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Measuring cell-generated forces: a guide to the available tools

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Abstract

Forces generated by cells are critical regulators of cell adhesion, signaling and function, and are essential drivers in the morphogenetic events of development. Over the past 20 years, several methods have been developed to measure these forces. Despite recent substantial interest in understanding the contribution of these forces in biology, implementation and adoption in the broader biological community remains challenging due to the inherently multidisciplinary expertise required to conduct and interpret these measurements. In this review, we introduce the established methods, and highlight the technical challenges associated with implementing each technique in a biological laboratory.

Introduction

Mechanical forces generated by cells not only drive the bending, stretching, alignment, and repositioning required for tissue development and homeostasis, but also regulate cell functions ranging from receptor signaling and transcription to differentiation and proliferation. Despite their importance, only a small fraction of such forces has been characterized. In contrast to the powerful and widely used array of molecular genetic tools to examine the expression, regulation, and activity of any specific protein, current understanding of the role of mechanical force in cell biology is based on only a handful of techniques. The methods vary significantly in their ease of use, assumptions, and in the technical and experimental overhead required for implementation. Here, we provide a critical and comparative review of the currently established methods for measuring cell-generated forces. Because more detailed treatment of each of these methods can be found, this report is meant to be a quick guide rather than in-depth review, and to serve as a technical resource for investigators looking to understand the available options to examine the role of cell-generated force in their own research.

In this review, we focus on methods for measuring forces applied by cells on the surrounding substrate. Active methods in which external forces are applied to cells to induce cellular signaling or to characterize mechanical properties (such as stiffness) are covered

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elsewhere[1]. The methods we discuss can be broadly categorized along three axes: 1) methods that measure forces generated by an entire tissue construct versus those generated by a single cell or small collection of cells, 2) methods that measure only deformation versus those that translate this deformation into cellular forces, and 3) methods that measure forces in two dimensions versus in three dimensions. We conclude with a perspective on how newer methods harness the cell's native force-sensing systems.

Measuring tissue deformation

The simplest methods to characterize the presence of cellular forces involve measuring deformations of cells, substrates, or tissues without attempting to relate these deformations to an actual force. For example, stromal cells embedded within collagen gels will compact the gel over a period of hours to days, likely mimicking the contractions that occur during wound closure[2–6]. Compaction, measured for example by the change in diameter of a cell-laden gel polymerized in a well, is driven in part by cellular forces and is substantially reduced upon inhibition of myosin-based contractile activity[7]. Similarly, laser ablation of cell-cell junctions in *Drosophila* embryos results in observable retraction of the ablated edges, thus providing a qualitative sense of the magnitude of contractile forces generated by neighboring cells[8–10]. The advantage to these approaches is that one does not need *a priori* knowledge of the mechanical properties of the material being deformed, or complex calculations to convert deformations to force (Box 1). In the most conservative sense, these approaches report the actual measured variable. However, deformation-based methods have major drawbacks. Implicit in the analysis is the assumption that more compaction or retraction means more cellular force, whereas fracture, plasticity, and viscoelasticity of the material can mean this assumption is not justified (Box 1). In addition, mechanical properties of living materials can change actively in response to perturbation, causing the tissue to compact more or less under constant force. Further, the time scales of these deformation assays (collagen compaction takes places over hours or days) do not allow measurement of force fluctuations, which are particularly important in the study of fast-contracting cells such as myocytes. Importantly, the reported deformation measurements cannot be compared across systems.

