Physicochemical Tools for Visualizing and Quantifying Cell-Generated Forces

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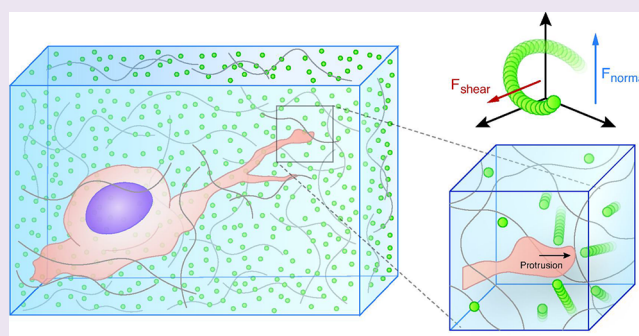
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ABSTRACT: To discern how mechanical forces coordinate biological outcomes, methods that map cell-generated forces in a spatiotemporal manner, and at cellular length scales, are critical. In their native environment, whether it be within compact multi-cellular three-dimensional structures or sparsely populated fibrillar networks of the extracellular matrix, cells are constantly exposed to a slew of physical forces acting on them from all directions. At the same time, cells exert highly localized forces of their own on their surroundings and on neighboring cells. Together, the generation and transmission of these forces can control diverse cellular activities and behavior as well as influence cell fate decisions. To thoroughly understand these processes, we must first be able to characterize and measure such forces. However, our experimental needs and technical capabilities are in discord—while it is apparent that we should study cell-generated forces within more biologically relevant 3D environments, this goal remains challenging because of caveats associated with complex "sensing—transduction—readout" modalities. In this Review, we will discuss the latest techniques for measuring cell-generated forces. We will highlight recent advances in traction force microscopy and examine new alternative approaches for quantifying cell-generated forces, both of individual cells and within 3D tissues. Finally, we will explore the future direction of novel cellular force-sensing tools in the context of mechanobiology and next-generation biomaterials design.

1. INTRODUCTION

Cells and subcellular components are constantly subjected to mechanical forces, and the physical cues they elicit act alongside soluble chemical signals and are important mediators of cellular communication and behavior. This complex interplay between aspects of the biophysical and biochemical is driven by a process known as mechanotransduction. It describes how the mechanical information in a cell's local microenvironment is converted into biochemical cues, which often feed into intracellular signaling cascades and relay instructions for changes to the transcriptional and translational programs operating in the nucleus of the cell.[1] In this way, the ever-evolving physical aspects of a cell's environment such as extracellular matrix (ECM) stiffness,[2−4] nanotopography,[5−7] and the contractile activity of neighboring cells[8,9] can have a profound impact on cell behavior and fate (Figure 1).

These external forces originate from a variety of sources and occur across a range of length and time scales. For instance, they may be the result of the body's physical movements during exercise or the perfunctory mechanical activity of the cardiovascular or respiratory systems. They may have tissue-specific origins, such as the shear stress of blood flowing across the endothelium, the pressure of which can regulate vascular physiology as it deforms endothelial cells lining the inner wall of blood vessels.[10] There are also microscale forces at play,

such as the interstitial flow of fluid through the ECM or the contractile forces exerted by cells as they migrate through or over tissues. While the effects of these biomechanical cues have not yet been as thoroughly characterized as their biochemical counterparts, it has already been established that mechanical stimuli regulate many fundamental cellular processes including adhesion,[11] migration,[12] proliferation,[13] and apoptosis.[14] Physical forces also help to orchestrate multicellular-level biological events such as embryogenesis and morphogenesis, which requires cells to generate enough force in order to move, bend, and deform both themselves and other cells as they self-assemble into tissues and eventually 3D organs. Not only do these forces drive physical development, but they can also influence developmental programming by modulating signaling pathways and early gene expression. For instance, it has been shown that mechanical pressure can ectopically activate or restore expression of the developmental regulator gene
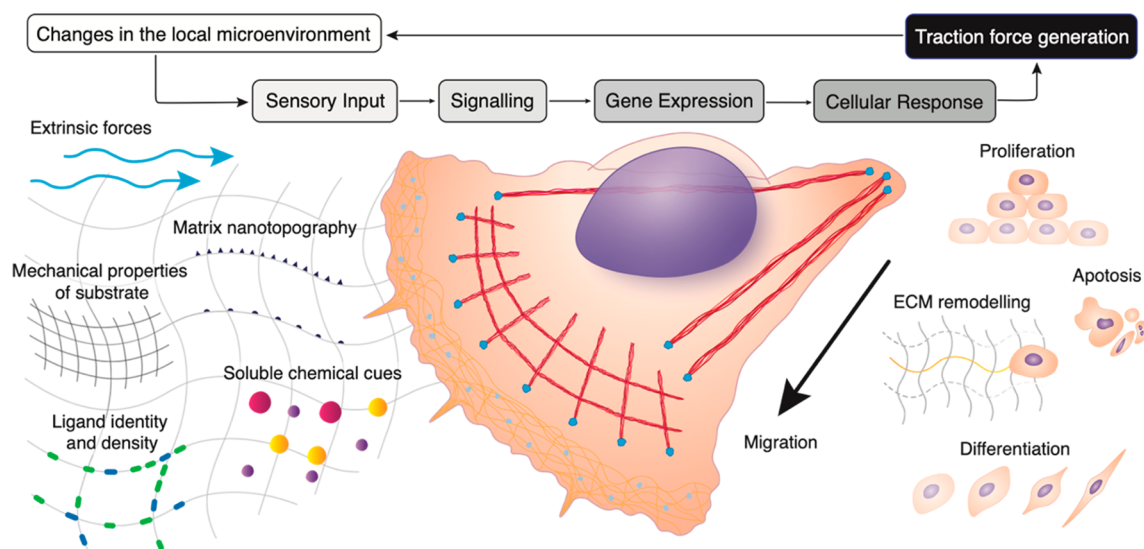
Figure 1. Relationship between local changes in the ECM, traction generation, and cellular output and behavior. As mechanical and chemical stimuli in the local microenvironment are registered by a cell, signaling pathways and gene expression programs are initiated within that activate traction force machinery. The cellular forces exerted regulate many fundamental cellular activities, some of which result in the introduction of new mechanical information, thus enabling complex coordination and cooperation between cells in tissue.
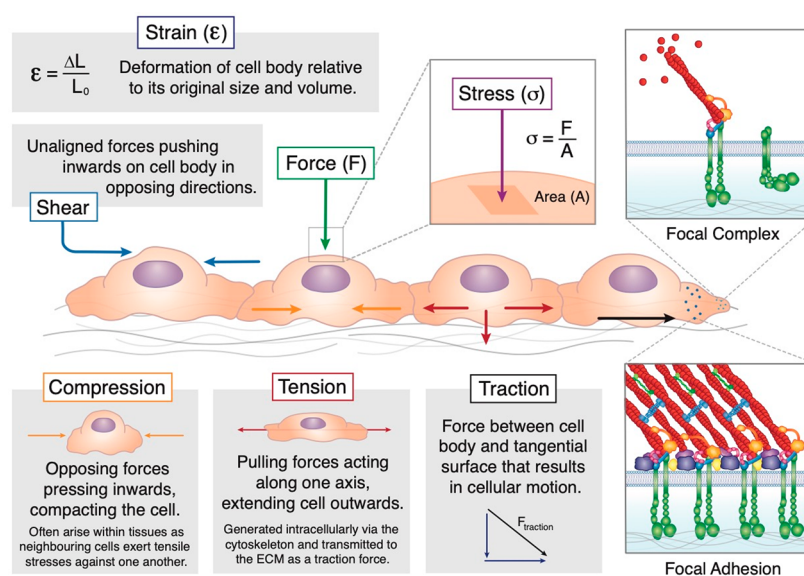

Figure 2. When force is applied to a region of tissue, mechanical stress (σ) is given as the ratio of the force (F) to the area (A) over which it is experienced. Compressive and tensile forces correspond to those aligned inwardly to and outwardly from the cell body, respectively. Shear forces arise when these inward forces are misaligned. Strain (also known as deformation; ε) is the relative change in size of the cell body after experiencing a force. It is a dimensionless quantity whereby the change in the length of the cell body (and L_0 is the original length) is positive for elongation and negative for contraction. Forces are reported in Newtons (N) and stresses in Newtons per square meter (N/m^2) or Pascals (Pa).

brachyury, in *Nematostella vectensis* embryos.[15] Cells are capable of responding to such stimuli using subcellular structures that double as mechanosensing machinery, for example, the nucleus,[16] the cytoskeleton,[17] the plasma membrane,[18] mechanosensitive ion channels,[19] and cell-adhesion complexes.[20] Our understanding of the molecular mechanisms underpinning cell-ECM adhesions and how forces arise and propagate at the molecular level remains rudimentary, but the growing collection of tools at our disposal for investigating cellular force generation make this an area increasingly accessible for scientific exploration. In this review paper, we explore the suite of techniques developed for monitoring cell and tissue generated traction forces, spanning

conventional traction force microscopy (TFM) techniques, designer macromolecular probes, and intra/extra- cellular transduction modalities.

