Colloquium series on Quantitative Cell Biology

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Measuring Cell Mechanics

Margaret Gardel
Patrick Oakes

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Measuring Cell Mechanics

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Colloquium Series on Quantitative Cell Biology

Editor

Wallace F. Marshall

University of California, San Francisco

A fundamental unsolved problem in biology is understanding how a living cell emerges from the multitude of molecular components. While cell biology has made great strides in enumerating all the components of the cell, this is only just the beginning, and the challenge we now face is understanding the cell as a complex, self-organizing system. To meet this challenge, we must take cell biology to a quantitative level, combining mathematical modeling with new methods in measurement and data analysis. The goal of this book series is to provide an overview of current approaches and challenges in the emerging field of Quantitative Cell Biology, in a way that will be accessible to readers both from the biological sciences as well as the physical and computational sciences. These state of the art volumes introduce readers to the cutting edge research in the field, including computational modeling and image analysis methods, while also discussing current understanding and open questions in the systems biology of cells. Each book is intended to be useful independent of the others, and the series as a whole will provide a comprehensive introduction for students and researchers who are new to the field.

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Measuring Cell Mechanics

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ABSTRACT

Cells are inherently physical entities that both experience mechanical forces from their external environment and generate their own internal forces to drive cell motion. Our particular aim here is to present the reader with an introduction to the primary tools used to measure these mechanical interactions and the material properties of cells that result from them. These approaches can be applied to a diverse array of physiological processes and systems, providing important insight into the regulatory roles of mechanical interactions in cells. We cover techniques at both the molecular and cellular scales, including those that actively and passively probe the system. Along the way we cover the fundamental principles of each approach, while emphasizing the relevant length and timescales, along with the typical magnitudes of the measurements that can be made. Each section ends by highlighting uses of the various techniques in recent relevant publications, illustrating the exciting future of cell mechanics in quantitative cell biology research.

KEY WORDS

cell mechanics, cytoskeleton, actin cortex, adhesion, contraction, tension, traction stress, FRET, AFM, TFM, optical tweezers, micropipette aspiration, magnetic tweezers, osmotic shock, laser ablation

Contents

CHAPTER 1

Introduction

As research in cell biology has evolved to incorporate more quantitative approaches it has become increasingly clear that mechanical interactions play a fundamental role in regulating a diverse array of physiological processes (Discher et al., 2005; Dufort et al., 2011; Iskratsch et al., 2014; Lecuit and Lenne, 2007; Schwarz and Gardel, 2012). These mechanical interactions complement and supplement traditional biochemical signaling pathways, and in the process link the molecular to the organismal length scales (Heisenberg and Bellaïche, 2013; Hoffman et al., 2011; Paluch and Heisenberg, 2009; Parsons et al., 2010). As we strive to understand the role of mechanical signaling pathways, we typically try to build realistic models that combine quantitative metrics with qualitative observation (Bergert et al., 2015; Brugues and Needleman, 2014; Oakes et al., 2014; Tee et al., 2015). In order for these models to be useful and, more importantly, predictive they must be informed by accurate and reliable measurements.

Specifically, we are often interested in making measurements of the mechanical properties of cells. This includes material properties such as stiffness or surface tension, and the ability to respond to applied stresses and strains. It also includes active processes such as measuring the amount of force cells can generate on their own, or how strongly they are adhered to a substrate. Making reliable quantitative mechanical measurements is, however, not always a straightforward task. Cells are complex ensembles of a vast array of proteins. They are also sensitive to their surroundings and capable of responding rapidly to perturbations, whether it is direct contact from a probe or a fluctuation in the properties of the extracellular environment. Complicating matters further, cells exhibit heterogeneous properties that can vary in both space and time, and often behave in nonlinear manners. Many of the tools we use originate from approaches in physics and engineering, and were adapted for use with cells. It is thus of vital importance that we not only understand the principles of the tools we use, but also the relevant length and timescales which can be accessed by each approach.