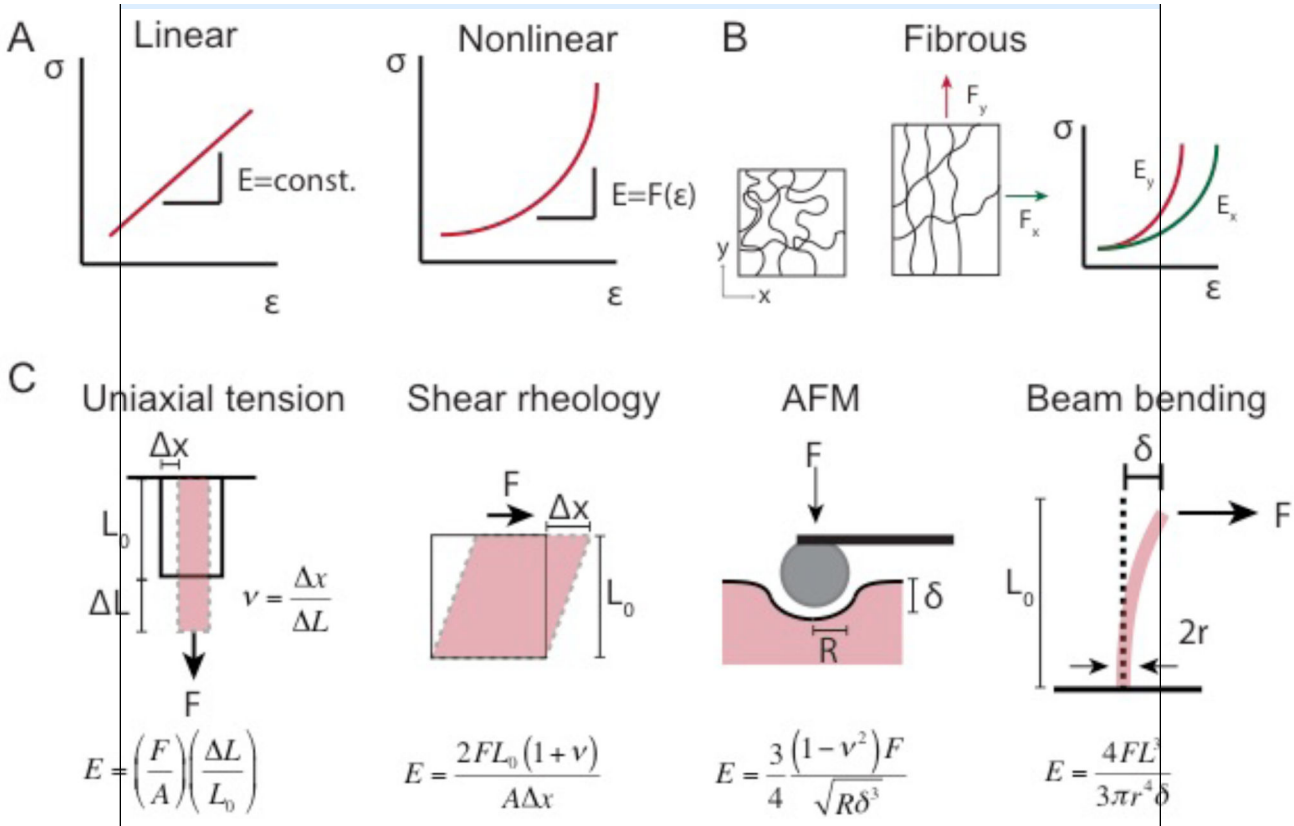
BOX 1

Traction measurements require understanding the mechanical properties of the ECM

The mechanical behavior of a solid material is defined by the manner in which it deforms under applied force, and the relationship between force and deformation is defined by a material constitutive equation. The effect of force on material deformation is dependent on the area over which the force is applied, so constitutive equations are defined in terms of stress, the force per unit area ($\boldsymbol{\sigma}$, units of Pascals), and strain, the fractional change in length of a material ($\boldsymbol{e}$, unitless). For linear elastic materials, stress increases linearly with increasing strain, and thus the relationship between stress and strain is characterized by a single parameter, E, known as the stiffness or elasticity of the material[24]. For nonlinear elastic materials, the relationship between stress and strain is a function of the magnitude of strain[24]. Most methods for measuring cell tractions assume that the substrate is both

linear elastic and isotropic, meaning the material properties are the same in every direction. Another common assumption, particularly with TFM methods, is that the substrate is infinite in size compared to the size of a cell and thus the deformation due to cell tractions does not depend on substrate geometry.

The ECM is a fibrous network of proteins, and these fibers introduce a length scale dependency to the mechanical properties of biological materials. That is, because individual fibers are much stiffer than overall aggregate hydrogel networks of fibers, the mechanical properties of the material are experienced differently depending on the area of contact between probe and material, and the amount that the probe is moved to take the measurement. Thus, properties measured by uniaxial tension testing and shear rheology (measured across millimeters or more of material) might not characterize properties relevant to cells that interact directly with fibers at the micrometer scale. Therefore, methods such as atomic force microscopy (AFM) are often used to characterize the material properties on cellular and subcellular length scales. Fibrous materials are also nonlinear (as fibrous materials are strained, the fibers align, increasing the resistance to further strain), and often anisotropic (stiffer in the direction of aligned fibers). Though a great deal of work has been devoted to measuring and characterizing the mechanical properties of biological materials (reviewed: [97]), the nonlinearity and length scale dependence of these materials greatly complicates measuring cell tractions in native ECM. In a linear material, a measured strain can be directly converted to stress through the linear elastic mechanical properties, but for a nonlinear material, the stiffness of the material at the observed level of strain and appropriate length scale first needs to be determined then used to relate measured strain to stress. The difficulty in determining tractions from measured strain in nonlinear ECM has motivated the development of a class of biologically active, synthetic materials that are isotropic and linearly elastic under the level of stress and strain that cells generate. These materials, including silicone, polyacrylamide, and polyethylene glycol have enabled measuring cell tractions, but the biological relevance of the tractions measured with these materials remains an open question as the contributions of the nonlinear, fibrous properties of biological materials to the tractions generated by cells have yet to be determined, though recent work estimating the forces from cells embedded in fibrous matrices has made some early advances[56].



