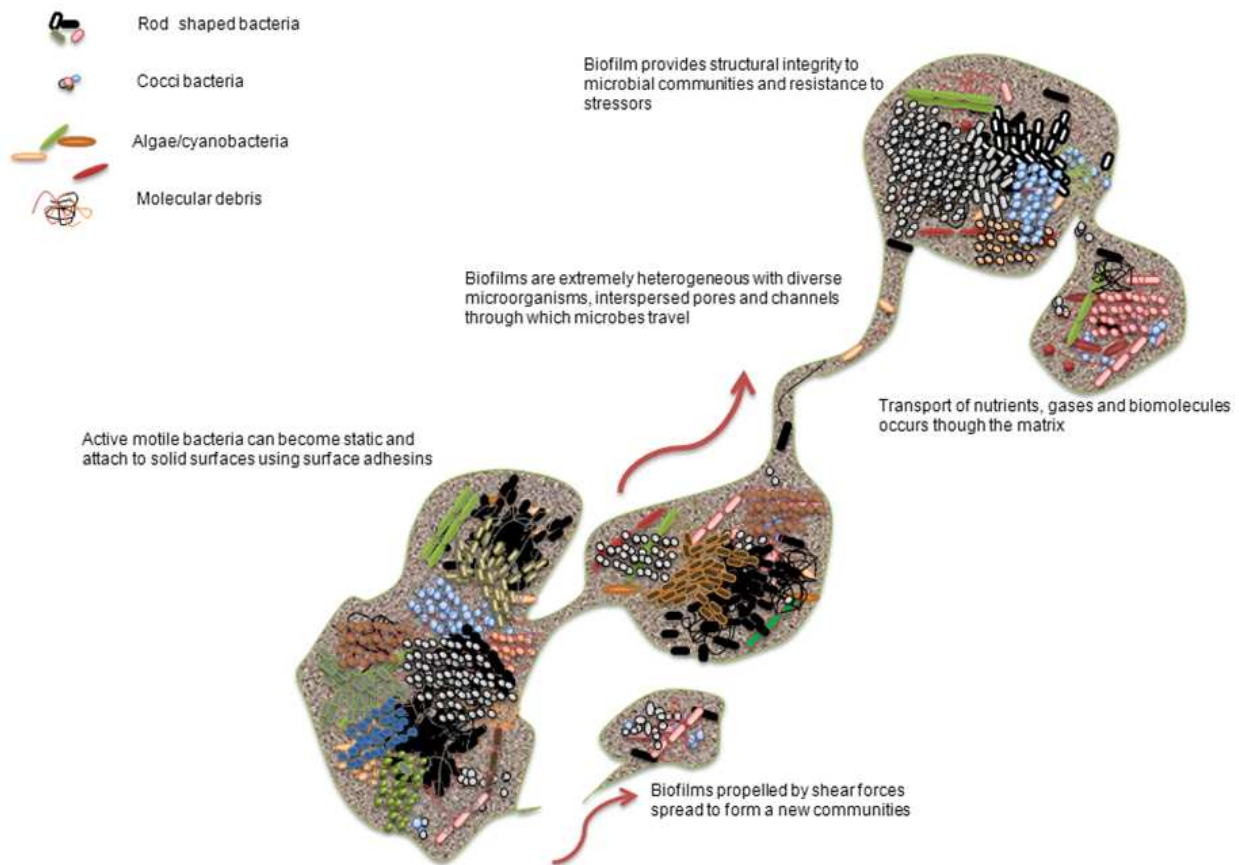
**Figure 8.** A model of biofilm growth and their dynamic behaviors.

Several attempts have been made to study the rheological properties of biofilms, yet they remain understudied, likely for the following reasons: (i) biofilms are extremely complex and heterogeneous with no defined geometry, making the use of conventional techniques difficult, (ii) their size and diverse nature make sample handling difficult and underscore the need to study biofilms in the natural environment to best represent their complexity (Figure 8). Studies have shown that biofilms formed under low shear conditions (laminar flow) are characterized by spherical micro-colonies divided by water channels [109]. It has also been shown that biofilms formed under higher detachment forces (*e.g.* shaking) produce more EPS to increase mechanical strength and to withstand shear stress [113].