1.1. Traction Force Generation. In addition to external forces acting on cells, an equally fundamental component of cellular biomechanics is the ability of cells to generate endogenous forces. Through their actin cytoskeleton, cells can also generate and exert nanonewton (nN)-scale forces[21] on their surroundings and other cells. A family of heterodimeric transmembrane receptors known as integrins are primarily responsible for coordinating the mechanical interactions between cells and the ECM by triggering the formation of focal adhesions, which physically link cells to their surround-
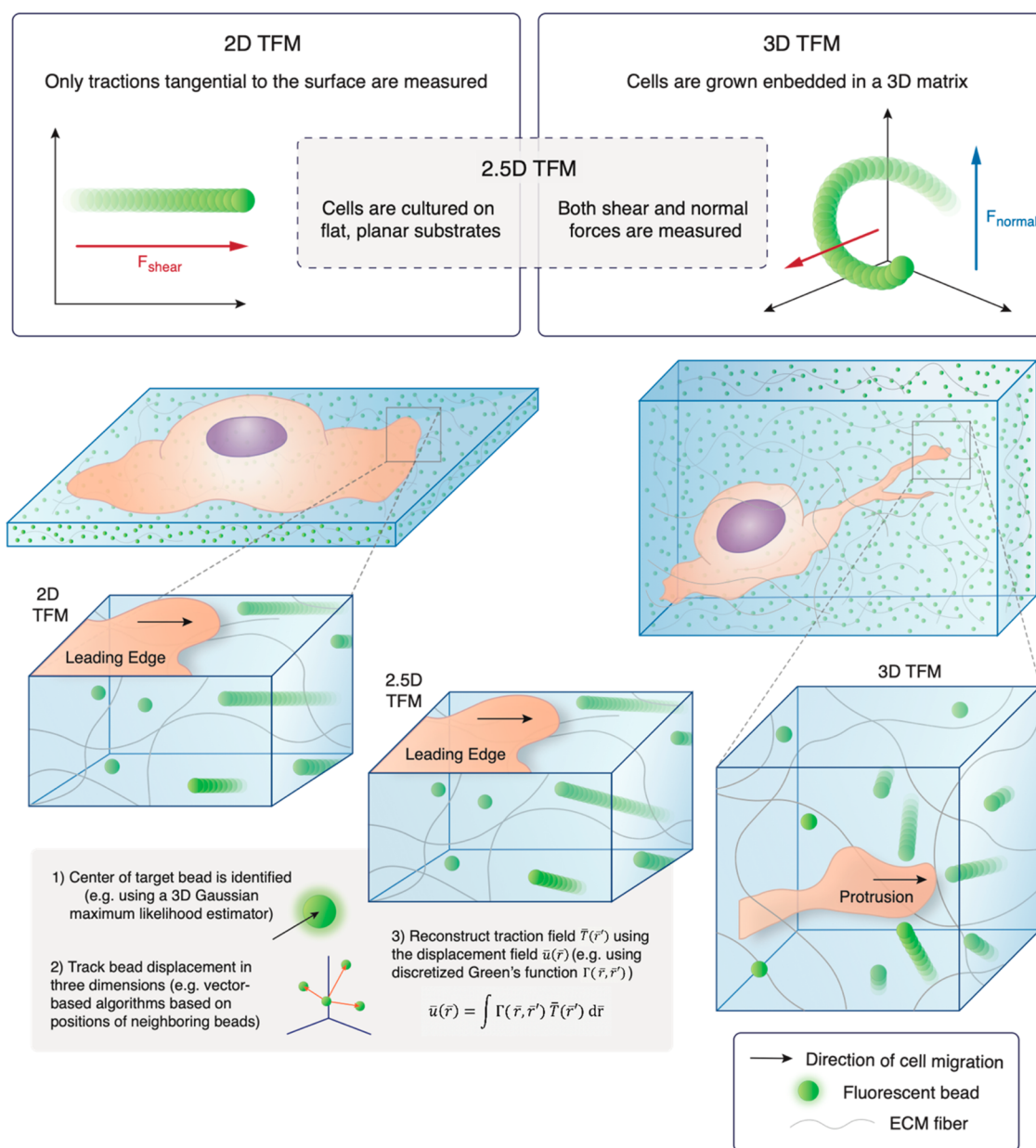
Figure 3. Comparison between 2D, 2.5D, and 3D TFM systems. In 2D TFM, the displacement of fiducial markers can only be captured in the x and y directions, allowing shear, but not normal forces to be measured. Cell-ECM adhesions are restricted to the basal surface, leading to unnatural cell morphology. In 3D TFM, the movement of fiducial markers can be tracked in all three spatial dimensions, allowing shear and normal forces to be reconstructed. In 2.5D TFM, cells are still grown atop flat substrates, but out-of-plane forces can still be detected.

ings. Binding to fibronectin causes integrins to activate, dimerize, and begin to cluster into nascent adhesion sites called focal complexes.[22] These structures mature into focal adhesions, as adaptor and scaffolding proteins such as talin, vinculin, and paxillin are intracellularly recruited to help link the actin cytoskeleton to integrin receptors. Thousands of integrins and upward of 50 helper molecules[23] including integrin-binding proteins and enzymes such as focal adhesion kinase (FAK) and Src cluster at these sites to assist in the polymerization of actin filaments into longer bundles of stress fibers. The subsequent pulling interactions between myosin motor proteins and the stress fibers of the cytoskeleton build up intracellular tension. Anchorage-dependent cells ultimately release this tension by exerting contractile forces that are transmitted to the ECM through the anchoring of focal adhesions in what is known of as a traction force, which may be on the order of anywhere from 100 to 1000 nN[24] (Figure 2).

These traction forces are essential to the mechanosensing and mechanoresponsive nature of cells. They serve as a mechanism by which cells can actively probe and interpret the mechanical characteristics of their local microenvironment, while concurrently allowing cells to physically react to any external stimuli. For example, cellular tractions can invoke remodeling of the surrounding matrix,[25] modulate cell morphology and polarity,[26] or enable long-range communication between distant cells.[27] Such outcomes can serve as new sources of physical stimuli for other cells, highlighting the dynamic and reciprocal nature of mechanoregulation at the cell-ECM interface. That is, cells can alter the architecture and

tensional conditions of the ECM and iteratively modify their own behavior in turn.

On the molecular level, traction forces can induce conformational changes in mechanosensitive proteins to expose cryptic binding sites,[28] alter the kinetics of receptor–ligand complexes,[29] or activate mechanosensitive ion membrane channels.[19] These events can trigger mechano-regulated signaling pathways, thus governing the expression of particular genes and phenotypes. Furthermore, traction forces are also responsible for the bending, stretching, alignment, and repositioning of cells required for significant tissue-level morphological events such as embryogenesis, wound healing, angiogenesis, and organ development. Cell-generated forces are also relevant in pathological settings such as the metastatic cascade, given changes in cytoskeleton shape and turnover rates of cell–ECM and cell–cell adhesions allow invading tumor cells to detach from a primary tumor site, infiltrate neighboring tissues, and penetrate the blood and lymph vessels to eventually colonize competent nearby organs.[30,31]

Physical cell–ECM interactions are prevalent in many areas of both physiological and pathological cell biology, and it is clear we need the ability to systematically characterize and quantify traction forces. Despite their importance, however, the role of cell-generated forces in directing tissue structure has yet to be thoroughly characterized, and much of the molecular interplay between mechanical stimuli and intracellular signaling remains obscure. In large part, this is due to a lack of experimental techniques that can adequately resolve nN (and by extension, pN) forces at submicron spatial resolutions. Thus, quantitative mapping of traction forces represents an ongoing challenge in the mechanobiology field.