ECM mechanical properties determine the relationship between force and deformation. (**a**) The elasticity, given here as the Young's modulus (*E*), determines the relationship between stress and strain in linear materials. In nonlinear materials, *E* is a function of strain. (**b**) The ECM is fibrous with anisotropic and nonlinear material properties. (**c**) Common methods for determining the mechanical properties of materials used to measure cellular forces.

To measure the forces generated in a compacting hydrogels, there are two general approaches that have been taken. The first is to use a gel that is large enough to attach to an external isometric force sensor[11–13]. These sensors are off-the-shelf devices that change resistance or voltage signals with force. They are effectively much stiffer than the tissue construct and undergo negligible deformation during the course of a measurement. Therefore, force, as opposed to displacement, is measured directly from the contractile tissue. Such systems have been used to measure the forces generated by cells from highly contractile tissues, including skin fibroblasts[11], cardiac myocytes[12], and skeletal myocytes[13]. Though these systems provide continuous and long-term measurement of tissue contractile forces, the signal processing required to convert the electrical signal output from the force sensor to actual forces might be beyond the expertise of a standard biological laboratory. Furthermore, these methods are limited in throughput because the lower bound operating range of the sensors is typically in micro- to milliNewtons, requiring the use of large tissues that need to be manually mounted to the force sensor.

The second approach is to incorporate cantilevers of known stiffness into the system, so that as the tissue contracts, the cantilevers bend (Fig. 1). The displacement of the free end of the cantilever can be imaged with optical microscopy, and these observed displacements are used to calculate the tissue contractile forces using beam theory[14]. An advantage of this system is that the deformation of many cantilevers can be measured simultaneously. The systems can also be made much smaller, requiring fewer cells and less extracellular matrix (ECM) material than the electronic assays, and do not require manual mounting of tissues to individual sensors[15–17]. More recently, vertical cantilevers have been microfabricated from silicone elastomer (polydimethylsiloxane, PDMS), enabling systems that can measure forces from constructs with as few as 100–600 cells[18–21]. These systems have become an increasingly important tool for measuring forces in cells such as cardiomyocytes, which cannot be isolated or propagated in large numbers[19,20,22].

Though measuring forces using these microfabricated constructs requires little more than a microscope with a suitably long working distance (the cantilever tips are ~300–500 μm from the coverslip[18]), fabricating the systems requires techniques that are not standard in biological laboratories. The cantilevers are fabricated by soft lithography[23], which involves replica molding of a patterned master silicon substrate. One such silicon master can be used to mold thousands of polymeric cantilevers, which can be performed with commercially available PDMS and a vacuum chamber. However, creating the original silicon master requires microfabrication facilities; although foundries will fabricate silicon masters for a cost, the technical designs required to specify the production process involve substantial expertise, which necessitates collaboration with a laboratory experienced in microsystem fabrication.

Measuring the net contractile forces generated by tissue constructs can provide quantitative information about the signals that drive tissue deformation, in particular the role of the ECM. However, ECM remodeling and cellular forces are coupled in the resulting aggregate measurement, which will therefore depend on the specific formulations for generating the cell-laden ECM gels. These factors make it difficult both to compare measurements across different studies, and to isolate the forces generated by individual cells.

Introduction to cellular tractions

Cells are mechanically attached to neighboring cells and ECM. Contractile forces generated by a cell through actomyosin contraction are transmitted to neighboring cells and ECM via cell-cell and cell-matrix adhesions. In general, forces between a body and a surface, such as the force that a car tire imparts on the road, are known as tractions[24]; cellular forces applied to the local microenvironment are known as cellular tractions. Cellular tractions are very small (pN – nN), and occur across small length scales (nm – μm), thus measuring them directly is difficult. However, forces applied to soft solid materials induce measurable changes in the material shape. Thus, cell tractions can be determined with 1) a quantitative map of material deformation, and 2) a well-defined constitutive relation of the substrate material (Box 1). A variety of techniques measure and map the forces generated by cells by culturing them on or in synthetic materials with well-defined mechanical properties that behave as isotropic linearly elastic solids under cellular deformation.

In general, any traction force generated by a cell can be decomposed into a component that acts parallel to the substrate surface, and a normal component, which acts perpendicular to the substrate surface (Fig. 1). Traction components parallel to the substrate surface induce deformation in the optical viewing plane and can be measured by conventional wide field microscopy. Most methods for measuring cell-generated forces measure only the in-plane component of cell tractions. However, more advanced microscopy techniques with 3D resolution, such as confocal microscopy, allow tracking material deformation perpendicular to the viewing plane and enable computing both the normal and in-plane components of cell tractions.