Our goal is to offer an introduction to tools designed to measure mechanical properties of cellular systems. While many of these techniques and approaches are also well suited to *in vitro* systems, we will focus primarily on their use in cells. As the cytoskeleton and its associated proteins are prominently involved in many of the mechanical properties and interactions of cells, a familiarity with their roles and functions is important. Although it is beyond the scope of this present work to delve into those details, a number of reviews are available as resources (Blanchoin et al., 2014;

Fletcher and Mullins, 2010; Gardel et al., 2010; Geiger and Yamada, 2011; Iskratsch et al., 2014; Lecuit et al., 2011; Parsons et al., 2010; Schwartz, 2010).

We begin with approaches aimed at making measurements at the molecular scale and then move to techniques that probe cellular scale properties. We then turn to methods that measure mechanical properties by directly perturbing the system and watching its response. Finally, we will end with a brief comment on the challenges and approaches that await the next generation. In each case, our goal will be to understand the fundamental principles at work with an emphasis on the relevant length, time, and magnitude scales of the measurements.

CHAPTER 2

Molecular Measurements

Cells exhibit rich molecular dynamics that ultimately combine to drive macroscopic cellular processes. Individual myosin motor proteins take steps of approximately 5–11 nm and generate 3–4 pN of force (Finer et al., 1994; Uyeda et al., 1990; Veigel et al., 2003). Actin filament polymerization generates forces on the order of a single pN (Footer et al., 2007). Similar values are found for microtubules and their associated motor proteins (Dogterom and Yurke, 1997; Gennerich et al., 2007). These forces are sufficient to stretch some proteins (del Rio et al., 2009; Grashoff et al., 2010; Jiang et al., 2003), although significantly smaller than the force required to fully unfold an extracellular matrix protein like fibronectin (Oberhauser et al., 2002; Puchner and Gaub, 2009). It is also important to consider that protein binding affinity is force sensitive, with traditional "slip-bond" models predicting enhanced dissociation under applied force, while "catch-bond" models predict reduced dissociation under applied force (Thomas et al., 2008). While slip-bonds are ubiquitous, catch-bonds can often be found in transmembrane adhesion proteins such as integrins (Kong et al., 2009), cadherins (Buckley et al., 2014), and selectins (Marshall et al., 2003) which can allow for stable association of molecules even under higher loads (~10 pN).

Addressing these molecular interactions requires tools that are sensitive to nm perturbations in distance and pN perturbations in tension. The following techniques in this chapter focus on measuring the mechanical interactions at these molecular scales. The Atomic Force Microscope (AFM) and optical tweezers are typically held as the gold standard approach for measuring interactions at these length and tension scales. Unfortunately, due to the fact that cells are living dynamic systems full of densely packed proteins that can act as physical barriers, these techniques are unable to probe individual molecules within cells. We thus look at techniques that are amenable to cellular systems. Interestingly, individual molecules actually exhibit surprisingly rich dynamics even when contained within more stable structures like adhesions (Margadant et al., 2011; Morimatsu et al., 2013; Rossier et al., 2012). Thus, one important caveat of these approaches is that although they report tension across individual molecules, they cannot be taken as proxies for measurements of tension across entire subcellular structures. Macroscopic measurement of cell properties will instead be addressed in the following chapter.