2. MEASURING TRACTIONS IN 2D ENVIRONMENTS

2.1. Traction Force Microscopy (TFM). To date, TFM remains the gold standard approach to measuring cell-generated forces, broadly referring to a collection of techniques that measure the tractions and stresses generated at the surface of a substrate by adherent cells. The defining trait of all TFM methods is that the cellular forces are not measured directly but rather, are mathematically derived based on the deformations made to the substrate by contracting cells. The technique was pioneered in 1980 by Harris et al. who demonstrated that fibroblasts were able to wrinkle a thin silicone rubber sheet as they spread across its surface.[32] Nowadays, standard TFM experiments make use of transparent, compliant materials such as hydrogels, in which a high density of fluorescent micro- or nanometer-sized beads have been randomly dispersed within. Adherent cells are made to interact with the gel through functionalization with adhesion proteins or short peptides. The fluorescent beads embedded within serve as fiducial markers; their lateral displacements from an initial position allow the substrate deformations caused by spreading or migrating cells to be detected. By culturing cells on a continuous elastic material, the cellular forces applied to it can be recovered by inverting a stress–strain constitutive relation using the known mechanical properties of the substrate in a theoretical model or finite element solver. Subsequent computational modeling of the substrate displacement field based on the known elastic properties of the gel gives rise to 2D vector maps of the traction forces produced beneath a cell.

Cells generate both contractile shear stresses (the force component acting parallel to the substrate surface) and compressive normal forces (the force component acting perpendicular to the substrate surface) on their surrounding microenvironment.[33] While 2D TFM successfully captures the shear forces cells apply to a surface because these induce lateral substrate deformations that can be observed in the optical viewing plane, the technique is unable to measure the out-of-plane, normal forces exerted by cells since these deform the substrate axially. This has led to many classical 2D TFM setups to be redesigned so as to enable measurements of these out-of-plane forces on flat substrates (known as 2.5D TFM).[34] However, the main focus in recent years has shifted to developing biomimetic 3D TFM systems which can resolve the traction forces exerted by cells embedded within a 3D matrix that approximates conditions of the native ECM much better than 2D surfaces (Figure 3). Not only does 3D TFM allow shear and normal force components to be extracted, but the encapsulation of cells provides a much more physiologically relevant system in which to study traction force generation. While 3D TFM is still not as commonly employed as 2D TFM since the recovery of traction forces in all three spatial dimensions is far more complex a computational process, a number of 3D TFM systems have emerged in recent years, as will be discussed further in section 3.1.

2.1.2. Approaches to Enhance the Resolution of Traction Imaging. One of the major pitfalls encountered in all forms of TFM is the limited spatial resolution, and by proxy, the limited force sensitivity of the technique. In TFM, the smallest force that can be resolved is dictated by the spatial sampling frequency of the displacement field, which is in turn determined by the density of fiducial markers and microscope optics. In practice, the need to track individual markers accurately competes with one's ability to acquire high-resolution force maps. Labeling the TFM substrate with the maximal bead density is desirable for capturing the complexity of the traction field created by a cell, yet too high a density of fluorescent markers interferes with the ability to accurately determine individual bead movements. Because this represents an inherent trade-off between spatial resolution and force sensitivity, a range of technologies have been introduced in recent years aimed at improving the resolving power of TFM such that it can better capture the processes of cellular mechanics that occur on the submicron length scale and over subsecond timeframes.

One such method involves the use of stimulated emission depletion microscopy (STED) to suppress the fluorescence of the outer regions of the PSF with a secondary laser beam.[35] The overall technique is known as super-resolved traction force microscopy (STFM) because, by reducing the intensity spread of the point source that is the fluorescent markers,[36] it can resolve traction forces at the submicrometer level (Figure 4, panel a). In effect, STFM can obtain such fine spatial detail—the authors report a 5-fold improvement in rendering marker displacements—because it permits a very high density of beads to be loaded into the gel without compromising the accuracy of marker displacement measurements. Yet, this method suffers from increased acquisition time since its point-scanning nature slows the imaging speed. The advent of 3D super-resolution fluorescent structural illumination microscopy (3D-SIM-TFM) has addressed this loss in temporal resolution.[37] SIM removes out-of-focus light to yield an optically sectioned image that presents only the image focal plane, thereby offering resolution doubling in all three spatial dimensions[38,39] (Figure 4, panel b). This technology allows TFM samples to be imaged at
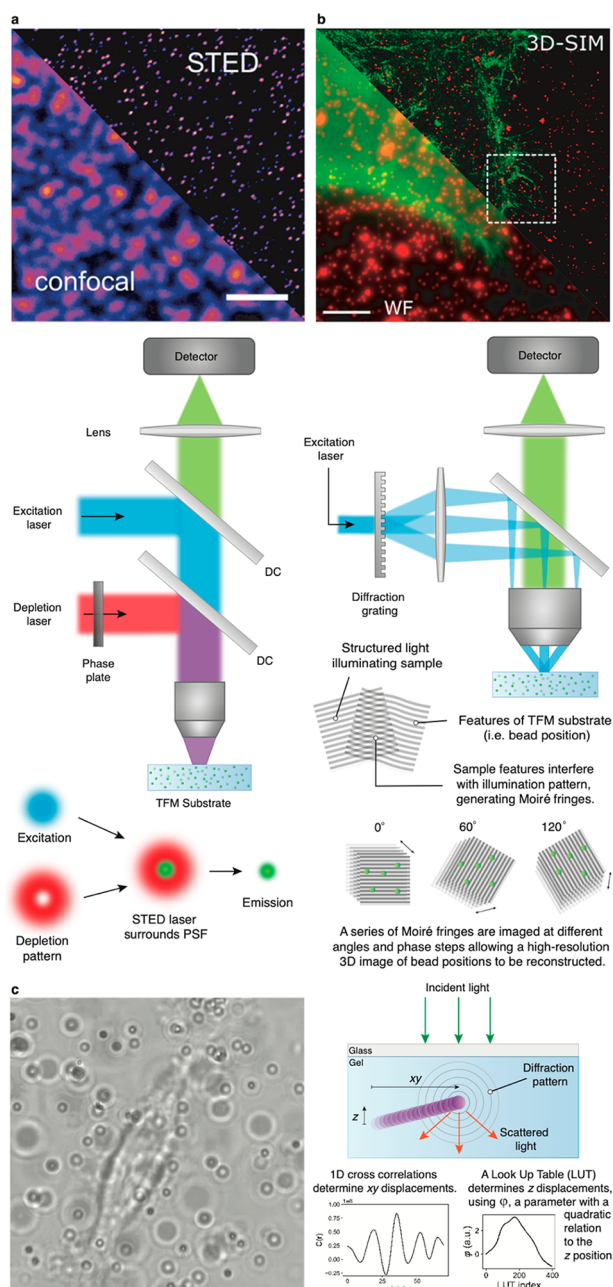
Figure 4. High resolution imaging of fiducial markers in TFM. (a) Super-resolved traction force microscopy using STED in comparison to confocal microscopy. Representative confocal and STED imaging of the distribution of beads at the top surface of the polyacrylamide gel. Reprinted with permission from ref 41. Copyright 2018 Elsevier. Schematic shows the working principle of STED microscopy whereby a second laser depletes fluorescence in the outer region of the PSF to bypass the diffraction limit. (b) 3D-SIM-TFM. Overlay of 3D-SIM and wide-field imaging of an adherent HeLa cell, indicating the improved resolution of both bead and cell imaging. Scale bars are 5 μm. Reprinted with permission from ref 37. Copyright 2019 American Chemical Society. Schematic shows the working principle of SIM microscopy whereby striped patterns of light are used to illuminate a sample. The high frequency of the illumination stripes overlapping with features of the sample produces Moiré patterns that can be used to reconstruct high-resolution 3D images. (c) Holographic TFM using the diffraction patterns of embedded particles to monitor traction. Reprinted with permission from ref 40. Copyright 2018 Springer Nature.

submicron resolution at the speed of a few seconds per field of view. Moreover, by taking a stack of the sectioned images in the z dimension, the technique can be used to quantify normal forces generated by the cell and, thus, reconstruct 3D stress fields at both high spatial and temporal resolution. Another recent approach to high-resolution TFM is the use of holographic tracking microscopy to measure the displacements of fiducial markers.[40] Known as holographic TFM, the technique can achieve nanometer-scale resolutions of marker displacements through the use of nonfluorescent particles at low spatial density (Figure 4, panel c). The technique relies on analyzing the diffraction patterns created by an LED light source shone on micron-sized polystyrene particles. A key advantage of this technique is its ability to compute marker displacements in the z dimension, done so by comparing the intensity profile of out-of-focus markers to a look up table of known z positions. This allows it to recover out-of-plane normal forces that are typically disregarded by conventional fluorescence-based TFM lacking confocal microscopes. However, this low-density marker approach is unable to characterize tractions at the focal adhesion level, making holographic TFM better suited to resolving larger-magnitude forces.

