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Single-Cell Mechanics: Structural Determinants and Functional Relevance

Marta Urbanska[1,2,3] and Jochen Guck[1,2,4]

[1]Max Planck Institute for the Science of Light, Erlangen, Germany; email: mu272@cam.ac.uk, jochen.guck@mpl.mpg.de

[2]Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

[3]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

[4]Department of Physics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

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Abstract

The mechanical phenotype of a cell determines its ability to deform under force and is therefore relevant to cellular functions that require changes in cell shape, such as migration or circulation through the microvasculature. On the practical level, the mechanical phenotype can be used as a global readout of the cell's functional state, a marker for disease diagnostics, or an input for tissue modeling. We focus our review on the current knowledge of structural components that contribute to the determination of the cellular mechanical properties and highlight the physiological processes in which the mechanical phenotype of the cells is of critical relevance. The ongoing efforts to understand how to efficiently measure and control the mechanical properties of cells will define the progress in the field and drive mechanical phenotyping toward clinical applications.

Contents

Mechanical phenotype: integrated physical representation of the cell as a mechanical object emerging from genetic, molecular, and structural determinants

Mechanical properties: physical properties of a material that define how the material behaves in response to applied forces

Mechanosensing: the ability of cells to perceive mechanical cues from the environment, realized by various mechanosensors and corresponding signaling pathways

1. INTRODUCTION

The single-cell mechanical phenotype is a valuable indicator of the functional cell state. Its changes accompany many physiological and pathological processes, such as malignant transformation of cancer cells (18, 72, 210, 213, 222), leukocyte activation (9, 14, 65, 184, 219), and cell fate specification (48, 65, 226). From the clinical standpoint, the mechanical phenotype can be utilized as a marker for disease diagnostics (39, 51, 68, 71, 103, 154), for identification of cells of interest for their downstream use in transplantation (94, 188), or as a therapeutic target (119). In terms of fundamental research, mechanical characterization of cells adds a biophysical perspective to their behavior and contributes to the functional understanding of tissue-scale processes such as development (117) and cancer progression (28).

In this review, we give a brief introduction to the definition of mechanical phenotype and methods for measuring it, summarize the knowledge of the various molecular and structural contributors to the mechanical phenotype of cells, discuss the most prominent examples of functional relevance of cell mechanical properties, and conclude by giving an outlook on future directions and challenges in the field. We focus on the mechanical properties of isolated, single cells and do not cover tissue-scale mechanical properties and the way in which single cells contribute to them, nor do we discuss mechanosensing or cell responses to external loads such as stretching.

2. PARAMETERIZING AND MEASURING THE MECHANICAL PHENOTYPE

2.1. Single-Cell Mechanical Phenotype

The mechanical phenotype is an integrated physical representation of the cell as a mechanical object that emerges from the underlying genetic, molecular, and structural determinants.

It generally describes the extent to which cells resist deformation when subjected to external forces. Depending on the probing modality and the assumptions about the underlying material model, various parameters can be extracted from the measurements to describe the mechanical phenotype of cells. This includes material properties such as the elastic modulus or viscosity and the resulting relaxation timescales (125). In this section, we introduce very basic definitions of these parameters that will help the reader to navigate the review (for a thorough introduction to mechanical properties, see 96, 108, 125, 144, 186).

The elastic modulus (or Young's modulus), E, is a measure of the resistance of a solid to deformation, i.e., its stiffness, and is defined by how much stress, σ, is necessary to deform an object to a certain degree (the deformation is measured by strain, ε) (108). For a linear elastic solid exposed to uniaxial stretch, the following holds true:

$$\sigma = E\varepsilon. \quad 1.$$

Viscosity, η, describes the resistance of a fluid to deformation at a given rate or, in other words, its resistance to flow. Contrary to elastic solids, which respond instantaneously to imposed loads and recover their initial shape immediately after the load is released, the response of a viscous fluid is not reversible and evolves over time, with a rate determined by η (96):

$$\sigma = \eta \frac{\mathrm{d}\varepsilon}{\mathrm{d}t}. \quad 2.$$

For reference, elastic moduli of cells typically have values in the range of 0.1–10 kPa (131, 224, 241), and viscosities have values in the range of 1–1,000 Pa·s (2, 48, 56, 231). It is important to note that the absolute values of the mechanical properties depend not only on the measured cell type, but also on the measurement method (241), as well as the rate and the magnitude of applied stress (see the sidebar titled The Blessings and Challenges of High-Throughput Methods) (53, 156, 241).

Cells are viscoelastic materials; i.e., they exhibit both a fluid-like time-dependent viscous and a solid-like elastic response. They are often modeled as a combination of viscous and elastic elements called dashpots and springs, respectively (186), or as power-law materials (102).

Deformation: quantitative measure of cell shape change under application of force; typically quantified by image-derived shape parameters such as 1− circularity or aspect ratio

Elastic modulus: the resistance of an elastic solid to reversible deformation; describes how much stress is required to deform an object to a certain degree

Viscosity: the resistance of a fluid to deformation at a given rate; determines the rate at which the material is deformed under stress

THE BLESSINGS AND CHALLENGES OF HIGH-THROUGHPUT METHODS

Mechanical phenotyping at the throughput of conventional flow cytometry—enabled by microfluidics-based methods (225)—brings about unprecedented opportunities. Short sample processing times prevent sample deterioration during measurement, and the ability to process thousands of cells gives the opportunity to capture rare cell populations. These enhanced capabilities make mechanical phenotyping amenable to high-throughput applications, such as screening for mechanical regulators (185), and much more applicable to clinical settings, for example, in blood-based diagnostics (219).

These advances, however, come at the price of rapid force application, intrinsically linked to the short measuring time. The rate of force application can influence the magnitude of the mechanical properties—cells are nonlinear mechanical objects and appear stiffer when probed faster (53, 121, 251). Moreover, as shown in microrheology measurements, the relative magnitude of loss (viscous) and storage (elastic) moduli can be completely reversed at very fast probing rates (3, 139, 180). Since actin networks can be fluidized at high strain rates (95, 138), the fastest-operating DC methods become insensitive to the perturbations of the actin cytoskelton (225). Finally, it is important to consider that, for some processes, such as cell migration, the properties measured at very high rates might not be relevant—in these cases, the cell fluidity on second to minute timescales is likely more important.

In the simplest scenario, a typical timescale, τ, over which a viscoelastic object can be deformed is defined by the ratio between its viscosity and elasticity:

$$\tau = \frac{\eta}{E}. \quad 3.$$

Viscoelastic: displaying a combination of fluid-like, time-dependent viscous responses and solid-like elastic ones

The viscoelastic response of cells can be influenced not only by the material properties of the structural components, but also by the flow of liquid through the porous meshwork of these structures—a phenomenon called poroelasticity (145).

2.2. Brief Overview of Classical Methods for Measuring the Mechanical Phenotype

Over the past few decades, a rich variety of methods for probing the mechanical phenotype of single cells has been established (for a review, see 84; for a comparative analysis of commonly used methods, see 225, 241). These methods can be categorized based on whether they probe the mechanical properties globally or locally, whether the probing is performed inside the cells or by applying stress from the outside, whether adherent or suspended cells are measured, and whether a step or oscillatory probing signal is applied (**Table 1**). In this review, we focus on the

Table 1 Methods for single-cell mechanical phenotyping

Method	Scale		Cell state		Applied mechanical signal			Applied force	Throughput
	Global	Local	Adherent	Suspended	Step	Oscillatory	None		
Micropipette aspiration	×	×	×	×	×	–	–	pN–μN	≤10 cells/h
Atomic force microscopy	×[a]	×	×	×[a]	×[b]	×[c]	–	pN–μN	≤40 cells/h[d]
Optical stretcher	×	–	–	×	×	×	–	pN–nN	10–100 cells/h[d]
Parallel-plate rheometry	×	–	×	×	×	×	–	nN–μN	≤10 cells/h
Magnetic twisting cytometry	–	×	×	–	–	×	–	pN–100 nN (166)	~100 cells/h
Particle tracking microrheology	–	×	×	–	–	–	×	None	~30 cells/h
Optical tweezers	×[e]	×[f]	×	–	×	×	–	fN–500 pN (166)	≤10 cells/h
Brillouin microscopy	–	×	×	×	–	–	×	None	1–200 cells/h[g]
Acoustic microscopy	–	×	×	×	–	–	×	None	~10 cells/h
Deformability cytometry	×	–	–	×	×	–	–	pN–μN	1–10,000 cells/s

[a]Wedged cantilever.
[b]Indentation.
[c]Microrheology.
[d]Based on the authors' experimental experience.
[e]Uniaxial displacement.
[f]Twisting.
[g]The lower bound corresponds to conventional implementation, and the upper bound corresponds to a line-scanning variant integrated with microfluidic sample delivery, introduced by Zhang et al. (248).
The estimates of applied forces and throughputs are based on Reference 84 unless otherwise indicated.