2.1 INTRAMOLECULAR TENSION: FRET TENSION SENSOR

One of the first tools developed for measurements at this length scale takes advantage of the natural behavior of fluorescent molecules. In traditional fluorescence microscopy, a fluorophore is excited by exposing it to a particular wavelength of light (Figure 2.1). The energy from the excitation photon is absorbed by the fluorophore causing an electron to move from the ground state to an exited state. As the electron relaxes back down to the ground state, the majority of the difference in energy between the two states is emitted as a photon. This emitted photon is what we observe as fluorescence. When two appropriately matched fluorophores are in close proximity (usually on the order of a few nm), however, that energy can be transferred between the molecules in a process called Förster Resonance Energy Transfer or FRET (Förster, 1948). During FRET the molecule being excited by the incident light is referred to as the donor and the molecule emitting the final photon is referred to as the acceptor. In this situation the energy released by the relaxation of the excited donor electron is transferred to an acceptor molecule causing one its electrons to move to an excited state (Figure 2.1). The excited electron in the acceptor molecule then undergoes a relaxation, emitting a photon with an energy that is characteristic of the difference between the ground and excited states of the acceptor molecule. Since this energy differs from the energy between the donor ground and excited states, the wavelength of the photon is also different. Furthermore, the probability of FRET between the donor and acceptor molecules is a decreasing function of the distance between them. Thus, by tracking the fluorescent emissions of both the donor and the acceptor molecules it is possible to infer changes in distance between the donor and acceptor molecules.

FRET alone has been used in a number of different systems to show that individual molecules are under tension. When genetically inserted into vinculin, a focal adhesion protein, it is possible to see that vinculin in protruding regions of the cell are stretched more than vinculin in retracting regions (Grashoff et al., 2010). A similar approach using cadherin, an adherens junction protein, revealed differences between the front and back of migrating border cells in *Drosophila* embryos (Cai et al., 2014). To date, a number of different probes have been developed that rely on different linkers, including spectrin repeats (Meng and Sachs, 2011), an α-helix (Meng et al., 2008; 2011) and an elastic domain derived from spider silk, referred to as flagelliform (Grashoff et al., 2010). Although their specific composition may differ, they all operate under the same general principles (Figure 2.2).

Changes in FRET efficiency only report the distance between the donor and acceptor molecules has changed. In order to convert FRET efficiency to tension, the elastic linker between the two molecules must be calibrated. For example, Grashoff et al. explicitly calibrated their probe using a combination of confocal microscopy and a laser tweezer (Grashoff et al., 2010). They were able to show that the 40-amino acid long chain they used as a linker between the donor and acceptor molecules behaved entirely elastically under applied cyclic stress and exhibited no hysteresis (Figure

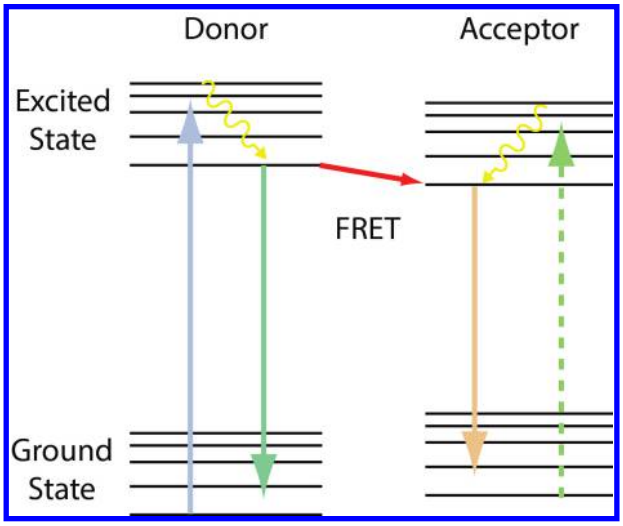


FIGURE 2.1: During typical fluorescence electrons are excited from the ground state to the excited state (blue arrow) where natural relaxation (yellow arrow) results in the emission of a photon of a given wavelength (green arrow). When an appropriate molecule is near enough, the energy from the excited electron in the donor molecule can be transferred to the acceptor (red arrow), which on account of its different ground states, emits a different wavelength photon during relaxation (orange arrow). This process is referred to as Förster resonance energy transfer or FRET. The dashed green arrow represents the excitation pathway for the acceptor molecule alone.

2.3). The FRET signal was most sensitive to forces from 1-6 pN and allowed them to show that the focal adhesion protein vinculin was under the highest tension during adhesion assembly and the lowest tension at the rear of migrating cells.