The schematic shows the working principle of holographic TFM whereby the diffraction patterns that originate from the intereference between the incident and scattered light are used to determine marker displacements in all three dimensions.

2.1.3. Reference Free Traction Force Microscopy. Another intrinsic shortcoming of conventional TFM is the need for a reference image of the same field of view in order to compare bead configurations in the stressed and unstressed states of the substrate. To obtain this "zero-force" or "load-free" image, cells must be detached from the substrate, which prevents the postprocessing of TFM samples for any parallel biochemical analysis, such as immunostaining for the localization of protein activity. The advent of reference-free TFM was made to bypass this experimental restriction, involving the fabrication of substrates whereby the fluorescent landmarks are not randomly dispersed within the gel but rather are deliberately arranged in specific and regular patterns such that the initial position of all fiducial markers is known before the occurrence of any cell-induced deformations.

A recent form of reference-free TFM has been that of a monocrystalline array of quantum dots (QDs) nanodrip-printed onto silicone surfaces (Figure 5, panel a).[42] Known as confocal TFM (cTFM), the method features a grid of fluorescent QD discs approximately 200 nm in diameter. As each disc acts as an individual fluorescent marker, multiple QD arrays can be overlaid on the same substrate, supporting the use of color multiplexing for increased spatial resolution.[43] Most notably, with each disc being only 30 nm in thickness or less, the technique avoids introducing any intrusive surface topography which may unwittingly alter the cellular responses and behavior observed.[44] The micropatterning of fiducial markers using lithographic approaches is also another popular method for fabricating repeating and regular arrangements in a reliable and high-throughput manner. For instance, Griffin et al. microcontact-printed circles onto highly elastic PDMS surfaces whereby the relative position of each circle in the uniform hexagonal pattern could be used to determine the deflection of the markers due to cellular tractions (Figure 5, panel b).[45] Fluorescent protein grids have also been micro-contact-printed on polyacrylamide gel surfaces to track cell-induced substrate deformations in a reference-free manner
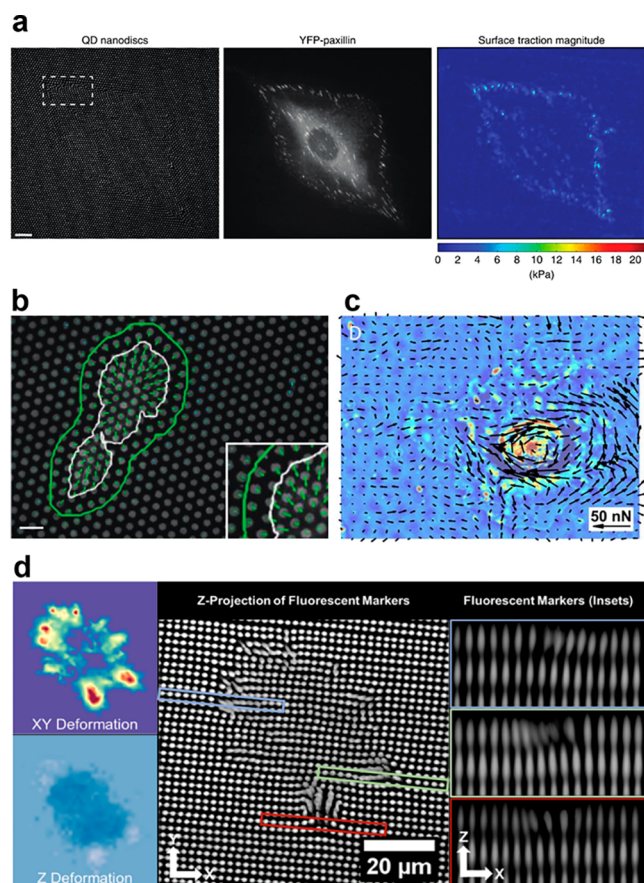
Figure 5. Reference free TFM technologies. (a) Confocal reference free TFM using red quantum dot nanodiscs spaced 1.5 μm apart. Surface traction peaks have been reconstructed and are seen localized at the cell circumference. Scale bar is 10 μm. Reprinted with permission from 42. Copyright 2016 Springer Nature. (b) Hexagonal arrays of microcontact printed protein islands on deformable PDMS substrates. Delaunay triangulation is used to measure their deflected position and thereby calculate displacement vectors (green arrows). A single cell is outlined in white, with its region of influence for deforming the substrate outlined in green. Scale bar is 10 μm. Reprinted with permission from ref 45. Copyright 2019 Elsevier. (c) The traction force field of a cell calculated using Hilbert Phase Dynamometry overlaid on the image of the cell. Reprinted with permission from ref 46. Copyright 2018 John Wiley and Sons. (d) 3D marker array using TP-LSL whereby the ability to observe substrate deformation in the x, y, and z dimensions allows for the measurement of cell-generated shear and normal components of traction forces to be reconstructed. Reprinted with permission from ref 48. Copyright 2019 American Chemical Society.

using a technique known as Hilbert Phase Dynamometry (HPD).[46] In HPD, off-axis holography is employed to calculate the in-plane displacement field, which can then be used to recover the shear forces produced by cells (Figure 5, panel c). A key advancement made by Weaver et al. through the application of HPD is the parallel measurement of cell growth alongside traction forces. Leveraging the reference-free nature of HPD, cells can grow undisturbed in culture allowing traction force measurements to be made over periods of time long enough to capture processes such as mesenchymal stem cell differentiation. This is achieved using spatial light interference microscopy (SLIM), which uses quantitative phase imaging to analyze cell dry mass densities.[47] Micropatterns can be fabricated not only atop surfaces but also internally within

TFM substrates, as demonstrated by Banda and colleagues. Their reference-free TFM system consists of a hydrogel substrate into which a 3D array of fiducial markers has been photolithographically patterned within using two-photon laser scanning lithography (TP-LSL).[48] The technique involves the incorporation of fluorescent poly(ethylene glycol) monoacrylates monomers throughout a poly(ethylene glycol) diacrylate (PEGDA) hydrogel in repeating rows and columns. Hence, this technique is compatible with 3D TFM analyses since the unstressed state of the gel can be digitally reconstructed by approximating the reference positions of displaced markers in deformed regions of the gel in all three dimensions based on surrounding nondisplaced markers (Figure 5, panel d). Moreover, a second round of TP-LSL can be used to photocouple PEG monoacrylates functionalized with the fibronectin derived integrin-binding peptide, arginine-glycine-aspartic acid (RGD), to the hydrogel surface to enable traction force analysis in respect to cell morphology and spread area.

2.2. Micropillar Arrays. Micropost or micropillar arrays can also measure the forces exerted by cells, specifically at individual focal adhesion sites.[49] The technique involves the use of a regularly ordered, vertical arrangement of elastomeric, micrometer-sized pillars (typically on the order of 0.5−10 μm in diameter[50]), the tips of which have been functionalized with ECM proteins in order to support cell adhesion. As cells spread and migrate across the tops of these arrays, the contractile forces they apply cause the pillars to bend laterally. Each pillar deforms independently of others, enabling them to serve as individual cantilevers that can report tractions exclusively at the site where the cell makes contact with pillar tips. Once micropillar deflections have been measured using optical imaging, traction forces can be straightforwardly calculated using classical beam theory and the spring constant, whereby deflection of the pillars is directly proportional to the applied force (Figure 6).[51]

Micropillar arrays were developed as an alternative to TFM because they present several distinct advantages. For instance, reference images are not required in micropillar-based studies since the position of undeflected pillars is already known based on the periodicity of the underlying grid. Furthermore, the
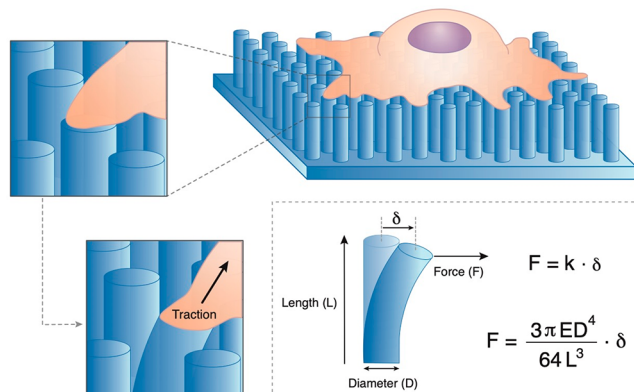
Figure 6. Working principle of micropillar arrays. The cellular forces required to deflect the elastomeric pillars can be calculated using Hooke's law whereby force is equal to the product of the spring constant (k) of the pillars and the sum of pillar deflection and substrate deformation (δ). The spring constant can be found empirically using parameters such as the Young's modulus (E), pillar diameter (D), and pillar length (L).

