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

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

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, cell-generated forces are not widely characterized. In contrast to the powerful and widely used array of molecular genetic tools for examining the expression and activity of any specific protein, current understanding of the role of mechanical force in cell biology is based on techniques typically implemented in only a few laboratories. The methods vary considerably in their ease of use, their assumptions, and 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 in other papers, this report is meant to be a brief guide rather than an in-depth review and to serve as a technical resource for investigators seeking to understand the available options for examining 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 elsewhere[1]. The methods we discuss here 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 a small collection of cells, (2) methods that measure only deformation versus those that translate 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 for characterizing the presence of cellular forces involve measuring deformations of cells, substrates, or tissues without attempting to relate those deformations to an actual force. For example, stromal cells embedded in 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

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by neighboring cells[8–10]. The advantage of these approaches is that they do not require *a priori* knowledge of the mechanical properties of the material being deformed or complex calculations to convert deformation to force (**Box 1** and **Fig. 1**). In the most conservative sense, these approaches report the actual measured variable. However, deformation-based methods also have drawbacks. Implicit in the analysis is the assumption that more compaction or retraction means more cellular force, but fracture, plasticity, and viscoelasticity of the material may invalidate this assumption (**Box 1**). In addition, the mechanical properties of living materials can change actively in response to perturbation, causing the tissue to undergo more or less compaction under constant force. Further, the time scales of these deformation assays (e.g., 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.

Two general approaches have been used to measure the forces generated in compacting hydrogels. The first is to use a gel that is

BOX 1 TRACTION MEASUREMENTS REQUIRE AN UNDERSTANDING OF 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 (σ, in pascals), and strain, the fractional change in the length of a material (ε, 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 the strain[24] (**Fig. 1**). 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 infinitely large 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 on 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 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 in ref. 100), the nonlinearity and length-scale dependency of these materials greatly complicates the measurement of 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 needs to be first determined and then used to relate measured strain to stress. The difficulty of 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 measurement of 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. Recent work estimating the forces from cells embedded in fibrous matrices has made some early advances[56].

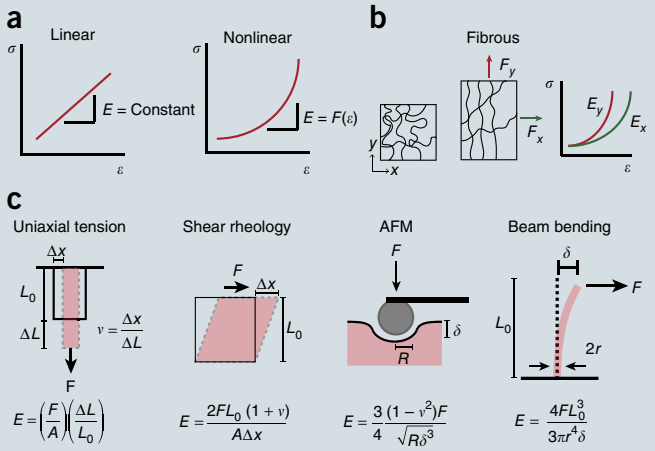

Figure 1 | ECM mechanical properties determine the relationship between force and deformation. (**a**) The elasticity, given here as the Young's modulus (E), determines the relationship between stress and strain in linear materials. In nonlinear materials, E is a function of strain. (**b**) The ECM is fibrous with anisotropic and nonlinear material properties. (**c**) Common methods for determining the mechanical properties of materials used to measure cellular forces. ν, Poisson ratio; F, force; A, cross-sectional area. All other variables not defined in the text are geometric parameters defined as in the schematics.