This particular calibrated linker has since been used widely in a number of different systems. The vinculin tension sensor has been used to look at tension in vinculin at cell-cell junctions (Kuriyama et al., 2014; Leerberg et al., 2014) and at focal adhesions following laser surgery (Chang and Kumar, 2013). Others have inserted the same elastic domain into E-cadherin (Borghi et al., 2012; Cai et al., 2014; Conway et al., 2013) and PECAM-1 in cell-cell junctions (Conway et al., 2013), and in ß-Spectrin (Krieg et al., 2014).

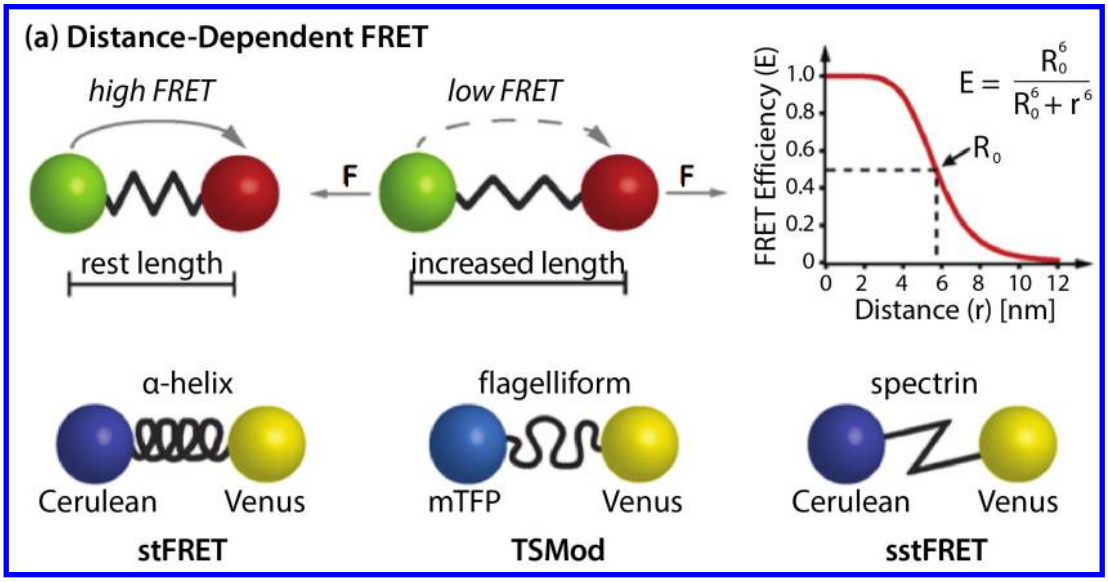


FIGURE 2.2: The basic principle of FRET-based biosensors is to insert flexible linkers into proteins that have been labeled with a pair of FRET fluorophores. The efficiency of the FRET signal is dependent on 1/r^6 and thus is sensitive to changes on the order of a nm. When these proteins are relaxed (i.e., under low tension) there will be a high FRET efficiency. As the proteins are stretched under tension the distance between the fluorophores will increase, resulting in a loss of FRET efficiency. A variety of different linker molecules can be used to connect FRET molecule pairs. Adapted from Cost et al. (2015).