process of recovering traction forces based on the deformation of discrete polymeric beams is far less computationally intensive compared to that of TFM, since the way deformations propagate on continuous substrates makes it difficult to decouple the displacement of one marker from its neighbors. Additionally, while the ability to vary hydrogel stiffnesses comes at the cost of simultaneously altering other bulk material properties of the gel such as porosity, ligand density, and wettability, the mechanical stiffness of the micropillars can be modulated simply by modifying the diameter[52,53] or height[54] of the pillars. This circumvents the issue of unintentionally changing other properties of the substrate material which might, in turn, influence cell behavior. Finally, increasingly sophisticated micro- and nanofabrication techniques have enabled a variety of micropillar-based systems to be developed to study traction force generation in respect to unique topographies, user-defined external stimuli, and pseudo-3D environments. Micropillars are typically fabricated with circular cross sections; however elliptical[49] and square[55] pillars have also been used to explore the role of substrate geometry on tractions. Another early reiteration of the arrays has been magnetically modified micropillars threaded with cobalt nanowires such that they can apply external forces to cells when in the presence of a magnetic field.[56] Micropillars have also been fabricated on thin stretchable PDMS membranes that apply equibiaxial stresses to adhered cells using a computer-controlled vacuum.[57,58]

As with TFM, developing technologies that improve the spatial resolution of micropillar-based systems is a primary goal. Reducing the size of micropillars down to nanometer dimensions can serve to enhance spatial resolution, with a number of nanopillars approximately 0.5 μm in diameter having been successfully used for traction force analyses.[52,59,60] However, as these have typically been fabricated using polydimethylsiloxane (PDMS), which is characterized by a Young's modulus (E) of 1−7 MPa,[61] it places a lower limit on what the size of the nanopillars can be in order to prevent collapse of the structures. Recently, Shiu et al. reported the fabrication of nanopillars 1.5 μm in height and 0.25 μm in diameter, composed of the much more rigid polymer SU-8[62] (∼600 MPa). With an image resolution of 50 nm per pixel, this SU-8 nanopillar system revealed that the tractions around the perinuclear region of fibroblasts were significantly larger than the adhesive forces acting at the cell's periphery, contrary to what is usually reported. Additionally, a novel plasmonic micropillar platform with self-organized gold nanospheres has also been used to enhance the spatial resolution.[63] Here, gold nanospheres implanted into the tips of the micropillars act as substitutes to the fluorescent molecules that are typically used to coat micropillar tips.[64] Pillar deflections were shown to be precisely reported without the need for high magnification and high numerical aperture objective lenses—the authors reported a spatial resolution of 30 nm using only a 20× objective lens.

As with most experimental platforms for measuring cell-generated forces, the trend in recent years has been the reappropriation of micropillar-based force measurement systems for studies in 3D. For example, as a way of investigating the effect of 3D physical confinement and cell contact area on traction force generation, double-sided micropillar arrays have been fabricated (Figure 7, panel a).[65] By inverting an additional micropillar array on top of an upright bottom array, cells can be cultured in the space created between the two opposing surfaces. PDMS micropillars have
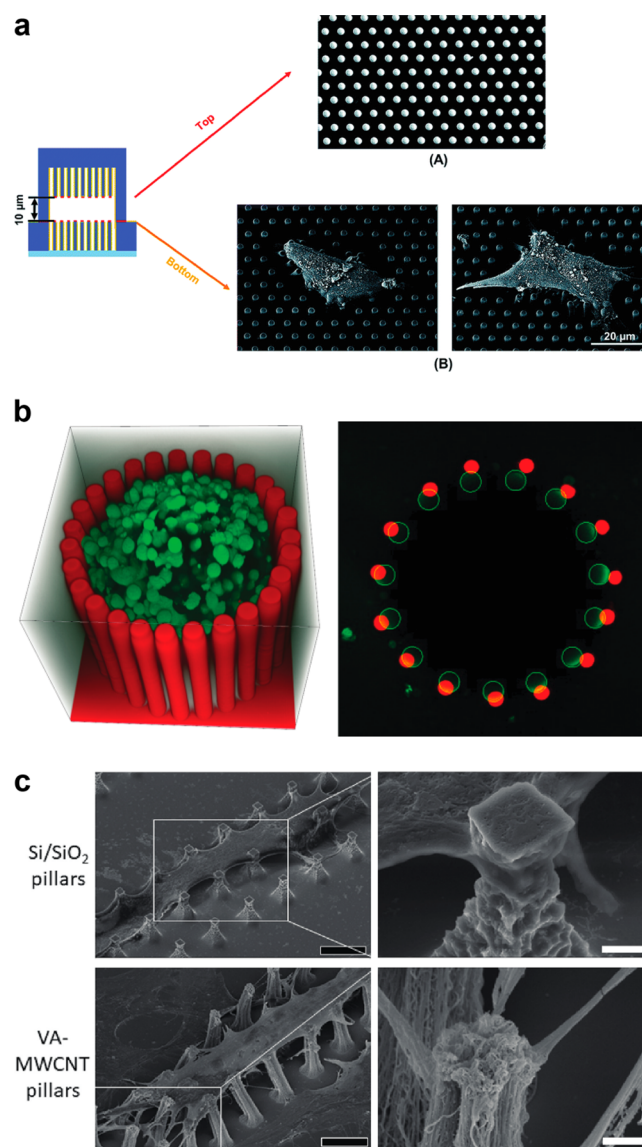

Figure 7. Recent advances in micropillar array platforms. (a) Scanning electron microscopy images showing cell adhesion and spreading on double-sided micropillar arrays. Micropillars are 3 μm in diameter, spaced 3 μm apart, with 10 μm separation between top (A) and bottom (B) arrays. Reprinted with permission from ref 65. Copyright 2019, The Royal Society of Chemistry. (b) The left image is a 3D confocal image showing a micropillar in a ring formation (red) surrounding an MCF-7-derived spheroid (stained green with the fluorescent dye CMFDA-SE). The right image shows the superimposition of the first and last images of the z-stack to indicate pillar radial displacement by the proliferating spheroid; the bottom of each micropillar is seen in green and the top of each micropillar in red. Reprinted with permission from 67. Copyright 2019 PLoS ONE. (c) Scanning electron microscopy images of Si/SiO$_2$ micropillars and vertically aligned, multiwalled carbon nanotube (VA-MWCNT) micropillar arrays on which chondrocytes have spread. Bending of the VA-MWCNT pillars is observed, as well as different anchorage patterns of the cells due to the unique nanostructure of the carbon nanotubes. Black scale bar is 10 μm and white scale bar 1 μm. Reprinted with permission from 68. Copyright 2020 Elsevier.

also been integrated into microchannels of varying cross-sectional areas[66] to enable measurement of the tractions exerted by cells as they migrated through unconfined (mimicked by wide channels 50 μm in width) and confined

microenvironments (mimicked by narrow channels 10 μm in width) as they would *in vivo* through 3D longitudinal channels formed between the connective tissue and the basement membrane of muscle, nerve, and epithelium.[31] Recently, micropillars have also been used to measure and characterize forces exerted by proliferating multicellular spheroids (Figure 7, panel b).[67] Arranged in a ring formation, PDMS micropillars deflected in response to the forces exerted by growing spheroids located in the center. Micropillar array technologies have also been adapted for use with unconventional materials such as carbon nanotubes in replacement of typical elastomeric materials like PDMS.[68] By mimicking the mechanical properties of native cartilage, Janssen and colleagues were able to use this platform to measure the contractile forces exerted by chondrocyte cells on the micropillars, as well as facilitate the unidirectional orientation of chondrocytes (Figure 7, panel c), which is normally a key obstacle in cartilage tissue engineering processes.

However, micropillar arrays are still inferior to TFM in some regards. Most notably, the unnaturally discontinuous surface cells experience on micropillar tips can affect the recruitment of integrins and other adhesion proteins, altering the distribution, size, and morphology of focal adhesions, thereby potentially distorting traction force measurements. Recent studies have shown that focal adhesion formation on nanopillars is fundamentally different from processes on micropillars.[52] More advancements in nanofabrication technologies are therefore necessary to produce arrays of submicrometer-sized pillars that more closely approximate a continuous adhesive surface for cells. In light of this, TFM studies have recently been conducted with magnetic micropillars embedded within a continuous layer of soft PDMS ($\sim$19.2 kPa).[69] Using this system, cellular tractions can be measured through both bead displacement and pillar deformation, as well as magnetic actuation of the micropillars as an additional tool to study the effect of mechanical stimulation on cells. Similar advancements are needed to continue improving the spatial resolution of micropillar-based systems and better resolve individual focal adhesions and early stage focal complexes.