Measuring cellular tractions in 2D

Cellular traction force microscopy (TFM) involves tracking the deformations of synthetic elastic polymer substrates that result due to the exertion of cellular force. This method, and its variations, remains the most widely used technique for measuring cell force. Cells are plated on flat, deformable synthetic substrates that are resistant to degradation, so that deformations due to force may be decoupled from changes in the mechanical properties of the local microenvironment caused by biochemical factors, including proteases, released by the cells[25,26].

In standard TFM, small (≤ 1um) fluorescent beads are mixed into silicone or polyacrylamide (PA) substrates to serve as fiduciary markers that can be tracked in space and time with optical microscopy[27,28]. A typical TFM experiment involves optically imaging the distribution of beads in a stressed state, releasing cell tractions via cell lysis[29], detachment[30], or myosin inhibition[31], and then imaging the beads again to determine their positions in the unstressed state. These two images (or sequence of images if dynamic forces are being measured) are passed through computational algorithms to determine the displacement of the beads caused by the cells and the forces required to cause such displacement (Box 1). Because the beads are much smaller than the size of a cell, TFM allows mapping cellular forces with subcellular resolution. Such measurements have enabled characterization of the force dynamics involved in a variety of cell biological processes including adhesion maturation[32,33], migration[28,34–36], differentiation[37], and malignant transformation[38]. Once the computational framework and imaging system are in place, measurements can be made quickly and repeatedly.

Silicone (12–100 kPa) and PA (1.2–100 kPa) are used as the cell substrate in TFM because their mechanical properties are well characterized and they behave as linear elastic solids under deformations typical in cell traction force measurements (Box 1)[39,40]. Unlike native ECM, silicone and PA are not degraded by cell proteases, so the mechanical properties of the substrate do not change significantly over the course of a measurement. While this is beneficial for quantifying cellular tractions, recent data suggests that degradation and ECM reorganization contribute to traction profile of cells *in vivo*[41,42]. To promote cell adhesion, the silicone and PA surfaces must be conjugated with ECM. This surface conjugation can be difficult to reproduce because the different reagents to crosslink the ECM proteins to the surface are labile and behave differently in different experimental conditions; also often only one ECM molecule, such as fibronectin[40], is used. The range of stiffnesses that can be

achieved with these materials only spans the higher range of native ECM (Table 1); it therefore remains unclear how well the tractions measured on these synthetic materials correlate with tractions generated *in vivo*.

Traditionally, the computational analysis required to calculate microparticle displacements and forces was a significant hurdle for laboratories looking to implement TFM, as the calculations are complex, nuanced, and difficult to validate. This is due in part to long-range elastic interactions between embedded beads, in which a force applied at a single point causes displacement of many surrounding beads due to the elasticity of the substrate, and because small errors in measuring the bead location can contribute large errors to the force calculations. The details, advantages, and disadvantages of the various computational techniques and algorithms are beyond the scope of this review and have been reviewed by others[43–45]. Recently, algorithms have been developed with sufficiently reduced computational cost that they can be implemented on standard desktop computers. There are also publicly available plug-ins for ImageJ and Matlab that compute cell tractions given stressed and unstressed images of fiduciary markers[44,46].

Nevertheless, tracking the beads and validating TFM measurements are challenging, and require techniques and equipment that might not be available to a standard laboratory. The size and spacing of the fiduciary markers and the optical resolution of the microscope determines the spatial resolution of the observed deformation field, and in turn, the spatial resolution of the computed traction field. Thus, mapping tractions with high resolution requires high-resolution imaging. A fundamental assumption in measuring tractions is that the mechanical properties of the cell itself do not influence the displacement field, which may not necessarily be the case. Furthermore, cells must be sufficiently sparse such that the displacement field generated by one cell does not overlap with that of a neighboring cell.

Validating the force measurements requires imparting a known, calibrated force on the substrate and comparing the computationally calculated force profile to the actual force[47] or simulating tractions with computational models[48,49]. This difficulty in validation, along with the many parameters in each measurement (bead size, bead density, substrate stiffness, cell density, cell relaxation method, imaging parameters), has thus far necessitated collaboration with groups that possess significant TFM experience and expertise. Even in adopting existing software plug-ins, the strengths and limitations of the different computational strategies can be difficult to sort through, and thus may require consultation with a laboratory with TFM experience to ensure that the calculations remain valid for particular types of studies.