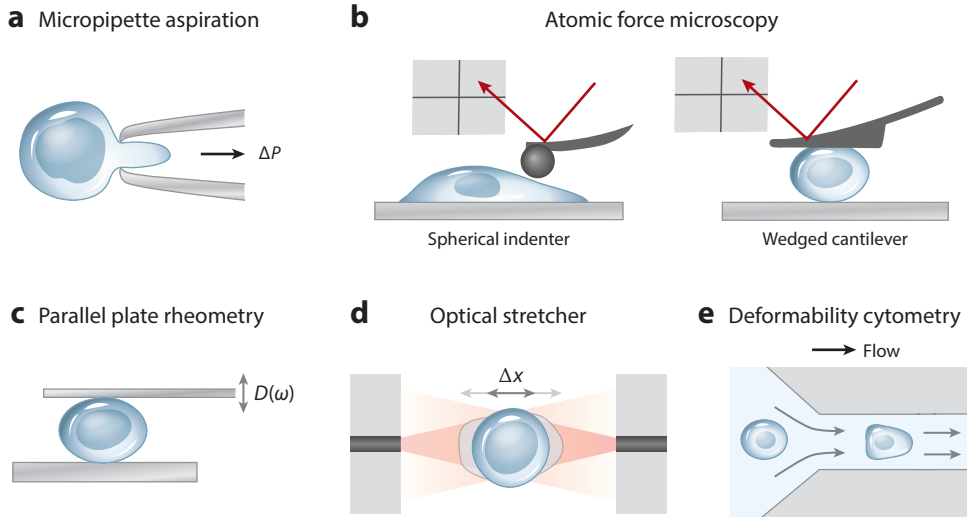

Figure 1

An overview of methods for measuring global mechanical properties of single cells. (*a*) Micropipette aspiration. A part of the cell is aspirated into a narrow capillary by a pressure difference, ΔP. Cell surface tension, bulk cell elasticity, and viscosity can be extracted by relating the aspirated cell extension and pipette geometry to the applied pressure. (*b*) Atomic force microscopy. A calibrated cantilever is used to indent the cell surface. The extent of the deformation is traced based on the position of the laser beam deflected from the cantilever surface onto a position-sensitive photodiode. The measurements can be conducted on adherent (*left*, in this case using a spherical indenter) or rounded (*right*, typically using a wedged cantilever, as depicted) cells. (*c*) Parallel plate rheometry. Compressive stress is applied to the cell by actuating the upper plate. $D(\omega)$ indicates oscillating displacement. (*d*) Optical stretcher. Two divergent, counterpropagating laser beams are used to trap and deform the cells. Δx indicates cell extension in the x direction. (*e*) Deformability cytometry. Cells are deformed by hydrodynamic stresses in a microfluidic channel. Figure adapted from Reference 223 (CC BY 4.0).

results obtained with methods that measure mechanical properties on the level of the whole cell (**Figure 1**).

The classical methods for probing the global mechanical properties of cells include micropipette aspiration (91), atomic force microscopy (AFM)-based indentation (175), parallel-plate rheometry (216), and optical stretching (69, 70) (**Figure 1*a–d***) (for a comparison of these methods, see 241). While many of these methods enable time-resolved measurements and extraction of full rheological properties, their throughput is limited to a few cells per minute (see **Table 1**). Additionally, their applicability beyond specialized laboratories is restricted by the need for expensive equipment and expert knowledge.

2.3. The Advent of High-Throughput Microfluidic-Based Approaches

Recently introduced microfluidics-based deformability cytometry (DC) approaches (**Figure 1*e***) overcome this bottleneck by offering astounding throughputs of up to thousands of cells per second and comparatively simple handling procedures (35, 65, 160, 225). While the traditional methods for measuring cell mechanics were instrumental in establishing basic ideas in the field, the new high-throughput methods are bringing these ideas toward the realm of applications and are fundamentally transforming the way cells are mechanically characterized (see the sidebar titled The Blessings and Challenges of High-Throughput Methods for a discussion of the unique opportunities and challenges of rapid mechanical phenotyping).

Deformability: ability of cells to change shape under force; quantified by cell shape parameters or an inverse of passage time through narrow constrictions

Deformability cytometry: a family of microfluidics-based high-throughput methods for measuring the deformability of cells

Migration: directional cell translocation in tissue that requires active generation of forces by the cell

There are several variants of DC that differ with respect to channel design, detection modality, and magnitude and type of applied stress, as well as the timescale at which the cells are deformed (for more details, see 225). In DC, a snapshot of maximum cell deformation can be used for real-time extraction of the elastic modulus of cells (142, 146, 160, 238a). More complete rheological information about the cells, including their viscosity, can be extracted from time-resolved measurements (56, 60, 112, 156, 238). Furthermore, the latest generations of DC offer a combination of mechanical readout with assessment of fluorescence (151, 185), integrated artificial intelligence–based image analysis (104, 153), and sorting capability embedded downstream of the analysis (152, 153).

3. INTERNAL STRUCTURES DETERMINING CELL MECHANICS

One of the central challenges in the field of cell mechanics is gaining a detailed understanding of the structural contributors to the mechanical phenotype. Such an understanding would guide the development of means for tuning the mechanical properties of cells on demand. Historically, the most-studied role in providing the mechanical robustness of cells has been that of the cytoskeleton (55). However, other organelles, cell surface organization, and intracellular packing can also contribute to the measured mechanical properties, and there are likely plenty of other players that remain to be identified. Below, we summarize the current knowledge about cellular constituents with known contributions to the mechanical phenotype of cells.

3.1. Cytoskeleton

The cytoskeleton is an interconnected network of protein filaments that gives structural stability and mechanical resilience to the cell (55). Its three major constituents are actin, intermediate filaments, and microtubules—each of which have unique properties, architecture, and distribution within the cell (**Figure 2**).

The actin network, as well as its contractility regulated via Rho signaling, is recognized as the most prominent contributor to the global mechanical phenotype of the cell (97). In suspended cells, actin is mostly organized into a thin, cross-linked meshwork underpinning the plasma membrane—the actin cortex (34) (**Figures 2*a*** and **3*a*,*b***). In adherent cells, actin additionally forms bundled cables spanning long distances—the stress fibers (220). The actin network is generally under tension generated by myosin motors. This tension, as well as the overall network stiffness, is further influenced by the network architecture: the amount of passive cross-linkers (e.g., α-actinin, fascin), actin nucleators (e.g., formins, Arp2/3), and actin–membrane linkers that connect the actin cortex to the plasma membrane (ezrin, moesin, and radixin, or myosin I protein families) (34, 101). On the regulatory level, actomyosin contractility is primarily governed by the signaling cascades mediated by the Rho GTPase RhoA and its downstream effector Rho-protein kinase ROCK (97) (**Figure 3*c***). Further Rho-family proteins, such as Rac and Cdc42, are involved in the regulation of the structure and branching of the actomyosin network (80). Destabilization of the actin cytoskeleton with chemical agents, such as cytochalasins or latrunculins, drastically reduces cell stiffness in both adherent and suspended cells (6, 63, 76, 156, 167, 230). Similarly, the inhibition of actomyosin contractility with myosin inhibitors, such as blebbistatin, or ROCK inhibitors, such as Y27632, results in a change of cell stiffness and surface tension (23, 54, 137, 218). When adherent cells are brought into suspension, the actin cytoskeleton reorganizes and the Rho signaling is triggered, leading to an increase in contractility and a consequent decrease in cell deformability over the course of approximately 30 min (136, 157). Optogenetic control of RhoA activation can be leveraged to locally control actin contractility (237). To date, such optogenetic tools have been exploited to study the influence of contractility on cell migration (86, 227), traction force generation (158, 227), cleavage furrow formation (229), and control of morphogenetic processes