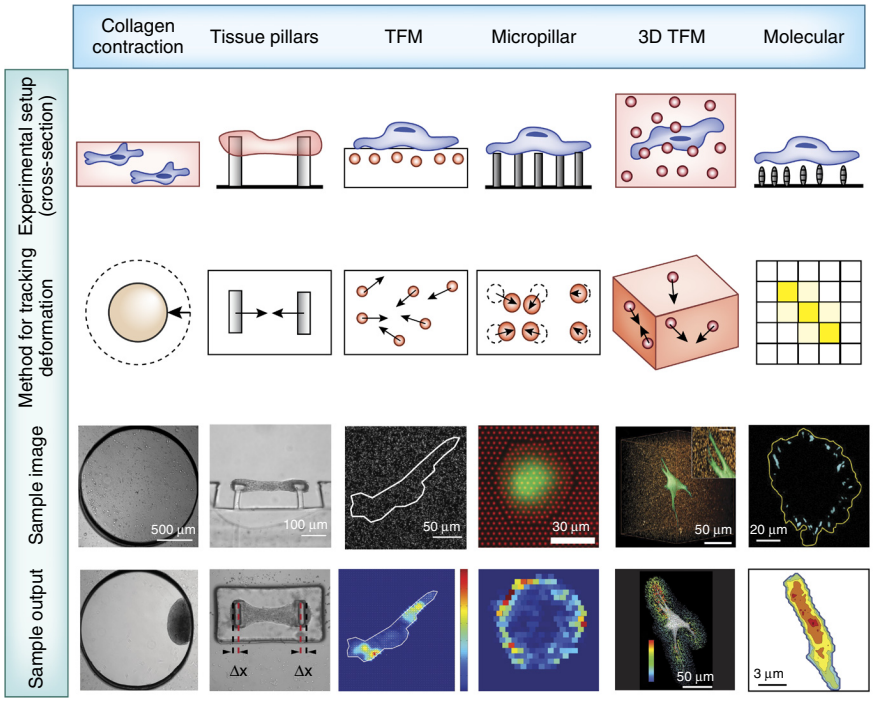

Figure 2 | Methods for measuring cellular forces. Adapted from refs. 57 and 81 with permission from Nature Publishing Group and from ref. 96 (http://dx.doi.org/10.1039/C0IB00156B) with permission of The Royal Society of Chemistry. TFM images courtesy of J.J. Fredberg[97].

large enough to be attached 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 the actual force might be beyond the expertise of a standard biological laboratory. Furthermore, these methods are limited in throughput because the lower bound of the sensor's operating range 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. 2**). The displacement of the free end of a cantilever can be imaged with optical microscopy, and the observed displacements can be 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 also can be made much smaller than the electronic assays mentioned above, which means they require fewer cells and less extracellular matrix (ECM) material, and they 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 the creation of 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]), fabrication of 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 done with commercially available PDMS and a vacuum chamber. However, microfabrication facilities are required for the creation of the original silicon master; although foundries will fabricate silicon masters for a cost, the technical designs needed 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 therefore depends on the specific formulation used 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 (in the range of piconewtons to nanonewtons) and occur across small length scales (nanometers to micrometers), and therefore 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 allow one to 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

Table 1 | Methods for measuring cellular forces

	Force and stress range	Cells per measurement	Spatial resolution[a]	Substrate and stiffness	Special requirements	Strengths	Major limitations	Reference(s)
Collagen gel	N/A	1×10^4 to 1×10^6	N/A	3D collagen type I Young's modulus: 0.01–0.1 kPa	None	Ease of implementation	Qualitative Cannot determine forces from single cells	2,11
Tissue pillars	1 μN–0.5 mN 0.02–2.5 kPa	100 to 2×10^6	4 mm	3D collagen type I, Matrigel, or fibrin with embedded PDMS pillars Pillar stiffness: 0.05–1.125 μN μm^{-1}	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	2–120 nN 0.05–0.6 kPa	1 to 1×10^3	2 μm	2D collagen type I; fibronectin; or arginine–glycine–aspartic acid (RGD)-coated PEG, PDMS, or PA Young's modulus: 1.2–1,000 kPa	Hydrogel or PDMS synthesis and functionalization Microparticle tracking algorithms	Uses standard lab equipment and fluorescence microscopy	2D substrates Synthetic substrates with limited biological relevance Computationally expensive Requires cell lysis or manipulation	27,31, 32,51,97–99
Micropillar	50 pN–100 nN 0.06–8 kPa	1–10	1 μm	2D collagen type I, collagen type IV, or fibronectin-coated PDMS Pillar stiffness: 1.9–1,556 nN μm^{-1}	Microfabrication PDMS functionalization	Ease of implementation and computation	Forces are independent for posts Fabrication	66–69,74
3D TFM	Not characterized 0.1–5 kPa	1	5 μm	3D RGD-conjugated PEG Young's modulus: 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 0.15–50 kPa	1	0.2 μm	2D RGD-conjugated DNA hairpin on glass Young's modulus: 50 GPa	DNA hairpin synthesis	High resolution with standard fluorescence microscopy	2D Currently limited to glass substrates Long sample-prep time	81,82