3. MEASURING CELL-GENERATED FORCES IN THREE-DIMMENSIONAL ENVIRONMENTS

3.1. 3D Traction Force Microscopy. Although tractions can be measured in all three dimensions even when flat, elastic substrates are used via 2.5D TFM methods, cells are nonetheless being grown on 2D planar surfaces, which poorly simulate the heterogeneous and dynamic 3D ECM of native tissue. In artificial 2D contexts, cells will adhere to a substrate only along their basal sides, yet in 3D environments, cells are supported by the fibrous architecture of the ECM and can be found bound on all sides. Consequently, when cells are encapsulated in a 3D matrix as opposed to atop a 2D surface, they can exhibit dramatic differences in shape and polarity, cytoskeletal architecture, and focal adhesion morphology.[70] For instance, while hMSCs cultured atop stiff gels show increased osteogenic differentiation, when embedded within gels of the same stiffness they undergo chondrogenesis.[71] Cancer cells in 3D culture present different gene expression patterns and display a higher level of resistance to the same chemotherapeutic agents compared to their 2D counterparts.[72−74] Many crucial physiological and pathological events such as morphogenesis, organogenesis, and tumorigenesis arise

exclusively in 3D contexts. Thus, to extract biologically meaningful results, traction forces should be measured when cells are embedded within biomaterials that resemble the 3D microenvironment. However, this is difficult not only because of the requirement to track fiduciary markers in all three dimensions but also because the mechanical characteristics of physiologically relevant 3D culture materials are much more complex than those of the synthetic materials used for 2D traction quantifications. To promote widespread adoption of 3D traction measurement systems, technologies are required that will (1) enable the imaging of submicron scale features in 3D, (2) further our understanding of the mechanical properties of *in vitro* bioinspired 3D matrices, and (3) improve experimental accessibility to the more rigorous computational algorithms required for extracting 3D stress fields.

Currently, the most popular ECM materials for 3D TFM studies include reconstituted collagen type I hydrogels;[75−77] however Matrigel[78,79] and fibrin[80,81] matrices have also been frequently reported. A significant caveat of collagen hydrogel use, however, is that the nonlinear, fibrillar nature of the gel prevents the calculation of traction forces from the measured deformations by classical mechanics approaches. Moreover, some cell types are capable of plastically deforming collagen by enzymatically degrading or actively secreting and depositing new collagen near the cell surface. For example, mesenchymal cells are able to remodel the collagen matrix either by realigning fibers by exerting tractions or degrading the matrix through proteolysis, further convoluting substrate deformation measurements. Synthetic polyethylene glycol (PEG) hydrogels have been specifically fabricated with the appropriate adhesion sites and proteolytically degradable capabilities for use with 3D TFM.[82] Additionally, Yip et al. recently measured the 3D traction stresses of fibroblasts using polyacrylamide substrates featuring grating grooves.[83] However, *in vivo*, cells do not exist in static environments such as these and are constantly reengineering and spatially reorganizing their local surroundings.

3D TFM experiments are also made challenging due to complex geometries of the matrix that can result from overlapping strain fields, which preclude straightforward calculation of cell tractions. For instance, depending on whether two close-neighboring traction vectors are aligned or opposed, they will generate displacements within the gel that could either add constructively or cancel each other out and negate part or all of the displacement. Further complicating this issue is the inherent heterogeneity and nonlinear mechanical responses of natural hydrogels. To sidestep these challenges, Stout et al. introduced the mean deformation metrics (MDM) approach, which is a kinematics-based method that quantifies the overall cell shape changes such as contractility, mean volume change, and rotation and correlates these parameters to cellular deformations.[76] Recently, Han et al. developed nonlinear stress inference microscopy (NSIM), which is able to infer stress fields in a 3D matrix using microrheology measurements taken with optical tweezers.[9] Because the contractile activity of cells produces dramatic increases in matrix stiffness, the technique is able to resolve stresses in regions surrounding a cell and investigate stress propagation inside 3D matrices by taking measurements of local stiffnesses on the cellular scale. Importantly, both methods have no reliance on knowing the material's constitutive stress−strain relationship, and further advances

like these are critical to making 3D TFM more amenable to longer term studies.

In order to continue developing increasingly realistic material models for 3D TFM that capture this, efforts must be made to better characterize the mechanical properties of collagen matrices, and consideration must be given to the dynamic process of matrix remodeling in order to accurately measure cell tractions in 3D systems. The fibrous and porous 3D polymers most useful as biomimetic ECM materials must be carefully assessed given that their mechanical properties can vary based on the spatial scale that is considered. At nanoscale or microscale dimensions, the Young's modulus E can become dependent on size or geometry and differ from its macroscopic equivalent. While the global stiffness of such materials is crucial to know since its purpose is to be reminiscent of native bodily tissue, individual cells within are interacting with only a miniscule section of the material. As such, mechanical characterization of ECM mimics should involve careful investigation of the bulk material, such as through rheometry-based methods, as well as the local stiffnesses using precise force mapping techniques such as AFM. For the reader interested in further discussion of these topics, we recommend this text.[84]

In addition to the considerations of biomimetic materials, 3D TFM requires imaging systems that can offer high spatiotemporal coverage alongside rapid volumetric acquisition rates. As it stands, confocal microscopy is the imaging modality of choice given that the optical sectioning provided by the confocal pinhole aperture provides high spatial resolution in all three dimensions. However, because it allows a depth range of only a few hundred micrometers, and is associated with photobleaching and phototoxicity effects, several limitations are imposed on the volumetric field-of-view, total imaging time, and maximum sampling rate that can be achieved in 3D TFM experiments. Recently, traction force optical coherence microscopy (TF-OCM) was developed for 3D TFM applications,[79,85] which relies on the interferometric principles of optical coherence tomography (OCT) to enable the quantitative reconstruction of 3D tractions with high temporal sampling. Using a Fourier domain OCM system, TF-OCM can measure traction forces with volumetric acquisition rates on the order of minutes. Additionally, by employing computational adaptive optics, an improved depth of penetration can be achieved. Finally, the label-free imaging nature of OCT at near-IR wavelengths helps to alleviate complications associated with scattering and photobleaching/phototoxicity. As of now, TF-OCM has only been used in traction force studies of single cells, however its ability to image over large volumetric fields of views suggests it could be an invaluable imaging system for multicellular constructs such as spheroids and cell networks.

3.2. Engineered 3D Multicellular Structures. Traction force measurements have mainly focused on isolated, migrating cells such as fibroblasts or cancer cells. However, landmark developmental events like embryogenesis and organogenesis are predominantly epithelial-driven processes, and epithelial cells rarely function individually. Rather, they are found in 3D polarized tissues connected to each other and the surrounding ECM.[86] Even traction force studies about single metastatic cancer cells lack the ability to elucidate the physical nature of cancer in the context of untransformed or tumorous tissues. Research in mechanobiology has also focused on measuring the forces exerted by small colonies or confluent monolayers of cells. While instrumental in furthering our knowledge of

collective cell behavior, these studies have been based on the flawed assumption that cell monolayers reflect the physiology of real tissues. Tissues are not static or homogeneous materials, nor are they comprised of ideal polymers. Developing tissues display striking variations in composition, geometry, and dimensionality as they are molded and shaped in the embryo. Thus, new techniques are called for that can quantify both the local and global stresses exerted by cells within native tissue constructs.