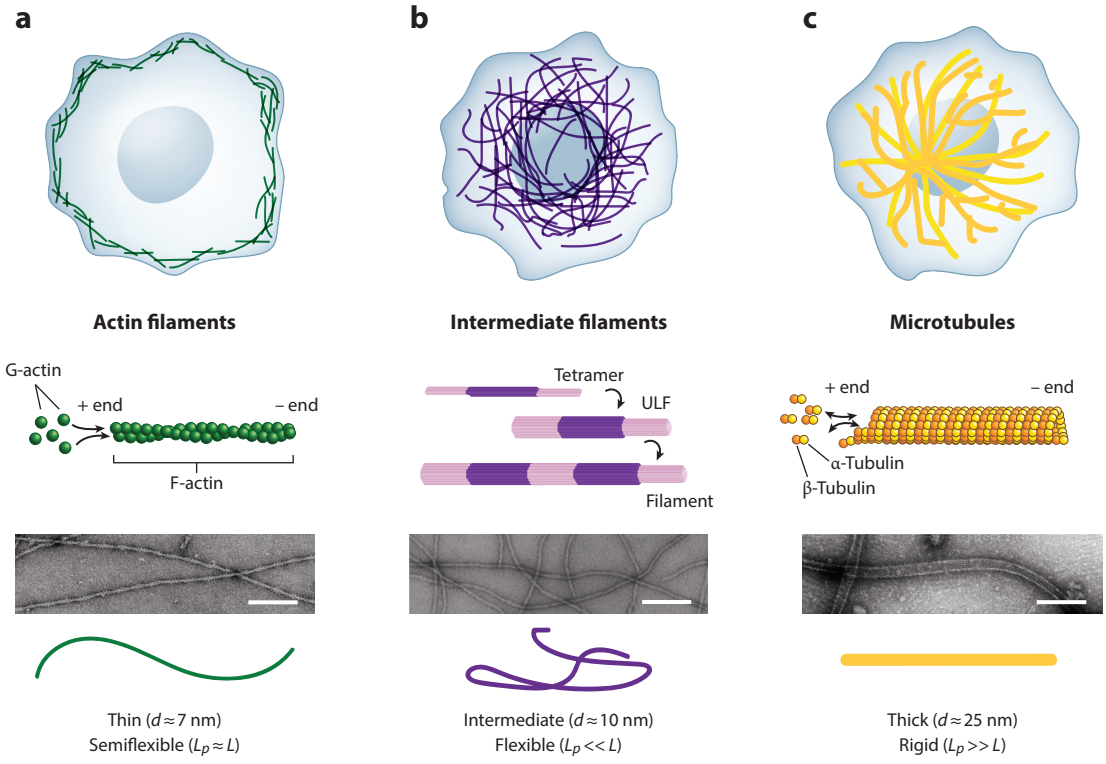

Figure 2

The three main cytoskeletal networks within the cell. (*a*) Actin filaments. (*b*) Intermediate filaments. (*c*) Microtubules. Shown are (from top to bottom): the typical distribution of the respective cytoskeletal filaments within a cell, the structure of the filaments, the transmission electron micrographs of their morphology when reconstituted in vitro, and the visualization of the relative flexibility of the filaments. Scale bars: 100 nm. Figure adapted from Reference 223 (CC BY 4.0). The embedded electron micrographs are adapted with permission from Reference 88. Abbreviations: *d*, diameter; *L*, contour length; L_p, persistence length; ULF, unit length filament.

(21, 93). Their implementation for the study of mechanical properties of cells remains to be explored.

Intermediate filaments, particularly vimentin and keratins, have been demonstrated to contribute to the mechanical properties of cells, especially at high strains (60, 78, 141, 165, 194, 232) and after actin depolymerization (60). Intermediate filaments form a dense meshwork located deeper inside the cytoplasm than the actin cortex (88). This meshwork, surrounding the cell nucleus and spreading toward the cell periphery, has been shown to protect the genetic material contained within the nucleus from damage during passage through narrow constrictions (165). Compared to actin and microtubule networks, which break at 20% and 50% strain, respectively, when reconstituted in vitro, intermediate filament networks withstand very high strains and demonstrate strain-stiffening behavior (27, 95). These properties support the notion that intermediate filaments have a load-bearing function at large cell deformations and protect the nucleus from structural damage.

In addition to the cytoplasmic intermediate filaments, a special class of intermediate filaments called lamins, comprised of A- and B-type lamins, is involved in the formation of the nuclear lamina that underlays the nuclear envelope (67). The nuclear lamina is connected to the cytoskeleton via the linker of nucleoskeleton and cytoskeleton (LINC) complex (100). The levels of lamins-A/C