Measuring 3D tractions

Contractile forces generated by cells impart traction forces normal to the substrate surface in addition to in-plane forces (Fig. 2). Tracking deformation in a 2D plane thus does not fully characterize the traction fields. To fully characterize the 3D traction field of a cell cultured on a 2D substrate (such methods are collectively referred to as 2.5D TFM, Table 1), TFM methods have been modified to track bead displacements in 3D with confocal microscopy[29–31,50–52]. Computing out-of-plane tractions also requires significant

computational resources, and many of the inverse computation methods for 2D TFM are not valid in 3D[43]. Overall, resolving normal tractions requires significant experimental and computational overhead.

In all of the methods for measuring cellular tractions discussed thus far, cells are cultured on 2D planar surfaces. However, *in vivo*, cells exist within 3D ECM, and the phenotype and shape of cells in 3D is strikingly different from cells cultured on 2D surfaces[53]. The nature of the cellular traction forces that underlie these phenotypic differences in 3D has been the subject of much interest recently; however, measuring tractions of cells in 3D is difficult not only because of the requirement to track fiduciary markers in 3D, but also because the material properties of biologically relevant 3D culture materials are much more complicated than the synthetic materials used for measurement of tractions in 2D (Box 1).

The most commonly used ECM material for 3D cell culture is reconstituted collagen type I hydrogel. Bead tracking techniques used for 2.5D TFM have enabled the measurement of deformations to pericellular collagen[54,55]. However, the nonlinear, fibrillar nature of collagen hydrogels (Box 1) prevents calculation of traction forces from these measured deformations using classical mechanics approaches. A recent report makes simplifying assumptions to estimate forces from cells embedded in fibrous matrices such as collagen, but additional investigation is required to determine whether these approaches will have widespread utility[56].

Synthetic, MMP-cleavable polyethylene glycol (PEG) hydrogels that are linearly elastic in the range of deformations induced by single cells, and tracking beads in these materials has enabled measurements of cellular tractions in 3D[57]. However, the computation of cell tractions from measured bead displacements is cumbersome, and resolving 3D tractions for cells in 3D remains a challenge for most laboratories.

Cells on microfabricated structures

Microfabricated platforms have been developed to measure cellular tractions directly in idealized mechanical environments. Microelectromechanical systems (MEMS) comprised of deformable silicon elements and integrated electronics allow cellular forces to be converted to electrical signals directly on chip[58,59] (reviewed: [60]). There are a variety of MEMS platforms, but generally cells are plated in close proximity to small (1–100 μm), compliant silicon elements, and as cells apply force to these elements, they deform, altering their electrical properties and causing a change in voltage or current across the element. The mechanical properties of silicon are well-known, so these electrical signals can be easily converted to a measurement of force. A major drawback to these systems currently is that typically only zero, one, or two probes are in contact with a cell at any one time so that spatial distributions of forces cannot be recorded. Though these systems promise the eventual development of a packaged cell traction tool that can measure tractions from hundreds or thousands of cells simultaneously, the expense and difficulty in fabricating the devices has prevented broad uptake in the biology community so far.

Similarly, microfabricated thin films that deform under coordinated contraction of multicellular sheets of cardiomyocytes have been implemented to measure changes in contractile force in response to drug treatment and with disease progression[61–63], but the films are difficult to fabricate and require collaboration with a laboratory with extensive microsystem expertise (for more information see recent reviews: [64,65])

In an analogous approach to the use of cantilevers for tissue constructs discussed above, silicone rubber cantilevers have also been developed to measure the forces of single cells[66]. Tissue-scale cantilevers are hundreds of microns in size and measure contractions of tissue constructs consisting of 100–600 cells mixed with ECM. Cellular- or subcellular-scale cantilevers are much smaller (0.5 – 10 μm) and fabricated in arrays (micropillar arrays). The tops of the cantilevers serve as the cell substrate with a single cell spanning tens to hundreds of cantilevers. The displacements of each cantilever in an array can be tracked and the applied force on the cantilever can be calculated using beam theory (Box 1). Because each cantilever moves independently of the others, this method allows direct computation of the forces applied to the surface of the cantilever, which dramatically simplifies the analysis required to measure cellular tractions and reduces the need for validation studies to verify the assumptions made in more complex computational methods. The unstressed position of the cantilevers is also known, which removes the requirement for cell lysis or release as in TFM measurements. By tailoring the length and width of the pillars, the stiffness can be controlled (Table 1)[67,68], and because the imaging and computational costs are low compared to traditional TFM methods, measuring tractions for multicellular populations is possible[69]. Computing the force balance between two neighboring cells on the micropillar substrate allows calculation of cell-cell forces[70–72]. Furthermore, the cantilevers can be made anisotropic to study the relationship between focal adhesion geometry and cell traction[73], and recently, cantilevers with dimensions smaller than a single focal adhesion (0.5 μm) have been fabricated to study the relationship between force and focal adhesion growth within single adhesions[74].