A decade ago it was demonstrated that biofilms are effectively viscoelastic, a property that plays a major role in the various stages of biofilm growth [109,112,114-117]. Biofilms, all of which exhibit classic viscoelastic behaviours [109,115,116,118,119], can grow in a wide variety of environments including laboratory conditions, under flow and even in hot springs, demonstrating their ability to absorb elevated changes in shear stresses. Biofilms are elastic for shorter periods, can resist shear stresses and detachment, and over longer periods of time they flow as viscous fluids and become streamlined [109,112,114]. Viscoelasticity of the biofilm matrix has been shown to determine its structural integrity, resistance to stress, and ease of dispersion [120]. Simple stress-strain and creep tests of mixed culture and single species biofilms showed that the deformation of individual cell

clusters was related to the fluid shear stress, and that both mixed species biofilms and those from pure cultures behaved like viscoelastic fluids [114]. It has been proposed that the viscoelasticity of biofilms allows them to resist detachment as demonstrated for *Staphylococcus aureus* biofilms [117]. Lieleg et al. [121] showed that high shear stress may transiently fragment the biofilm but does not cause it to detach from the surface. This resilience has been attributed to the viscoelastic behavior of the bacterial biofilm. Biofilms of various *Pseudomonas aeruginosa* strains subjected to physical deformations in fluid were shown to be viscoelastic fluids, which behave like elastic solids over periods of a few seconds but like linear viscous fluids over longer times. Therefore in several studies, bacterial biofilms have been reported to behave as viscoelastic materials [38,122,123], while in other studies they are described as elastic [124,125]. Chemical perturbations can reduce biofilm viscoelasticity and hence slow down recovery to their original state [126].

Several experimental models have been generated to help understand the rheology and mechanical behaviour of biofilms. Rheology of undisturbed dental plaque biofilms made up of *Streptococcus mutans* and those from pond water showed a linear viscoelastic behaviour for which the Burger model was successfully applied to study creep compliances [127]. There are a large number of studies in the literature that have used different techniques to measure various material properties of biofilms, each of which provide information about their mechanics. Some of the most common methods used to measure the tensile strength of biofilms include cone and plate rheometry [128] and later the centrifugation method [125]. Particle-tracking microrheology has been successfully used to measure the strength of single species biofilms of *S. aureus* and *P. aeruginosa* [129]. The strength and apparent viscoelastic modulus of *P. aeruginosa* biofilms grown on membrane filters has also been investigated using a uniaxial compression experimental device and a film rheometer [118]. Directly applied and controlled loading forces have been used to quantify various biofilm viscoelasticity parameters, usually without a hydrodynamic flow, for example microbead FS [120], the micro-cantilever technique [130,131], indenters [122] or T-shaped probes [75], which are used to pull (tensile testing under a normal load) or push (compression testing under a normal load) the biofilm (see review 38 and references therein). On the other hand, there are several methods that use hydrodynamic loading, where biofilms are subjected to a fluid flow in flow cells [109,112,132], or Couette–Taylor type reactors [133,134]. Real time monitoring of cell growth and proliferation corresponding to viscoelasticity changes within a biofilm have been investigated in *Streptococcus mutans* biofilms using a Quartz crystal microbalance with a dissipation monitoring device [135].

Although there have been a large number of techniques developed to measure various biofilm parameters, a fully effective method is pending. The diverse magnitude of viscoelasticity parameters might reflect diverse biofilm properties, growth environments and source organisms. The viscoelastic properties of biofilms are adaptations to stress factors and shear forces, and are achieved through modifications to the secreted EPS. A better understanding of biolfilm mechanical properties and viscoelastic behaviours may inform effective strategies for biofilm removal or control.

## 4. Factors affecting viscoelasticity of biological materials

Cell components imparting viscoelastic properties to the entire cell are well studied, but there are very few examples in the literature in which external factors are shown to affect cell viscoelasticity. The structural integrity of biological systems is partially dependent on the degree of hydration. Humidity affects the mechanical properties of biopolymers, but the intensity of this effect depends on the type of biopolymer. For example, effects of humidity on cellulose are much less severe than on peptidoglycan. Thwaites et al. [136] demonstrated that the viscoelastic behavior of *Bacillus subtilis* depends on humidity, which affects the viscoelasticity of the peptidoglycan layer. Increasing humidity gives rise to cell wall hydration, allowing water to form hydrogen bonds with peptides and other cell wall components, making the cell wall more pliable [136]. Bacterial and fungal spores are very rigid under dry conditions, but increased humidity leads to removal of the outer spore hydrophobic layer, making it softer than under dry conditions and leading to changes in viscoelasticity and eventually germination [20, 136].