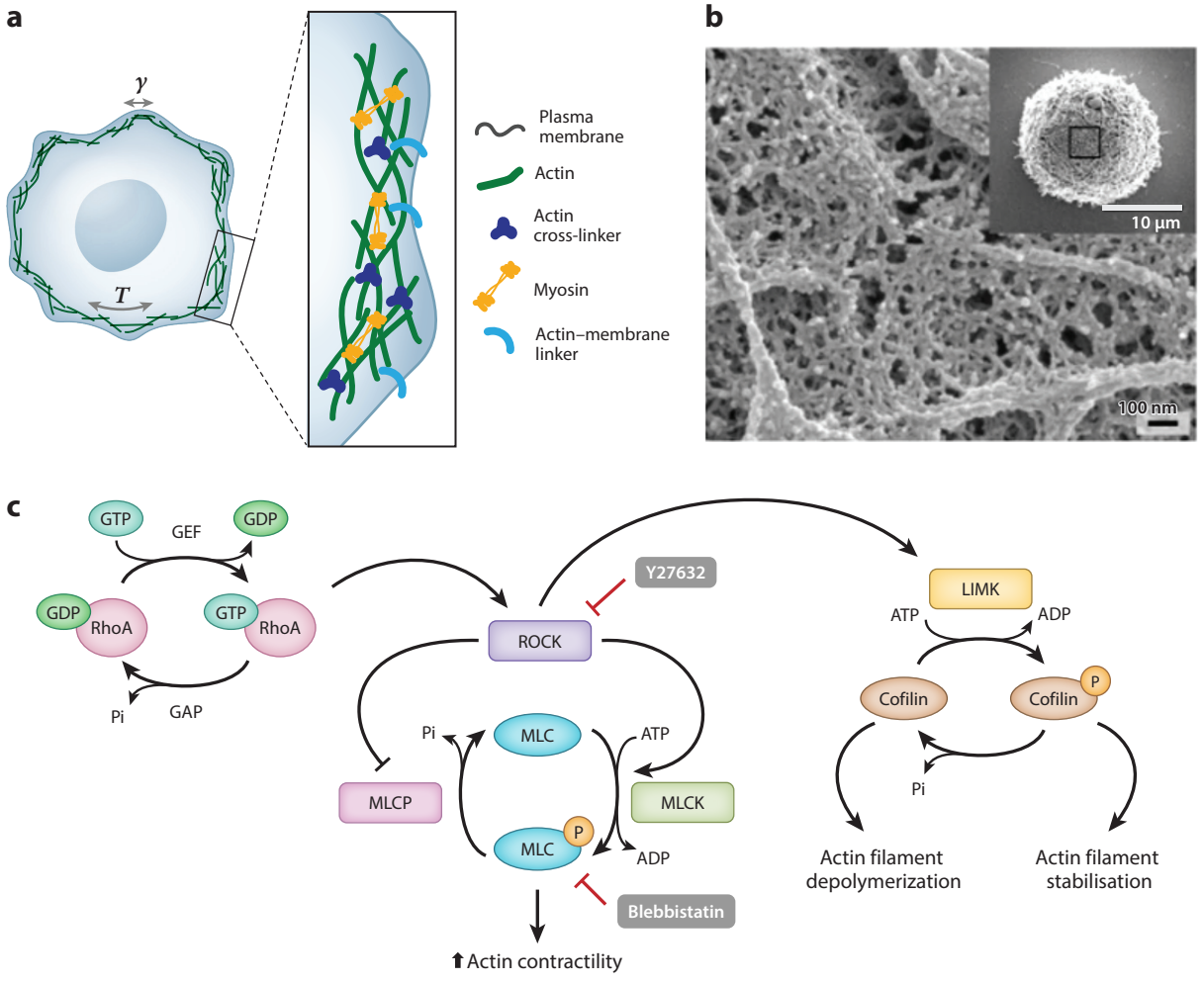

Figure 3

Mechanical properties of the cell surface are dominated by the actin cortex and its contractility regulated by the Rho-ROCK signaling pathway. (*a*) The actin cortex (*green*) directly underlies the plasma membrane and is under tension, T, due to contractility generated by myosin motors. The membrane tension, γ, is small compared to T. The inset on the right depicts actin-interacting proteins and the membrane–cortex attachments responsible for the network architecture. Panel adapted from Reference 223 (CC BY 4.0). (*b*) Scanning electron micrographs of the actin cortex of a membrane-extracted interphase HeLa cell. Panel adapted with permission from Reference 33. (*c*) The regulation of actin contractility via the Rho-ROCK signaling pathway. The small GTPase RhoA is active when associated with GTP. The cycling between its active and inactive states is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). ROCK, the effector protein of RhoA, increases the phosphorylation of the myosin light chain (MLC) in two ways: by directly phosphorylating the MLC, in parallel with MLC kinases (MLCKs), and by inhibiting MLC phosphatases (MLCPs). The phosphorylation of the MLC leads to an increase in actin contractility. ROCK also has a stabilizing effect on actin filaments through activation of LIM domain–containing protein kinase (LIMK), which inactivates the actin-severing protein cofilin by phosphorylating it. The commonly used myosin inhibitor (blebbistatin) and ROCK inhibitor (Y27632) are indicated in gray boxes. The schematic is based on concepts presented in References 80 and 179.

were shown to reflect the stiffness of the tissue of origin (211) and the level of cell contractility (200). Furthermore, perturbations of nuclear laminas and the constituents of the LINC complex result in compromised cellular stiffness (13, 83, 111, 118) (the contributions of the nucleus are further discussed in Section 3.3).

Microtubules withstand compressive forces generated by actomyosin contractility and the environment (12, 122). They are comparatively stiff, straight rods that constitute tracks for intracellular transport and are typically organized into a branched network spreading from the cell center to the cell periphery (**Figure 2*c***). Although there is some evidence of the contribution of microtubules to cell stiffness at high strains (107), their role has been difficult to address directly because pharmacologically induced microtubule disassembly causes a reinforcement of the actin cytoskeleton and cell contractility (26), obscuring the direct effect of microtubule disassembly on cell stiffness.

Beyond these three canonical networks, depletion of septins—increasingly recognized as the fourth cytoskeletal component (148)—has been shown to reduce cell stiffness in cultured cells (149). In nondividing cells, septins form a filamentous network at the cell cortex that is involved in functions such as bleb retraction and lateral compartmentalization of the plasma membrane (20, 62, 148). Septins are known to interact with membranes and other cytoskeletal networks (202) and have a role in maintaining cell shape and cortical stiffness (61). More extensive insights into their role in cell mechanics have yet to be established.

There is growing evidence suggesting that the cytoskeletal networks interact more universally with one another via direct physical links, as well as at the regulatory level, than previously thought (25, 41, 202). Thus, a holistic approach to studying the impact of the entire cytoskeleton on cell mechanics is desirable. It is also important to acknowledge that the cytoskeleton is a rather dynamic structure that turns over at the expense of energy on the timescale of seconds to minutes. Thus, mechanically, it contributes an apparent elastic stiffness at short timescales but behaves like an active liquid on longer timescales. This becomes relevant in the discussion of the functional relevance of cell mechanics in Section 4.

3.2. Plasma Membrane Structures

Apart from the cytoskeleton, another prominent structural component of the cell surface is the plasma membrane: an asymmetric lipid bilayer composed of a fluid mosaic of lipids and proteins (235). Reconstituted lipid bilayers are easy to bend (their bending modulus is on the order of 10^{-19} N m^{-1}) but very difficult to stretch (their area expansion modulus ranges from 0.1 to 1 N m^{-1}) and typically rupture at 2–4% lateral extension (81, 193). The plasma membrane, however, is not a flat lipid bilayer, but instead an extensively wrinkled surface with actively maintained topological features such as membrane folds, caveolae, clathrin-coated pits, blebs, microvilli, and membrane ruffles (58, 115) (**Figure 4**). The membrane excess gathered in these structures can buffer an increase in the membrane tension and account for up to fivefold volumetric expansion of cells (corresponding to twofold surface area expansion). Further membrane reservoirs, accommodating a total of up to 10-fold volume increase, are available for recruitment from intracellular membranes via exocytosis (66). In particular, caveolae, characteristic cup-shaped invaginations formed by the membrane proteins caveolin and cytoplasmatic cavin, have been shown to react robustly to membrane tension increase and act as a first responder when cells are subjected to stress (199). It is noteworthy that the functions of caveolae, and caveolins in particular, are multifaceted, as apart from structuring the membrane, they also engage in signaling functions and cross-talk with cytoskeletal networks (44, 163). In recent studies, caveolin-1 has been shown to affect cellular stiffness (114, 126, 129, 224). Whether this effect is connected to the structural functions of caveolin in caveolae formation or to its involvement in signaling remains to be resolved.

The effective tension of the plasma membrane in nonstressed cells ranges from 0.03 to 0.3 mN m^{-1} (115) and is dictated by an interplay between in-plane tension in the lipid bilayer and the contributions from the connections between the membrane and the underlying cytoskeleton, as well as by the intracellular pressure (58). Plasma membrane tension is important for regulating processes such as vesicular trafficking and the signaling connected to it (58). With respect to the