One approach is the insertion of deformable materials of known mechanical properties into the tissue of interest (Figure 8, panel a). For instance, fluorescent oil microdroplets coated with adhesion ligands have been inserted into 3D cell aggregates and live embryonic tissue with their measured deformations used to characterize the local anisotropic normal
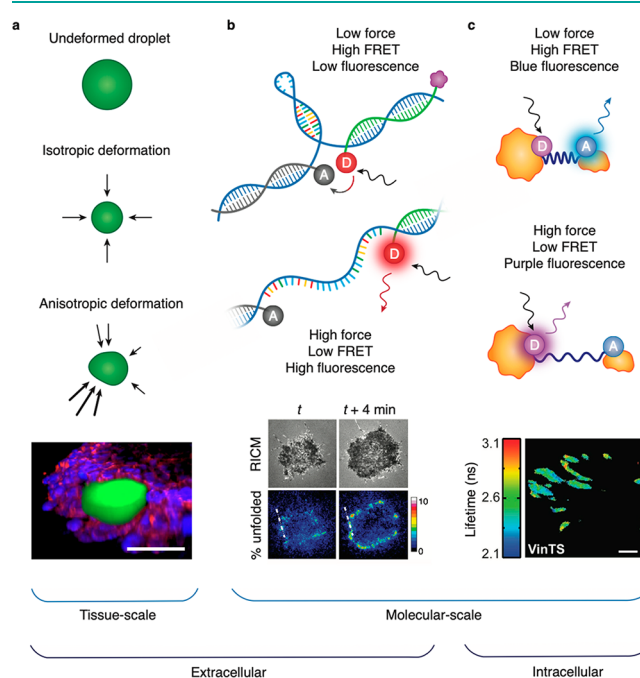

Figure 8. Forms of tissue- and molecular-scale tension sensors. (a) Deformable droplets can be embedded in multicellular structures, such as spheroids, whereby their deformation due to compressive cellular forces is used to map stresses within the tissue. The bottom image is a 3D confocal reconstruction of a deformable polyacrylamide sphere (green) embedded at the edge of a spheroid at day 2 of the culture. F-actin is stained in pink, and cell nuclei stained in blue. Scale bar is 50 μm. Reprinted with permission from ref 90. Copyright 2019 Springer Nature. (b) DNA hairpin probes unfold in response to a threshold amount of cellular force. Strategic placement of a fluorophore and quencher pair at the base of the hairpin allows hairpin unfolding events to be fluorescently tracked. The bottom image shows representative RICM and 22% GC content hairpin probe time-lapse images showing the initial cell spreading and adhesion. The % unfolded channel specifies the fraction of the hairpins that have been unfolded within each pixel. Reprinted with permission from ref 101. Copyright 2014 Springer Nature. (c) GETS are expressed by the cell directly, thus cell-generated forces are reported directly from within the cell. They utilize a peptide spring element that extends in proportion to applied force, in combination with a FRET pair to monitor extension of the spring, to thereby determine the magnitude of cellular force. The bottom image is a fluorescence lifetime image of HEK293 cells expressing a vinculin tension sensor (VinTS). Scale bar is 2 μm. Reprinted with permission from ref 111. Copyright 2010 Springer Nature.

forces occurring during tissue remodelling.[87] Due to their incompressibility, however, these oil droplets are unable to deform isotropically, precluding measurements of compressive stress components. Compressible, elastic microdroplets fabricated from polyacrylamide gels have been introduced to bypass this issue. Stiffer hydrogel microdroplets (~15 kPa) that are able to deform isotropically have been fabricated in order to map variations in tissue rigidity and show instances of nonuniform stress distributions in tumor spheroids.[88] However, the force-sensing range of these stiffer microdroplets is still too coarse for measurements of single-cell forces, which can be 3 orders of magnitude smaller than the external loads used to validate these sensors. Softer alginate microgel sensors (~1.5 kPa) have been reported,[89] however these are complicated to use due to the nonlinear behavior of calcium-cross-linked alginate, as well as the material's sensitivity to external calcium ion fluxes. Most recently, Lee et al. developed even softer polyacrylamide hydrogel microspheres (~0.15 kPa) known as microspherical stress gauges (MSGs) to measure the compressive and tension stresses that arise during tissue remodeling processes.[90] By analyzing their axial or radial deformations, they were able to report the spontaneous formation of a tensional "skin" around spheroids, which is believed to play a role in keeping the tissue intact and under mechanical compression. Similarly, Vorselen and colleagues developed microparticle hydrogel stress sensors to investigate the mechanics of macrophages during phagocytosis.[91] These sensors were designed to undergo IgG-mediated phagocytic engulfment in order to report the cell-generated forces exerted on phagocytic targets, which can be derived following super-resolved 3D analysis of the particle shape before and after cell-induced deformation events.

The norm for traction force measurement systems has been fabricating precise microscale patterns in 3D biomaterials for generating tissue-like scaffolds in which to study cell behavior. However, the rise of 3D bioprinting technologies in recent years has enabled experimenters to quickly and systematically recreate 3D cellular microenvironments and tissue-mimetic architecture in a controlled and high-throughput fashion. For instance, 3D microbeams made from living cells[92] have been bioprinted to investigate how collective cell-generated forces direct the physical evolution of multicellular structures over time. By measuring the stress threshold values required to observe beam buckling, beam contractions or total beam failure and adjusting for the cell volume fraction of each beam, stresses correlated to single cells could be estimated. Other 3D bioprinted tissue constructs have been used to explore how vessel-like networks and vessel lumens form differently in the presence of traction inhibitors blebbistatin and Y27632.[93] While no force measurements were made in this particular study, the qualitative characterization of cellular tractions lay important groundwork for the coupling of 3D bioprinting to TFM studies.

4. MEASURING CELL-GENERATED FORCES ON THE MOLECULAR LEVEL

Tension is transmitted through individual integrin receptors, warranting the development of molecular-scale force sensors to better resolve forces in the low pN regime and study molecular mechanotransduction systems in live cells. A sentiment exemplified best by the work of Han et al., they recently found that the commitment of hMSCs to osteogenic or adipogenic differentiation was dictated only by a 5 pN

difference in tension force exerted through focal adhesions.[94] Estimating single-molecule forces by quantifying substrate deformation, however, is indirect since TFM and micropillar arrays do so by approximating the local density of receptors and averaging substrate stress across micrometer areas. Single molecule force spectroscopy (SMFS) techniques can achieve this but are often low throughput. Further reading on SMFS is recommended here.[95,96] Additionally, because the cellular machinery involved in mechanosensing and force generation is reliant on the nanoscale operation of protein assemblies, there is an outstanding need for bespoke force-sensing tools that can be integrated into living cells and tissues and be operated on the nanometer length scale. To meet this need, a new class of molecular force-sensing probes, collectively known as molecular tension sensors, has been engineered to measure the mechanical forces borne by discrete proteins in living cells and report the traction stresses generated by cells locally and in real time.

4.1. DNA-Based Force Probes. A powerful development in the field of traction force studies has been that of DNA nanotechnology. By exploiting the Watson–Crick base pairing properties of DNA, this technology can be used to create highly discrete and user-defined nanostructures that can readily self-assemble given the appropriate conditions and the rational design of their nucleotide sequences.[97] These qualities make them ideal for use in biosensing applications whereby a suite of DNA nanostructures can be generated easily and rapidly. In particular, the advent of DNA-based tension-sensing devices has transformed the way in which traction forces can be explored. DNA-based force probes utilize a donor/acceptor pair separated by a tension-sensing component, which is comprised of strands of DNA (Figure 8, panel b). Such probes reveal information about the forces generated by individual receptors, through the use of small adhesive peptides to encourage the binding of receptors to the probe. Subsequent tension applied by the receptor extends the tension-sensing component of the probe and fluorescence from donor/acceptor pair to be released. An early predecessor to DNA-based probes featured a flexible PEG linker as the spring element in place of a DNA strand.[98−100] However, probes employing PEG linkers were only able to provide a graded "analogue" response from an aggregate of receptors, making it difficult to reliably measure single-molecule forces. Thus, DNA hairpin probes have been deployed to bypass such limitations.

There are several benefits of using DNA as the molecular building block for measuring and monitoring cell traction. First, DNA is capable of adopting a secondary structure called a hairpin, comprised of a double-stranded "stem" region and single-stranded "loop" region. The hairpin allows DNA probes to behave more like a digital switch because the force-induced unfolding of this hairpin structure is highly cooperative. Thus, these probes abruptly unfold, or become "activated," in response to a threshold amount of force. Second, the force-response threshold of the DNA hairpin can be rationally tuned by simply modifying the hairpin stem in GC content and length; a library of DNA hairpin tension probes can be rapidly generated and enable sensing over various forces of interest (e.g., ~1−20 pN[101]). DNA hairpin probes have been successfully synthesized and used to mechanically investigate not only integrins[101,102] but podosome-based integrins,[103] platelet integrins,[104] and T cells.[105,106] Most DNA hairpin probes lack the ability to provide any directional force information, however a recently developed form of these