Restricting cell adhesion to the surface of an array of cantilevers greatly simplifies traction computation, but cell adhesive ligands are necessarily constrained to the micropillars, which presents a unique surface topography that influences cell adhesion structure, and could impact the magnitude and distribution of cellular traction forces. Functionalizing the micropillar surface with ECM ligand to promote cell adhesion is also difficult. Furthermore, fabrication of these systems is sophisticated and requires equipment that is not standard in biological laboratories[75]; however, as with the cantilevers for microtissues, fabrication of the PDMS devices is possible in a standard laboratory if the silicon master is available.

Next generation methods

Traditional methods for measuring cell traction require measuring deformations of synthetic cell substrates, and thus the sensitivity of the measurement is coupled to the stiffness of the substrate. Over the last decade, a class of probes that measure strain in molecular springs have been developed that allow high resolution imaging of tractions on stiff substrates by conjugating these sensors onto the cell culture surface. These molecular tension sensors consist of either a fluorophore and quencher or a Förster resonance energy transfer (FRET)

fluorophore pair separated by an entropic polymeric molecular spring, arranged such that the emission spectra of the fluorophores shift as a function of strain in the spring[76–79]. Though these sensors are able to report changes in traction at single adhesion complexes, difficulty in calibration prevents straightforward conversion of shifts in emission spectra to absolute forces.

These limitations have motivated the development of a new class of DNA hairpin force sensors by our lab and others[80–82], which couple a fluorophore-quencher pair such that when the hairpin unfolds under force, the emission of the fluorophore can be measured with conventional fluorescence microscopy. Unlike the protein-based force sensors, these hairpins can be rationally designed to unfold under a variety of forces. Furthermore, because they can be conjugated to many materials, one can use these sensors to measure cell-generated forces on glass, plastic, or other polymers where traditional TFM methods would fail. As with all fluorophore-based sensors, there are limitations due to bleaching and optical sensitivity in using and calibrating the sensors, and unlike TFM-based methods, these methods only provide the magnitude and not direction of forces. These probes are not yet commercially available, but have the potential for more widespread adoption.

Another category of FRET-based force sensor detects forces within single proteins. In contrast to the sensors discussed above, which are used to coat substrates and report forces applied by cells to the substrate, these new sensors are force-sensitive proteins that can be used to measure forces within cells. Proteins within the native cellular mechanotransduction cascades have been engineered with fluorophore pairs such that force-induced deformations in the protein impact the separation distance between fluorophores, and thus impact FRET efficiency. Therefore, FRET emission levels vary as a function of force. These proteins can then be expressed in living cells to provide measurement of the forces across single molecules in real-time. A vinculin tension probe has allowed measurement of the forces in cell adhesion complexes[83,84], and similar force probes have been developed to sense the tension in VE-cadherin[85], PECAM-1[85], E-cadherin[86], α-actinin[87], and fibronectin[88].

These molecular methods hold great promise for measuring cellular forces *in situ*, but the process of developing new molecular probes is prohibitive for most groups[89]. Furthermore, the range of sensitivity to force is specific to each probe and difficult to manipulate, and the perturbations to cell biology due to the insertion of the probe are poorly understood. Fundamental questions also remain about the interpretation of forces measured in single molecules as they relate to traction stresses or stresses in larger adhesion complexes. For example, in a given measurement, it is unknown how many unlabeled proteins and other force-bearing elements are acting in parallel to the probes, and thus remains unclear how one calculates the total forces exerted.

Challenges and outlook

Measuring cellular forces in physiologic context and understanding their contribution to biological processes is a formidable challenge. Current methods measure the forces between a cell and a single material. But *in vivo*, cells are connected to a host of materials and other cells, all of which contribute to the generation and propagation of cellular forces. For

example during embryogenesis, forces are required for proper tissue development and patterning[90], but these forces cannot be measured directly without isolating the cells and culturing them on a synthetic substrate that is sufficiently compliant to allow measurement of deformation by small cell-generated forces. It remains unclear how forces measured *in vitro* on such mechanically simplified materials relate to forces in living tissues. Although the development of injectable liquid droplets has provided some insight into the cellular forces in living embryonic tissue[91], understanding the mechanisms by which cellular tractions and cell-cell forces regulate tissue patterning and development still requires substantially improved tools.