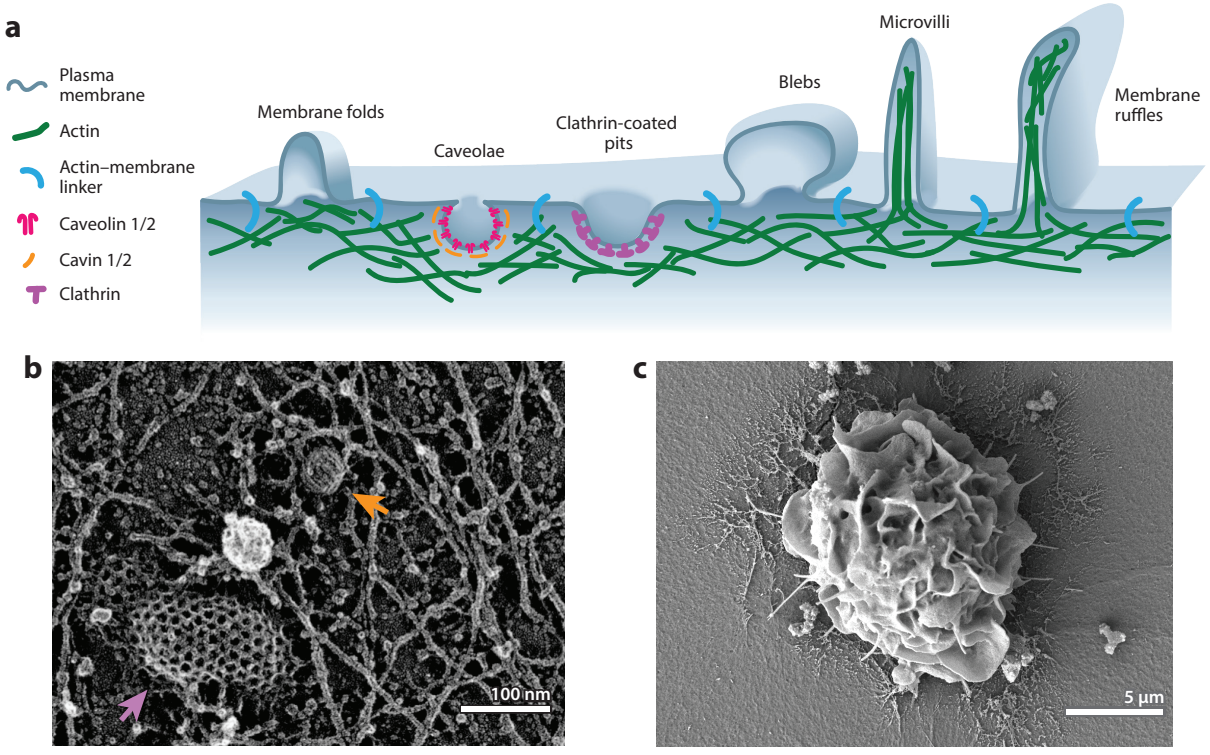

Figure 4

Cells maintain membrane reservoirs in topological structures. (*a*) A schematic overview of the structures through which the cell maintains membrane reservoirs. Panel adapted from Reference 223 (CC BY 4.0). (*b*) Scanning electron micrograph of membrane invaginations (caveola: *orange arrow*; clathrin-coated pit: *purple arrow*) on the cytoplasmic surface of the plasma membrane of a rat keratinocyte. The fibrous network is the actin cytoskeleton. Panel adapted with permission from Reference 147 (CC BY-NC-SA 4.0). (*c*) Scanning electron micrograph of membrane ruffles on the surface of a mouse macrophage. Panel adapted from Reference 52 (CC BY 4.0).

global mechanical properties of the cell surface, plasma membrane tension is considered to be dominated by the cortical tension (40, 218).

In terms of altering the plasma membrane composition, recent studies have shown that depleting cholesterol from cells with methyl-β-cyclodextrin (MβCD)—in contrast to the effect observed on membranes—causes an increase in cell stiffness (15, 17, 119) and membrane tension (10, 99). Markedly, the increase in stiffness upon MβCD treatment was corroborated by AFM, optical tweezers, and DC measurements on murine B16F10 and human Me275 cancer cells (119); AFM measurements on HEK cells (15); and micropipette aspiration of bovine aortic endothelial cells (17). An increase in the cholesterol levels, in turn, had no effect on mechanical properties in bovine aortic endothelial cells (17) but was reported to cause a decrease in cell stiffness (119) and membrane tension (99) in other cell lines. It is unclear whether the change in stiffness is caused by the increase in the cholesterol level or by secondary effects connected to membrane organization and signaling. In the presence of actin-disrupting drugs, the effects of the cholesterol depletion on the cell stiffness were abrogated (17), suggesting that cholesterol depletion indeed affects cell stiffness by altering the properties of the actin cytoskeleton or its attachment to the membrane.

Overall, the contribution of the plasma membrane to the mechanical phenotype rests mostly in its resistance to being stretched. Since any deformation away from a spherical shape requires

an increase in surface area, the membrane area available in microstructures that can be added to the surface—further moderated by the dynamically maintained membrane tension—limits the extent and rate of cell deformation and, thus, its apparent elastic modulus and viscosity. Secondary effects include the signaling that happens in and through the membrane, which can also change the mechanical phenotype, if only after the relevant times required for the signaling to take effect.

3.3. Contribution of the Nucleus and Other Organelles

In addition to the structures at, or close to, the cell surface, the organelles situated deeper within the cell, such as the cell nucleus, can also contribute to the measured mechanical properties. The stiffness of the nucleus relative to that of the whole cell is controversial. Most of the recent studies indicate that the nucleus is softer than the whole cell (176, 207, 233); AFM indentation measurements performed on apically exposed nuclei within cells yielded Young's moduli lower than those obtained for whole cells (207), and the theoretical models of nuclear contributions in micropipette aspiration (176) and AFM indentation experiments (233) indicate lower nuclear stiffness. Additionally, AFM probing of enucleated cells revealed that removing the nucleus does not decrease cell stiffness (45). In contrast, many early studies suggested that the nucleus is stiffer than the whole cell (19, 73, 75). These studies were conducted either on nuclei inside the cell (19, 73) or on isolated nuclei (19, 75)—both approaches have their limitations, as inside the cell, the nucleus is surrounded and supported by the cytoskeleton (see **Figure 2**), and in the case of nuclear extraction, the nuclear properties can be compromised by the chemical or mechanical isolation procedures. Nonetheless, there is compelling evidence that modifying the mechanical properties of the nucleus—either by perturbing the nuclear laminas, which limit the increase in surface area of the nucleus under deformation (13, 83, 111, 112, 118), or by altering the chromatin compaction (22, 112)—can lead to changes in the mechanical properties of the entire cell. Thus, the nucleus is an important structural element in the context of the mechanical properties of the cell and can likely influence them either directly or by interfering with other cellular components. Among all cellular components, the nucleus comes the closest to displaying purely elastic behavior, and it does not flow even on longer timescales (24, 90), which plays an important role in cell migration (see Section 4 below). Other, smaller organelles (the endoplasmic reticulum, the Golgi apparatus, various functional vesicles, lipid droplets) likely contribute to the time-dependent resistance to deformation by their physical presence within a confined space. As an example, lipid droplets in adipocytes mature during differentiation to eventually take up most of the cell volume, and their effect on the overall stiffness of the cells increases with the maturation (1, 198). Since the presence and relevant properties of such organelles are difficult to modify and very cell type dependent, their specific contribution to cell mechanics, while it undoubtedly exists, has been little investigated.

3.4. Cytoplasmic Packing

Finally, macromolecular packing in the cytoplasm can have an impact on the overall cell stiffness. The cytoplasm is a densely packed environment, filled to near capacity with macromolecules (49). The level of cytoplasmic packing in the cell can be increased either by water efflux (74, 79, 145, 205, 250) or by overexpression of proteins and macromolecular complexes, such as ribosomes, that account for a large fraction of the cellular volume (37). The increase in the volume fraction occupied by macromolecules leads not only to a decrease in the ability of small molecules to diffuse around in the cell, but also to a steep increase in the apparent stiffness of the cytoplasm (37, 250), analogous to the glass transition observed in colloidal mixtures (250). This increase in cytoplasmic stiffness leads, in turn, to a decrease in whole-cell deformability and affects most of the global mechanical measurements of the cell (74, 79, 145, 205, 225, 250). In yeast, it has been

demonstrated that a decrease in intracellular pH and a consequent solidification of the cytoplasm can lead to increased cell stiffness (150).

Circulation: passive advection of cells through the vasculature that is driven by blood flow and does not require active force generation by the cell

3.5. Conclusion

In this section, we discuss the main subcellular contributors to the mechanical properties of cells that are generally considered to be relevant and have been investigated to date (in order of decreasing prevalence in the literature). The field, however, is still far from a comprehensive understanding of how these contributors, and some additional ones, combine to make up the mechanical phenotype of a cell. We return to this point in Section 5, but first we turn to the relevance of cell mechanics to the cellular functions.

4. FUNCTIONAL RELEVANCE OF SINGLE-CELL MECHANICS

The power of cell mechanics as a marker of cell state changes rests in the fact that the cytoskeleton (as the likely main contributor to cell mechanics) and the overall mechanical properties of cells are intricately involved in certain cellular functions that depend on the ability of cells to change shape. Some of the most prominent examples of the functional relevance of single-cell mechanical properties are discussed below.