Table 1. Techniques for Measuring Cell-Generated Forces

	spatial resolution	force/stress regime	force recovery	reference
conventional 2D TFM	1–2 μm	2–120 nN	shear	129–132
STFM	40–80 nm	<1 nN	shear	35
3D-SIM-TFM	100 nm (lateral), 300 nm (axial)	<1 nN	shear and normal	37
holographic TFM	6.7 μm	~1–10 nN	shear and normal	40
cTFM	0.75–3 μm	~1–30 nN	shear and normal	42
HPD	9 μm	~1–100 nN	shear (demonstrated), normal (theoretical)	46
TP-LSL	2.12 μm (lateral), 3.5 μm (axial)	~100–300 nN	shear and normal	48
TF-OCM	18 μm (demostrated), 10 μm (theoretical)	~1–200 nN	shear and normal	79
micropillars	~1 μm	50 pN–100 nN	shear	49, 52, 133
nanopillars	50 nm	<1 nN	shear	62, 134
oil microdroplets	2–40 μm	~1–4 kPa (nN/μm^2)	normal	87
polyacrylamide microdroplets	~750 nm	0.2–3.5 nN (or 10–1000s Pa)	shear and normal	88
DNA hairpin probes	~200 nm	4.7–19.3 pN	single receptor forces	101, 102, 107, 108
GETS	<100 nm	1–11 pN	single adhesion protein forces	111, 119, 121

probes overcomes this limitation by leveraging excitation-resolved fluorescence polarization microscopy.[107] Since the fluorescence intensity is maximal when the polarization angle of the excitation light and the fluorophore align, the 3D orientation of the probe can be determined by varying the polarization angle and measuring total fluorescence. DNA hairpin probes have also been attached to larger cylindrical DNA origami bodies to allow multiplexing of several probes and increased force-sensing range.[108] Notably, DNA probes are considered reversible reporters. That is, they refold within microseconds once no longer under force, restoring the probe's quenched state and making imaging of transient or short-lived mechanical events with a lifetime below the fluorescent acquisition time window (>100 ms) difficult. Recently, however, DNA probes that can "store" and "erase" a history of mechanical tension have been developed.[105] This system uses a complementary "locking" strand that prohibits reformation of the hairpin to maintain the probe's open fluorescent state, and an "unlocking" strand that displaces the locking strand via toehold mediated strand displacement to reset the probe to its closed quenched state. In essence, this grants experimenters the ability to switch between the locked and unlocked states of the probe and selectively maintain different time frames for the integration of the force signal. DNA hairpin probes also do not exclusively function via the FRET mechanism. Another modality of DNA probes is those that rely on nanometal surface energy transfer (NSET).[105,106] Gold nanoparticles as energy acceptors offer superior quenching capabilities due to their multidipole nature, as well as more efficient energy transfer over longer distances compared to FRET-based probes, offering enhanced signal to background ratios.

However, there is an inherent trade-off between the chemical stability of DNA probes and the need to support normal cell metabolism with the use of serum. Cell culture often requires 2–20% of media to be enriched with serum proteins to provide essential nutrients to cells.

However, serum is also abundant with nucleases that can degrade DNA nanostructures over time. Additionally, cells themselves may secrete proteases and/or nucleases that can further damage DNA-based probes. Zhang et al. reported degradation of their three-stranded probe within 1–2 h of incubation with fibroblasts.[101] Coating the probes with an oligolysine-PEG copolymer has been shown to provide DNA nanostructures with upward of 1000-fold protection against nuclease digestion, as well as stabilize DNA nanostructures in the low salt conditions of cell media,[109] however this is at the expense of longer and more complicated syntheses procedures.

4.2. Genetically Encoded Tension Sensors. The second subset of molecular tension sensors is known as genetically encoded tension sensors (GETS), which are used to provide information about mechanotransduction events in adhesion proteins. Synthesized using recombinant protein engineering techniques, GETS are mutant proteins that have been genetically engineered to include a force sensing component, otherwise known as tension sensor module.[110] This module is comprised of a spring-like peptide linker between a donor/acceptor Förster resonance energy transfer (FRET) pair. The FRET pair is designed to separate when force is applied to the sensor, and the increased distance between the donor and acceptor causes a decrease in FRET efficiency, yielding a fluorescent signal in response to intracellular force (Figure 8, panel c). Cells can be engineered to directly express GETS in host proteins involved in force generation and transmission, which was first demonstrated with vinculin.[111] GETS have since been integrated into other key adhesion proteins, such as talin,[112] E-cadherin,[113–115] α-actinin,[116] desmoplakin,[117] as well as the nuclear lamina.[118] A form of GETS that responds to angular orientation instead of FRET pair distance has also been developed, affording this version of the probe a much wider dynamic range.[119] Typically, the dynamic range, force response, and sensitivity of distance dependent GETS can be calibrated by varying the peptide linker and FRET pair. In order to do so, however, many protein mutants need to be created and tested to assess the best site within the protein to insert the tension sensing module to where avoids or minimizes interferences with normal protein function. Hence, while the attraction of these probes lies in the seamless *in vivo* integration they offer, the biggest drawback of GETS is that it is laborious to design and engineer probes that will be both efficient reporters and preserve the biological functions of the host protein. Furthermore, under the constraint that they can only be tested postsynthesis, *in vitro*, the tailoring of these probes can be a rather low-throughput process.

Additionally, GETS are often criticized for a lack of sensitivity, primarily as a result of a weak FRET signal that is further obscured by the autofluorescence of cells during imaging.[120] Thus, the recent work of LaCroix et al. represents an exciting advancement in molecular mechanobiology, involving a redesigned GETS and proposed computational model for predicting the performance of the peptide spring element within cells prior to synthesis.[121] By using unstructured or "softer" synthetic polypeptide linkers, vinculin sensors nearly 3-fold more sensitive than previous iterations were generated. This is because the mechanical behavior of unstructured polypeptides can be precisely estimated using well-established models of polymer extension,[122] hence the force and extension responses can be determined without the need for lengthy *in vitro* calibration experiments. Second, by using a novel calibration model which describes the relationship between FRET efficiency and applied force as a function of polypeptide length and persistence length (determined empirically), LaCroix and colleagues developed a system to identify the optimal peptide length to measure forces in vinculin. Notably, it is expected that this model will lessen the trial and error burden of GETS engineering and ultimately contribute to the facile and rational design of these probes.

Other forms of DNA-based force sensors include linear double-stranded DNA (dsDNA), such as the tension gauge tethers (TGTs)[123] and, more recently, the integrative tension sensor (ITS).[124] In contrast to DNA hairpin probes, the dsDNA shears irreversibly once having experienced a threshold amount of force, and the probe remains in its fluorescent state leading to signal integration over time and allowing traction force analyses even after cells have been detached from the substrate. Several more insightful reviews detailing the role of DNA nanotechnology in mechanobiology studies are listed here for further reading (Table 1).[110,125−128]

5. CONCLUSIONS AND OUTLOOK

Measuring cell-generated forces is pivotal to understanding some of the most fundamental questions in biology: how are the ultimate architectures of tissues and organs determined? What physical cues determine the fate of an individual cell within the developing tissue or bring about the onset of diseases like cancer? In order to answer these questions, continued efforts to enhance the spatial and temporal resolution of traction force measurements, disseminate more advanced computational models for extracting traction force fields from the raw experimental data and improve the likeness of lab-fabricated biomaterials to better emulate the chemical and mechanical properties of the natural ECM are needed. It is expected that such insight will greatly promote the engineering of biofunctional and mechanoresponsive biomaterials for purposes such as clinical diagnostics and tools for regenerative medicine, since understanding the spatiotemporal generation of cellular forces during biological processes can serve to guide biomaterials design in the laboratory. For example, parsing out the molecular details that define both normal and abnormal mechanotransduction events could deepen our understanding of underlying disease mechanisms to expose new diagnostic or therapeutic approaches. Likewise, studying traction force interaction among populations of cells may allow us to deconstruct some of the mechanical processes underlying tissue organization and development. To achieve these things will require the interdisciplinary efforts of the cell and developmental biology, material science, and engineering communities.

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Notes

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■ KEYWORDS

Traction force: Tension exerted by adherent cells to their surroundings that is tangential to the extracellular matrix or the underlying substrate

Extracellular matrix: The noncellular, three-dimensional network composed of extracellular macromolecules such as proteoglycans and fibrous proteins like collagen, as well as water, minerals, and polysaccharides

Focal adhesion: Supramolecular protein complexes that provide adhesive contact between a cell and the extracellular matrix

Cytoskeleton: The internal framework of a cell comprised of protein filaments and tubules in the cytoplasm

Stress strain constitutive relation: A relation that describes how a solid deforms over time in response to stress

Micropatterns: The fabrication of microscale patterns comprised of adhesive ligands that dictate which areas of a substrate cell adhesion is permitted

Spheroids: Three-dimensional cultures of cells that have self-aggregated into sphere-shaped 3D structures

Elastic modulus: A measure of the stiffness of a solid material, or its resistance to deformation under elastic load; the ratio of stress (force per unit area) to strain (proportional deformation)

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