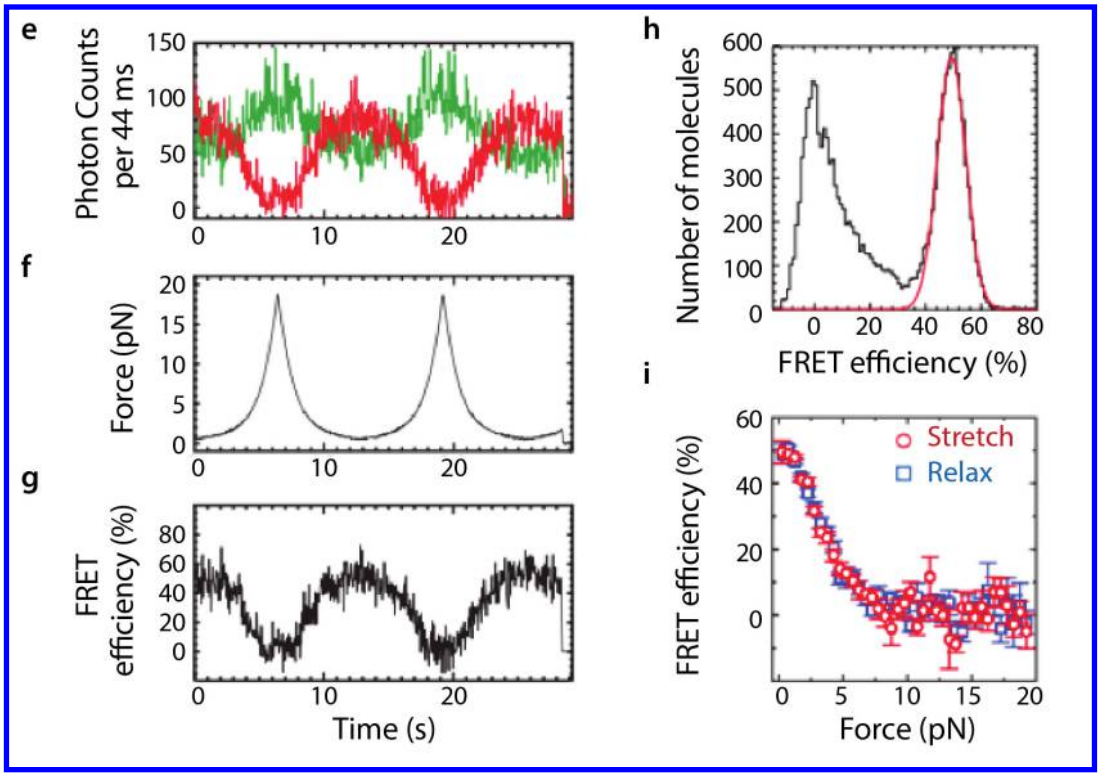


FIGURE 2.3: A representative time trace of (e) donor (green) and acceptor (red) fluorescence, (f) applied force, and (g) FRET efficiency for the TSModCy sensor as an optical tweezers pulls on it. As the force increases the donor fluorescence increases and acceptor fluorescence decreases, resulting in a concomitant drop in FRET efficency. (h) The zero-force FRET efficiency of the TSModCy sensor illustrating the peak FRET efficiency for the construct. (i) Average traces of FRET efficiency as a function of applied force for repeated stretching and relaxation of the TSModCy sensor demonstrating that the linker behaves elastically. This calibration curve allows the user to directly relate FRET efficiency to applied force and illustrates that it is most sensitive from 0-6 pN. Adapted from Grashoff et al. (2010).

2.2 INTERMOLECULAR TENSION: MOLECULAR TENSION-BASED FLUORESCENCE MICROSCOPY (MTFM)

A similar class of sensors has been developed to measure interactions between cells and their external environments. Since these probes do not need to be genetically encoded they can take advantage of organic fluorophores, which tend to have increased photostability. Morimatsu et al. (2013) took the same flagelliform linker used in Grashoff et al. (2010) to link two organic dyes, one of which is tethered to a surface that has been passivated to prevent nonspecific adhesion. This probe, referred to as a Molecular Tension Sensor (MTS), is sensitive to forces in the 1-6 pN range and was used

to demonstrate that tension is not uniformly distributed across integrins in focal adhesions (Morimatsu et al., 2013, 2015).