4.1. Circulation through Microvasculature

An obvious example of the cell mechanical phenotype being important for a physiological process is the circulation through the microvasculature. Microcapillaries, the smallest vessels of the cardiovascular system, can measure as little as 2 μm in diameter (124, 170), much less than the diameters of blood cells—8 μm for red blood cells (RBCs) (143) and between 6 and 30 μm for leukocytes (43). Consequently, cells need to deform to pass through the vasculature (124, 170) (**Figure 5**). There are both pathological consequences and interesting physiological aspects to be considered in relation to the necessity for shape change.

Impaired deformability of blood cells can perturb physiological blood flow and lead to poor organ perfusion, decreased oxygen transport, and vascular occlusions, all of which have detrimental effects on patients' health (127). RBCs stiffened by malaria infection were found to more readily obstruct small capillaries in vitro than uninfected RBCs (87, 196); in patients, the reduced deformability of RBCs was correlated with fatal disease outcomes (42). Similarly, in sickle cell anemia, less deformable RBCs were more prone to cause occlusions of the capillary system (16). In the context of white blood cells, the stiffening of leukocytes observed in leukemias (110, 123, 183, 184, 249) and as a response to chemotherapy (109) is associated with leukostasis—a formation of leukocyte plugs in microcapillaries. The accumulation of leukemic cells in the blood vessels of the brain or lungs can result in severe conditions such as intercranial hemorrhage or respiratory failure, respectively (109, 110). Other conditions in which decreased deformability of leukocytes was reported to contribute to vascular occlusions include acute respiratory distress syndrome (172, 173), sepsis (155), and pneumonia (245). In a recent publication from our group, Kubánková et al. (106) reported a very drastic change of the mechanical phenotype of almost all different blood cell types during acute COVID-19 infections, a result that is likely to have implications for the circulatory abilities of these cells and the concurrent thrombotic events being reported. In contrast to the cell stiffening observed during chemotherapy treatment, the leukemic cells that survive exposure to anticancer drugs for prolonged periods of time were reported to have higher deformability than control cells (133).

Another area in which the deformability of cells is crucial in the context of circulation is the efficient delivery of stem cells to target organs after intravenous transplantation. In cell-based

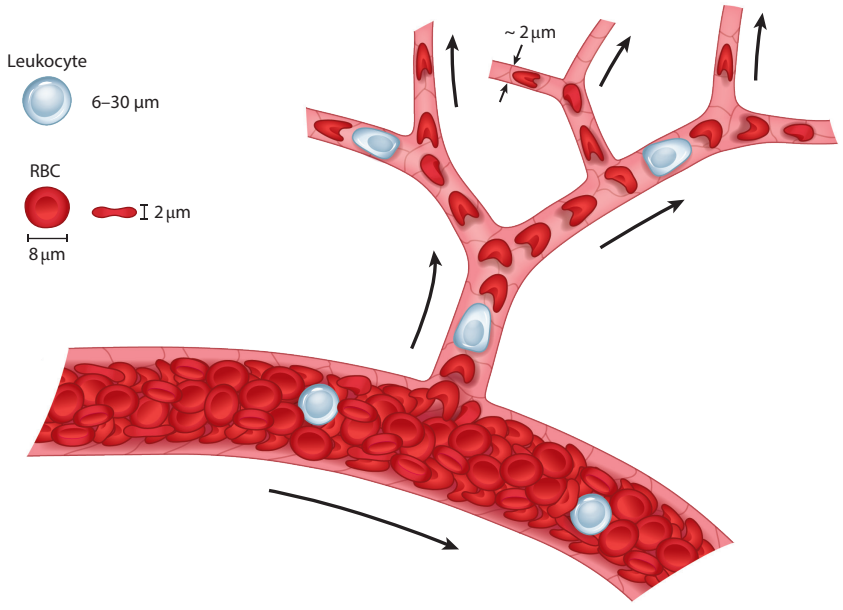

Figure 5

Blood cells need to deform to pass through the microvasculature. As cells pass through the vasculature, they come across increasingly narrow capillaries. When the encountered vessels are smaller than the cells' diameter, cells need to deform to traverse them. The reference dimensions of undeformed red blood cells (RBCs) and leukocytes are provided in the upper left corner. Figure adapted from Reference 223 (CC BY 4.0).

regenerative therapies, mesenchymal stem cells, hematopoietic stem and progenitor cells, and other tissue-specific progenitor stem cells can be transplanted into patients for immunomodulatory or regenerative purposes (134). When administered intravenously, the cells need to pass through the pulmonary capillary bed before arriving to target organs, which carries a risk of substantial sequestration of cells in the lungs and diminished or delayed homing (46, 50, 192, 234). It has recently been demonstrated that expanding mesenchymal stem cells in 3D spheroids rather than in 2D plastic-adherent cultures results in a smaller and more deformable phenotype of cells (217). This phenotype was connected with faster passage through microfluidic channels mimicking microcirculation; decreased trapping in the lung; and increased delivery to organs such as the liver, heart, spleen, or kidney upon transplantation into mice (217), suggesting that engineering the biophysical properties of stem cells could foster their capability to pass through the microcirculation and enable efficient transplantation. Similar cell-mechanical considerations are likely relevant, but at present unexplored, in the administration of Car-T cells and natural killer cells as novel anticancer therapies.

There are also interesting physiological aspects to be considered in how leukocytes in circulation change their mechanical phenotype to eventually leave the blood vessels. For example, circulating neutrophils are activated at sites of infection by various biochemical agents, such as cytokines and bacterial products, which triggers profound morphological and mechanical changes (140). While several studies showed that in vitro activation of neutrophils led to stiffening (47, 161, 182, 240), others reported softening (38, 65). These seemingly opposing observations were reconciled as consecutive phases in recent time-resolved studies, which revealed that, after an initial phase of stiffening and compaction (the first 15 min upon stimulation), neutrophils become

larger and more deformable at longer timescales (9, 219). The latter is also the state of neutrophils found circulating in the blood of patients with active infections (9, 219). It stands to reason that the initial stiffening is part of how the cells manage to go from circulating, to being marginated toward the vessel wall, to rolling along the endothelial surface with increasing adhesion, to then being able to stop and extravasate (191). In smaller capillaries, the increased stiffness could help slow the neutrophils down to enable extravasation. The extravasation step as an active migratory process will then have its own mechanical necessities, of which the later softening described for the second activation phase could be a signature.

Compliance: the degree of deformation with time given a constant applied stress; a measure of deformability and the inverse of elasticity

4.2. Migration of Immune Cells

Immune cells extravasate and migrate through tissues to reach the sites of infection or inflammation within the body (132, 228). For example, in the innate immune response, neutrophils and macrophages are recruited to infection sites to eliminate pathogens from the body (171) (**Figure 6**). As discussed above, neutrophils circulate in the bloodstream and extravasate into tissues when they are captured by adhesion molecules displayed by activated endothelium (191). Macrophages are either recruited locally from the population residing in the tissues or differentiated from monocytes recruited from the bloodstream (132, 197).