[a]Minimum distance between which two point forces can be resolved.

and a normal component, which acts perpendicular to the substrate surface (**Fig. 2**). 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 one to track material deformation perpendicular to the viewing plane and enable the computation of both normal and in-plane components of cell tractions.

Measuring cellular tractions in 2D substrates

Cellular traction force microscopy (TFM) involves tracking the deformations of synthetic elastic polymer substrates that result from 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 can 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 (≤1 μm) 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 the following: 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. Computational algorithms are used to analyze the resulting two images (or sequence of images, if dynamic forces are being measured) 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 cellular forces to be mapped with subcellular resolution. Such measurements have enabled characterization of the force dynamics involved in a variety of cell biological processes such as adhesion maturation[32,33], migration[28,34–36], differentiation[37], and malignant transformation[38].

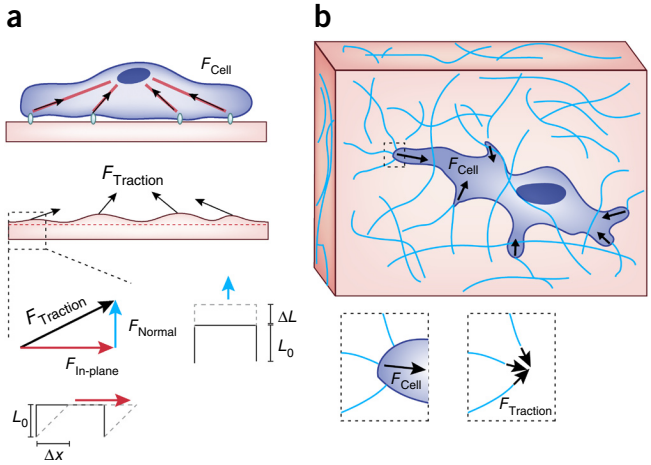

Figure 3 | Cellular tractions on 2D and in 3D substrates. (**a**) Traction forces applied by cells induce deformation to the cell substrate and are balanced by reaction stresses within the substrate (not shown for clarity). TFM and micropillar assays measure the component of cellular traction forces 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.

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 cell substrates in TFM because their mechanical properties are well characterized and they behave as linear elastic solids under deformations typical in cell traction force measurements[39,40] (**Box 1**). 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. Although this is beneficial for quantifying cellular tractions, recent data suggest that degradation and ECM reorganization contribute to the 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 used to cross-link 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 spans only the higher range for native ECM (**Table 1**); it therefore remains unclear how well the tractions measured on these synthetic materials correlate with tractions generated *in vivo*.

The computational analysis required to calculate microparticle displacements and forces has been 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 the displacement of many surrounding beads because of 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 to 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 tasks 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 determine 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 might 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, and imaging parameters), has thus far necessitated collaboration with groups that possess significant TFM experience and expertise. Even when one is adopting existing software plug-ins, the strengths and limitations of the different computational strategies can be difficult to sort through, and thus consultation with a laboratory with TFM experience may be required 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. 3**). 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**), we and others have modified TFM methods to track bead displacements in three dimensions with confocal microscopy[29–31,50–52]. Computing out-of-plane tractions also requires considerable computational resources, and many of the inverse computation methods for 2D TFM are not valid for 3D measurements[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 environments are strikingly different from those of cells cultured on 2D surfaces[53]. The nature of the cellular traction forces that underlie these phenotypic differences has been the subject of much interest recently; however, measuring tractions of cells in three dimensions is difficult not only because of the requirement to track fiduciary markers in three dimensions, but also because the material properties of biologically relevant 3D culture materials are much more complicated than those of the synthetic materials used for the measurement of tractions in two dimensions (**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 in pericellular collagen[54,55]. However, the nonlinear, fibrillar nature of collagen hydrogels (**Box 1**) prevents the calculation of traction forces from these measured deformations by 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]. The use of synthetic, MMP-cleavable polyethylene glycol (PEG) hydrogels that are linearly elastic in the range of deformations induced by single cells, along with bead tracking in these materials, has enabled measurements of cellular tractions in three dimensions[57]. However, the computation of cell tractions from measured bead displacements is cumbersome, and resolving 3D tractions for cells in a 3D environment 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) comprising deformable silicon elements and integrated electronics allow cellular forces to be converted to electrical signals directly on chip[58,59] (reviewed in ref. 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, which alters their electrical properties and causes 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 of these systems currently is that typically only zero, one, or two probes are in contact with a cell at any one time, so spatial distributions of forces cannot be recorded. Though these systems promise the eventual development of a packaged cell-traction tool able to measure tractions from hundreds or thousands of cells simultaneously, the expense and difficulty of fabricating the devices have prevented broad uptake in the biology community so far.