In addition to the biological expertise needed to frame questions related to cellular forces, expertise in microfabrication, polymer chemistry, and/or computation are needed to implement most of the methods described here. The multidisciplinary nature of many of these techniques has itself been a barrier to adoption, but the packaging of system components—analogous to the packaging of reagent kits for molecular biology—promises greater adoption by the broader biological community. For example, the multiple startup companies founded to commercially distribute prefabricated microtissues, and the Matlab scripts and ImageJ plugins for converting images of fiduciary markers to cellular tractions[44,46] are enabling more investigators to measure cellular forces in their own laboratories. One caveat to such ‘standardized’ software is that it cannot verify when the experimental conditions satisfy or violate assumptions required in the force calculations. Furthermore, studies have demonstrated that changes in substrate mechanics, cell shape, and multicellular architecture can lead to changes both in cell structure and contractile forces[66,72,92–96]. Thus how one compares forces exerted by cells in one context versus another remains challenging.

There remains an inherent tradeoff between force resolution and cost of implementation and analysis (Table 1). The macroscopic methods (collagen contraction, microtissues) are more straightforward to implement, but resolving the contribution of individual cellular contractile forces to observed tissue contraction has not been possible. On the other hand, smaller sensors (TFM, micropillars) provide a more direct measurement of cellular forces but require complicated equipment and methods for implementation, and reduced overall throughput. The newly developed molecular probes shift the burden of implementation to more widely used biological techniques, but interpretation and validation of the forces measured with these probes remains a significant challenge.

The development of molecular biology tools required interdisciplinary collaboration and innovation in multiple fields, from chemistry to physics and mathematics. We expect such collaboration will be needed for major advances in cellular biophysical tools as well. A growing community of scientists and engineers is supporting the continual development of methods to address current shortcomings in measuring cellular forces. Further integration with new biological tools to control intracellular signaling will allow the field to reach a point where we can control cellular forces from the inside-out, in addition to measuring their magnitude and direction. Although we are still in the early stages, as these methods mature, the focus will shift from tool development to understanding forces as an effector and regulator of cells and tissues.

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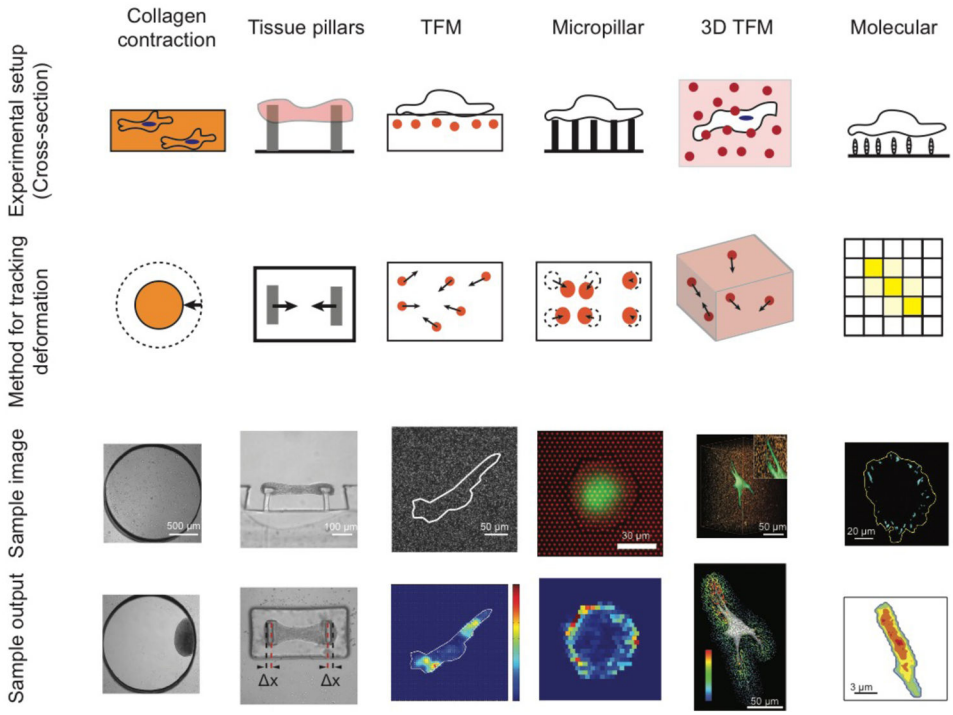

Figure 1.

Methods for measuring cellular forces (adapted from refs. [578198]. TFM images courtesy of J.J. Fredberg[99]).