Differentiation of myeloid precursor cells toward neutrophils is accompanied by an increase in cell deformability and a decrease in viscosity, both of which seem to be useful when migrating through dense tissues (48, 113). Neutrophils further soften after activation (8, 38, 204), which could additionally promote their migratory capability. Contrary to neutrophils, macrophages appear less compliant than their precursor cells on short timescales (<1 s); however, they do show decreased viscosity and increased compliance at longer timescales (>10 s) (48). Since macrophages do not circulate through the vasculature, they may not require high deformability at short timescales, and their long-timescale properties likely support tissue infiltration (48). On the other hand, an

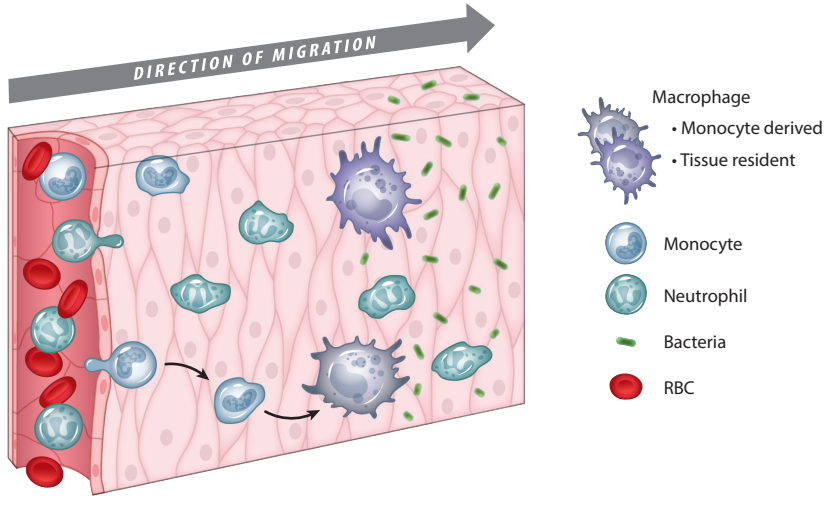

Figure 6

Immune cells navigate complex environments when migrating to bacterial infection sites. Neutrophils and monocytes leave the blood vessels and migrate toward the infection site through dense tissues, which requires them to deform and squeeze through narrow spaces. After extravasation, monocytes differentiate into macrophages. Simultaneously, the tissue-resident macrophages are recruited locally. Abbreviation: RBC, red blood cell.

increased elastic modulus of macrophages (measured on short timescales) was reported to facilitate phagocytosis and increase with activation of macrophages (164). Other immune cells that migrate into tissues, such as B and T lymphocytes, natural killer cells, or dendritic cells, experience similar obstacles, and their successful trafficking may also be influenced by their dynamic mechanical phenotypes (130). The changes of the mechanical properties of the immune cells during pathological events in the body can be used as label-free diagnostic biomarkers (219).

Cell migration is a process that happens within a timeframe of minutes or longer; on such timescales, cells mostly behave like liquids and can adapt their shape to any form. The only structure within the cell that is relatively elastic is the nucleus (24, 90). The elastic nature of the nucleus has important implications for cell migration through tight spaces, where it is the nucleus that limits the ability of the cell to flow easily through narrow gaps (36, 57, 85, 239). In fact, the migration of neutrophils through narrow spaces was shown to be limited by the deformation of their nuclei (36), especially given that their cytoplasm was shown to become transiently softer when entering constrictions (244). As a side note, it is speculated that neutrophils—cells that need to shuttle quickly between the vasculature and tissues by transendothelial migration (while being postmitotic and as such not needing to maintain the integrity of their genetic material)—have a multilobed nucleus rather than a spherical one to facilitate squeezing through narrow gaps. However, this is at present still an unproven hypothesis.

4.3. Invasion and Migration in Cancer Metastasis

Another process in which the mechanical properties of cells have been implicated is invasion and migration in the context of cancer malignancy. Metastasis—a sequence of events that leads to development of secondary tumors—is the major cause of cancer-related deaths (203). It involves multiple steps, including detachment of the cells from the primary tumor, migration through surrounding tissues, endothelial transmigration during both entry into and exit from the blood or lymph vessels, and finally colonization and secondary tumor formation (236) (**Figure 7**). Metastatic cascade recapitulates some of the aspects discussed above for the circulation and migration of immune cells.

While tumorous tissues are typically characterized by increased stiffness (120, 169), malignant transformation of cells was shown to be correlated with cell softening in the overwhelming majority of studies, and the cancerous cells become softer with increasing invasive potential (for reviews, see 4, 209). The softer phenotype of cancer cells, frequently associated with high invasiveness, has been hypothesized to facilitate squeezing of cells through narrow spaces (72, 128, 243), and there is some direct evidence that cell stiffness plays a role at various stages of the metastatic cascade (59). For example, during detachment from the primary tumor, cells undergo an epithelial-to-mesenchymal transition (EMT), a process in which epithelial, polarized cells undergo cytoskeletal remodeling and reduction of intercellular adhesion to acquire a mesenchymal, motile phenotype (178). EMT was shown to reduce cellular stiffness and promote invasion (31), and softening of cells during their detachment and migration from breast cancer spheroids has been directly observed (82, 89). Interestingly, while interphase cells become softer during EMT, the mitotic cells increase their cortical stiffness, which could have implications for successful cell division in crowded environments (92). The ability of cells to deform is also crucial for transendothelial migration during intra- and extravasation (29), and the deformability of the cell nucleus is rate limiting for passing through narrow pores (57, 85, 239), both when crossing epithelial barriers and when invading the tissues. Cancer cells were shown to soften their nuclei during transendothelial migration (181) and when entering narrow constrictions (177), suggesting that they can adapt their mechanical properties to the encountered migratory challenges. While cell softening is generally thought to

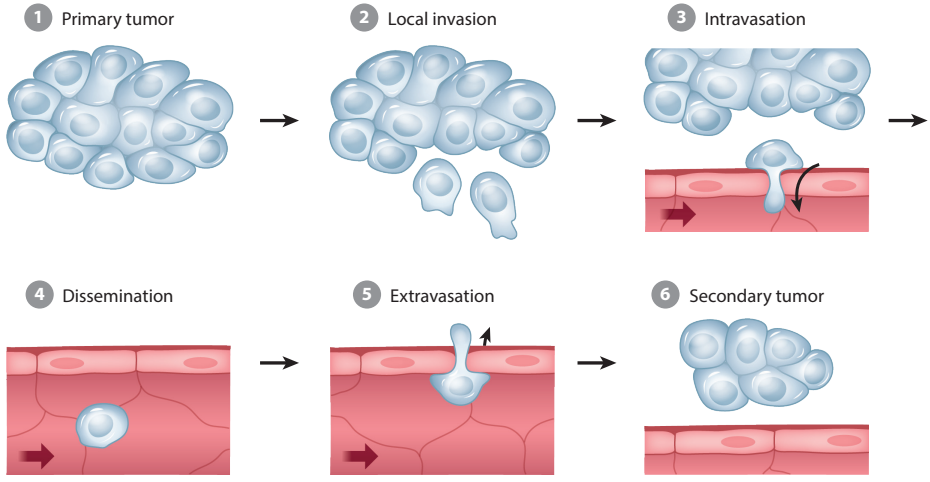

Figure 7

Stages of cancer metastasis. Distant metastasis is a multistep process during which cancer cells are carried via the bloodstream to new locations in which they proliferate and ultimately form secondary tumors. Initially, the cancer cells are contained within the primary tumor (①). In the local invasion step (②), cells detach from the primary tumor and explore the surrounding tissue. Next, the detached cells enter nearby blood vessels (③, intravasation) and are carried with the bloodstream throughout the body (④, dissemination). Finally, some of the circulating cells exit the blood vessels at a distant location (⑤, extravasation), migrate through local tissue, and proliferate to form a secondary tumor (⑥). Each step of the metastatic cascade poses unique mechanical challenges to the disseminated cells. The dark red arrows in panels ③–⑤ indicate the direction of blood flow.

promote migration, stiffer cells may have an advantage in resisting cell damage during exposure to forces, for example, in circulation. Thus, it is likely that, for successful metastasis, cancer cells need to dynamically and repeatedly adapt their mechanical properties (59). Since cell softening and mechanical adaptability of the cells appear to be crucial for the progression of metastasis, mechanical properties of cancer cells could be targeted to prevent cancer spreading, a possibility that is being actively explored (162, 208). In addition to reducing metastasis, modifying cancer cell mechanics might also enable the immune system to fight them more efficiently: Cancer cells stiffened by high MRTF expression (214) or cholesterol depletion (119) were much more vulnerable to efficient T-cell-mediated killing, which opens up an exciting new avenue for the exploration of cell mechanics in cancer treatment.