Similarly, microfabricated thin films that deform under the 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 such 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 approach analogous 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 micrometers 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 are 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 a 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 a 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, one can control their stiffness[67,68] (**Table 1**), and because the imaging and computational costs are low compared to those of traditional TFM methods, it is possible to measure tractions for multicellular populations[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 for studies of 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 enable study of 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 can potentially affect the magnitude and distribution of cellular traction forces. Functionalizing the micropillar surface with ECM ligand to promote cell adhesion is also difficult. Furthermore, the 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 PDMS devices is possible in a standard laboratory if the silicon master is available.

Next-generation methods

Traditional methods for measuring cell traction require measurement of the deformations of synthetic cell substrates, and thus the sensitivity of the measurement is coupled to the stiffness of the substrate. Over the past decade, a class of probes that measure strain in molecular springs have been developed that allow high-resolution imaging of tractions on stiff substrates through conjugation of the sensors to the cell culture surface. These molecular tension sensors consist of either a fluorophore and a 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, the difficulty of their calibration prevents straightforward conversion of shifts in emission spectra to absolute forces.

These limitations have motivated the development by our lab and others[80–82] of a new class of DNA hairpin force sensors that 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 with which traditional TFM methods would fail. As with all fluorophore-based sensors, there are limitations

to the use and calibration of the sensors owing to bleaching and optical sensitivity, and unlike TFM-based methods, these methods provide only the magnitude and not the direction of forces. These probes are not yet commercially available, but they have the potential for more widespread adoption.

Another category of FRET-based force sensor detects forces in 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 in cells. Proteins in native cellular mechanotransduction cascades are engineered with fluorophore pairs such that force-induced deformations in the proteins affect the separation distance between fluorophores, and thus the 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 (ref. 85), E-cadherin[86], α-actinin[87], and fibronectin[88].

These molecular methods hold great promise for the measurement of 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 it remains unclear how one calculates the total forces exerted.

Challenges and outlook

Measuring cellular forces in a physiological 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 one cannot measure these forces 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 is 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, along with the Matlab scripts and ImageJ plug-ins 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 for the force calculations. Furthermore, studies have demonstrated that changes in substrate mechanics, cell shape, and multicellular architecture can lead to changes in both cell structure and contractile forces[38,66,72,92–95]. Thus how one compares forces exerted by cells in one context versus another remains a challenging question.

There remains an inherent tradeoff between force resolution and the cost of implementation and analysis (**Table 1**). The macroscopic methods (e.g., collagen contraction and microtissues) are more straightforward to implement, but resolving the contribution of individual cellular contractile forces to observed tissue contraction has not been possible. Smaller sensors (as used with TFM and micropillars) provide a more direct measurement of cellular forces, but they require complicated equipment and methods for implementation and have lower 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 that 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 the measurement of cellular forces. Further integration with new biological tools to control intracellular signaling will allow the field to reach a point where scientists can control cellular forces from the inside out, in addition to measuring their magnitude and direction. Although the field is still in the early stages, as these methods mature, the focus will shift from tool development to understanding forces as effectors and regulators of cells and tissues.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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