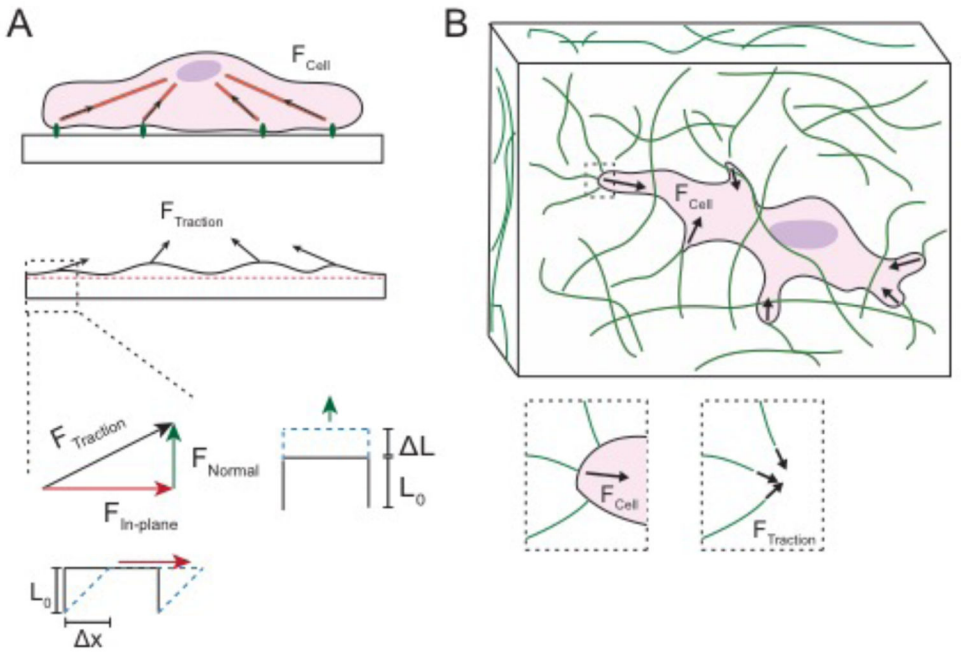

Figure 2.

Cellular tractions on 2D and in 3D substrates. (**a**) Traction forces applied by cells induce deformation to the cell substrate and are balanced by reaction stresses within the substrate (not shown for clarity). TFM and micropillar assays measure the component of cellular traction forces acting in the imaging plane ($F_{In\text{-}plane}$), parallel to the substrate surface. 2.5D TFM enables quantification of the traction components normal to the field of view (F_{Normal}). (**b**) In 3D ECM, cellular tractions are distributed throughout the 3D space, and traction forces propagated along ECM fibers cause remodeling of the ECM, altering local mechanical properties.

Table 1

Methods for measuring cellular forces.

	Force/stress range	# cells per measurement	Spatial resolution*	Substrate/stiffness	Special requirements	Strengths	Major limitations	Sources
Collagen gel	N/A	1×10^4–1×10^6	N/A	• 3D collagen type I • 0.01–0.1 kPa	• None	• Ease of implementation	• Qualitative • Cannot determine forces from single cells	2,11
Tissue pillars	• 1 uN – 0.5 mN • 0.02 kPa – 2.5 kPa	100–2×10^6	4 mm	• 3D collagen type I, Matrigel, or fibrin with embedded PDMS pillars • 0.05–1.125 μN/um	• Tissues <10 mm require microfabrication	• High throughput • Ease of computation	• Requires highly contractile cells • Cannot determine forces generated by single cells	12,15,16,18,20
TFM	• 2 nN – 120 nN • 0.05 kPa – 0. 6 kPa	1 – 1×10^3	2 μm	• 2D collagen type I, fibronectin, or RGD coated PEG, PDMS, or PA • 1.2 – 1,000 kPa	• Hydrogel or PDMS synthesis and functionalization • Microparticle tracking algorithms	• Uses standard lab equipment and fluorescence microscopy	• 2D substrates • Computationally expensive • Requires cell lysis or manipulation	27,31,51,99,32,100,101
Micropillar	• 50 pN – 100 nN • 0.06 kPa - 8 kPa	1 – 10	1μm	• 2D collagen type I, collagen type IV, or fibronectin coated PDMS • 1.9 – 1,556 nN/um	• Microfabrication • PDMS functionalization	• Ease of implementation and computation	• Forces are independent for posts • Fabrication	66–69,74
3D TFM	• Not characterized • 0.1 kPa – 5 kPa	1	5 μm	• 3D RGD-conjugated PEG • 0.6—1 kPa	• Confocal microscopy • 3D mesh editing and finite element software • 3D, MMP-cleavable synthetic hydrogels	• Fully resolved 3D tractions in physiologic 3D environments	• Currently limited to single cells • Computationally expensive	57
DNA hairpin	• 4.7 pN – 2 nN • 0.15 kPa – 50 kPa	1	0.2 μm	• 2D RGD-conjugated DNA hairpin on glass • 50 GPa	• DNA hairpin synthesis	• High resolution with standard fluorescence microscopy	• 2D • Glass • High sample prep time	81,82

*Minimum distance between which two point forces can be resolved