4.4. Cell Fate Specification and Morphogenesis

The transition from undifferentiated to more specialized cell types is often hallmarked by changes in the mechanical phenotype. In in vitro studies, stiffness changes have been shown to be an indicator of early differentiation of murine and human embryonic stem cells (ESCs) (32, 65, 168, 226) and cardiac (212), as well as chondrogenic (159), differentiation of human ESCs. Apart from studies on pluripotent ESCs, human mesenchymal stem cells have been shown to change their mechanical phenotype during differentiation toward osteogenic (11, 30, 246) and adipogenic lineages (246), and the differentiation potential for a given lineage of adipose-derived stem cells was shown to correlate with cell stiffness (64). Human myeloid precursor cells soften during differentiation toward neutrophils and monocytes but stiffen during differentiation toward macrophages (48, 113). Finally, human hematopoietic stem and progenitor cells (94) and human skeletal stem

cells (242) were shown to be distinguishable from other cell types found in bone marrow based on their size and deformability. Perturbation studies will be necessary to gain more knowledge on whether changes in cell mechanics play a causative role in cell type specification; to understand better the functional roles of such changes, studies in vivo or in multicellular assemblies are necessary.

There are also strong hints of the importance of mechanical properties of cells for development and cell-fate specification in vivo. In the early murine embryo, the compaction of the 8-cell-stage mouse embryo is driven by pulses of cortical tension (135), and subsequent lineage specification and sorting of the trophectoderm and inner cell mass are triggered by differences in cell mechanical properties (187). During gastrulation in zebrafish, the three cell lineages acquire distinct mechanical phenotypes, with the ectoderm showing the highest cortical tension, the mesoderm showing intermediate tension, and the endoderm being the softest (105). These differences in cortical tension are sufficient to guide cell sorting in multicellular assemblies, with the stiffest ectoderm cells being sorted toward the aggregate center when mixed with mesoderm cells (105). Apart from being involved in cell migration and sorting, mechanical properties of individual cells can also contribute to the stiffness of the local microenvironment and influence the behavior of neighboring cells. One prominent example of this phenomenon is the migration of neural crest cells in *Xenopus laevis* embryos, which is driven by myosin-dependent stiffening of underlying mesoderm cells (7, 215).

5. FUTURE DIRECTIONS

With the mounting evidence that changes in cell mechanical properties reflect physiological and pathological transitions in cell state (39, 51, 71, 154), and the increasing efforts to understand and model living tissues as physical systems (116, 206), the research on mechanical phenotyping of cells is more relevant than ever. The progress of the field in the years to come will depend on addressing several key challenges.

First, it will be necessary to give more attention to why mechanical properties are changing in diverse processes, as opposed to merely reporting the correlation. This will lead to a mechanistic understanding of these changes and help determine whether the mechanical properties are instructive factors or simple by-products of the studied processes. Quantification of the relevant mechanical properties will serve as the necessary basis for physical models, which can then make falsifiable predictions. To test such predictions on the experimental side, it is imperative to identify the knobs that can be used to tune the mechanical properties of cells at will. Such control of mechanical properties would facilitate exploration of the impact of cell-mechanical perturbations on cell and tissue function, in particular in in vivo experiments. It would further enable the targeted modification of mechanical properties as a corrective intervention in various diseases. For example, slowing down the dissemination of metastatic cancer cells and increasing the efficiency of T-cell-mediated cancer cell killing (119) by artificially stiffening cancer cells are exciting prospects.

While one motivation for this review was the discussion of the origins of the mechanical phenotype, the future will also include an unbiased search for as-yet unknown regulators using the possibilities enabled by new high-throughput techniques (see the sidebar titled The Quest for Novel Regulators of Cell Mechanics). This will further instruct the specific modification of mechanical properties that, in the long term, will turn cell mechanics into a controllable property. One of the challenges to this effort is that any chosen perturbation not only affects the mechanical properties, but also likely interferes with other processes and signaling networks. This ambiguity can be addressed by using multiple methods of achieving the same mechanical alteration to isolate

THE QUEST FOR NOVEL REGULATORS OF CELL MECHANICS

There are several ways in which the search for knobs for tuning cell mechanics can be approached. First, with emerging high-throughput technologies (225), it is becoming increasingly feasible to perform screens to identify regulators of cell mechanics. To date, in the context of cell mechanics, several RNA interference screens have been performed with preselected lists of targets using methods such as AFM (33, 221) and DC (185). Screens encompassing a broad selection of targets, and other approaches such as CRISPR (195) or chemical compound libraries, will likely be realized through further increases in throughput, parallelization, and automation of sample loading in methods such as DC. Second, to elucidate which molecules govern the mechanical phenotype, one can sort the cells based on their mechanical properties and perform downstream -omics analysis (transcriptomics, proteomics, or even lipidomics) in bulk or at the level of single cells. Also in this case, the microfluidic technologies that allow sorting for cell mechanics at reasonable throughputs (152, 153) will come in handy. Finally, data-driven approaches that take advantage of available -omics profiles for cellular states with diverging mechanical properties and machine learning for data analysis can be used to deduce which players are involved in regulating cell mechanics (224).

the mechanical change as the one causal factor for the consistent effect observed. For example, stiffer cells might always migrate slower, irrespective of which secondary biological processes are simultaneously impacted.

In the context of circulation and migration, we need to understand better how (active) viscoelastic properties of cells determine their migratory potential, in addition to simply knowing that they correlate with it. Ultimately, on the relevant timescales of minutes, cells behave as liquids due to their ability to reorganize their cytoskeleton and rebuild themselves. Thus, any measurement of an apparent elastic modulus on a (sub)second timescale, which constitutes the majority of reports on cell mechanics to date, will at best only reflect a shift in the ratio between elastic and dissipative processes and in the worst case be a nonrelated epiphenomenon. There seems to be an increasing appreciation that we need to measure the full viscoelastic properties of cells. It is conceivable that it is actually the viscosity that is the relevant property for the determination of how cells flow through the tissue, and even of how cells circulate through constrictions in the vasculature. While circulation is the process where the short-term elasticity of cells matters most (to quickly deform and pass constrictions while being advected along), once they are retained in capillaries for times longer than a few seconds, it is the viscosity that determines whether the cells will make it through or whether they are terminally stuck. The relevant consideration might be whether the mechanical relaxation timescale is shorter than the time required to form adhesions. If we want to fully understand the functional relevance of cell mechanics, especially in the context of migration, we need a comprehensive picture that goes beyond passive viscoelastic properties and includes the active nature of cells.

Yet another challenge is posed by the fact that cells have to date mostly been probed in an artificial in vitro setting. They are taken out of their context, which is known to affect their mechanical properties. The high-throughput methods that measure cells in suspension are directly relevant for liquid samples such as blood (219) or pleural effusions (222), and rapid extraction methods for isolating cells from solid tissues that better preserve their biophysical properties are becoming available (201). However, there is also a pressing need for reliable characterization of cell mechanics in situ. This capability will likely come from the advance of optical techniques because light can penetrate cells, tissues, and small organisms and deliver information with diffraction-limited, and thus subcellular, resolution. Current approaches include various forms of optical elastography, in which the optical imaging is used to map out the strain fields after application of static or dynamic

stresses (98). A variant of optical elastography, Brillouin microscopy, analyzes the inherent density fluctuations inside the sample to infer the elastic and viscous properties in a confocal volume (5, 174, 189). Even though mechanical measurements with such optical techniques are conducted in a localized manner (as opposed to whole-cell measurements) and at high frequencies, they already contribute to answering important open questions, such as what the mechanical properties of subcellular compartments inside an intact cell are (190, 247) and how the mechanical properties of a cell change while the cell migrates inside a tissue (181).

While cell mechanics and the broader field of mechanobiology are becoming mainstream in areas such as developmental biology, the ultimate success of cell mechanics research will depend on proving its use in diagnostic or therapeutic applications. With the new high-throughput methods becoming commercially available and the substantial effort in pushing forward such applications [by companies such as Artidis (169) and Cytovale (77)], this will likely be only a matter of time.

DISCLOSURE STATEMENT

J.G. declares competing financial interests, as he is the co-founder of the company Rivercyte GmbH, which commercializes deformability cytometry products and applications. M.U. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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