Cells also require optimal temperature conditions for their survival. Temperature affects the proper function and conformation of biomolecules, and hence indirectly affects cell mechanical properties. The rigidity of *E. coli* has been found to increase as a function of temperature, attributed to the folding of lipoproteins in the outer membrane leading to an increase in turgor pressure [136]. An earlier study showed that a temperature sensitive mutant of *B. subtilis* (indole⁻ and thymine⁻) was converted to a spherical shape from the wild type rod shape, attributed to loosening of the cell wall strength [137]. Hochmuth et al. [7] demonstrated that for red blood cells the time dependent viscoelastic behavior was reduced with increasing temperature.

The elastic behavior of the bacterial cell surface depends on the cell volume and ionic strength of the surrounding medium, which is related to its osmolarity. Abu-Lail and Camesano [138] observed that the elasticity of *E. coli* increased with reduced solvent polarity, and that bacteria in the least polar solvent have the highest Young's modulus [138]. Further, the spring constant of bacteria in a high ionic strength solvent of is higher than that in low ionic strength.

## 5. Summary

In summary, the viscoelastic properties of biological structures are responsible for their mechanical behavior which in turn is required for normal cell function. Viscoelasticity of whole biological cells is the combined contribution of cellular components, and several creative methods have been put forward to measure the associated parameters. Emerging experimental tools enable quantitative deformation studies of individual cells, biological polymers and macromolecules, which have led to understanding the relationship between mechanical properties and function. Nonetheless, studying the mechanical behavior of cellular components remains challenging. There are several theoretical models to determine the mechanical properties of cells and their components, but based on diverse findings and the different cell types used to determine viscoelastic parameters, it is difficult to compare

cell rheology measurements. There are uncertainties associated with the methods developed to determine mechanical properties, and any single method cannot be used for all cell types. To date, atomic force microscopy appears to be the most effective method for measuring the viscoelasticity of biological materials.

It is certain that cellular viscoelasticity plays a great role in normal cell function such as cellular homeostasis, cell-cell communication, stress adaptive mechanisms, tissue formation, and locomotive functions. The most basic requirement of cells is their mechanical strength, which has potentially led to the evolution of complex multicellular organization in higher animals and even molecular evolution in the most primitive prokaryotes. Vital components of any given cell, such as its envelope, cytoskeleton and EPS, are devoted to maintaining a unique viscoelasticity, making the significance of this property in biological systems of great importance. Considering the diversity of living cells in nature, viscoelasticity remains universal, making its study exceptionally important, but nonetheless the study of cellular viscoelasticity remains in its infancy. The contribution of the cell cytoskeleton to viscoelasticity remains the most well studied, but there are still unresolved issues regarding its contribution, such as how mechanical force propagates through the cell cytoskeleton without a change in its composition. Although it is widely thought that the mechanical characteristics of cell components are significantly affected by external physical factors, our knowledge in this area is inadequate.

## 6. Conclusions and outlook

Through constant adaptation and survival, cells have acquired sophisticated structures made up of simple biomolecules which have remarkable mechanical integrity. Recent progress in the development of novel experimental techniques provides almost unlimited opportunities in the field of cell mechanics. By applying the basic rheology principles of non-living materials to live cells we can establish strong connections between cellular mechanics and function. More emphasis on the viscoelastic materials of cells such as the cell membrane, wall, envelope, and elaborate structures adopted by multiple cells including biofilms and tissues, will provide further insight into their contribution to cell mechanics. Combining the powerful experimental techniques discussed in this chapter, the wealth of knowledge from biochemistry with theoretical models (not discussed here, see [139] for a review) will allow us to further explore the importance of cellular viscoelasticity. In future, the resolution of several remaining gaps will lead to a fundamental and novel understanding of cellular function associated with cytomechanics.

## Author details

Supriya Bhat, Dong Jun, Biplab C. Paul and Tanya E. S Dahms[*]
*Department of Chemistry and Biochemistry, University of Regina, Regina, SK, Canada*

[*] Corresponding Author

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