A slight variant to this approach was used in the development of Molecular Tension-based Fluorescence Microscopy (MTFM) sensors. Where FRET-based sensors use two fluorophores, MTFM sensors use only a single fluorophore and rely on a quenching molecule to reduce the fluorescence signal under low tension (Chen et al., 2010). Quenching has been shown to be possible using the QSY21 molecule (Jurchenko et al., 2014; Stabley et al., 2012) and gold nanoparticles (Liu et al., 2014, 2013). These systems make use of flexible PEG linkers which behave like entropic springs and are sensitive to forces ranging from 0–25 pN and changes in distance from 5–25 nm (Figure 2.4). Liu et al. used this system to study the tension across integrin molecules during cell spreading, finding that the ability to cluster allows integrins to support a 2–3 fold increase in tension during adhesion maturation (Liu et al., 2014).

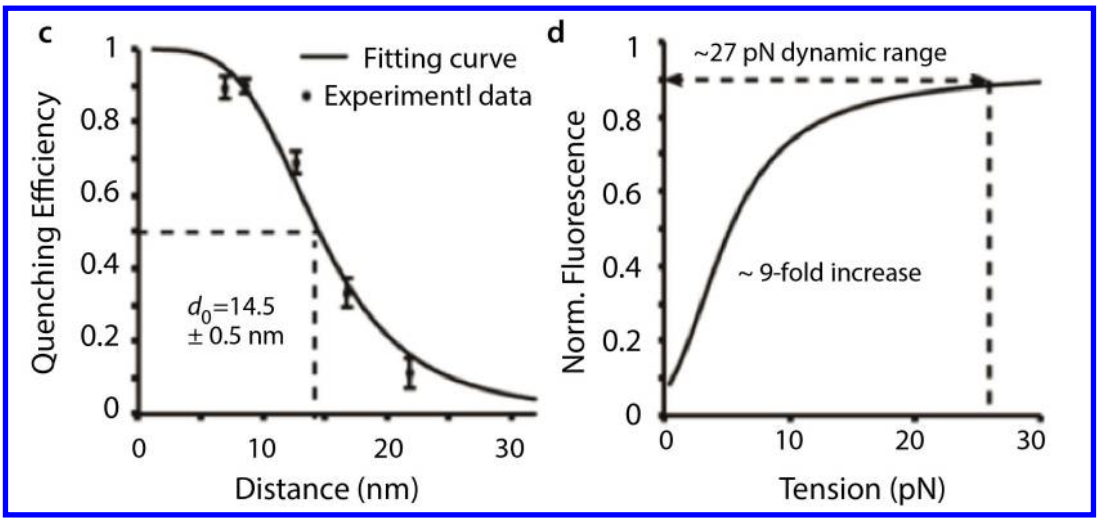


FIGURE 2.4: (c) The quenching efficiency of gold nanoparticles based on the distance of the fluorophore from the particle. As the sensor is stretched the quenching efficiency decreases. (d) The predicted change in fluorescence based on applied tension using a worm-like chain model. Adapted from Liu et al. (2014).

Typically, these sensors use biotin-streptavidin bonds to secure the sensor to the substrate surface. It should be noted that recently Jurchenko et al. suggested that the tension generated by cells could actually be strong enough to dissociate biotin-streptavidin bonds (Jurchenko et al., 2014). This could thus lead to artificially high fluorescence values and only provide a lower bound estimate on the tension across the molecule.

2.3 MOLECULAR ADHESION: DNA ZIPPERS

It is also possible to create a sensor that does not rely upon fluorescence at all. Wang and Ha took advantage of DNA zippers to construct what they termed Tension Gauge Tethers (Wang and Ha, 2013). These tethers consisted of double-stranded DNA bound on one end to the surface, and on the other with a ligand of interest. The geometry of the system then determines whether the tether ruptures through low level forces via unzipping, or high forces via shearing (Figure 2.5) (Hatch et al., 2008). By varying the geometry they were able to create tethers with tolerances of 12–56 pN. Using this system they demonstrated that cells were unable to spread on tethers that ruptured at less than 43 pN and that less than 12 pN of tension was required to activate Notch receptors (Wang and Ha, 2013).

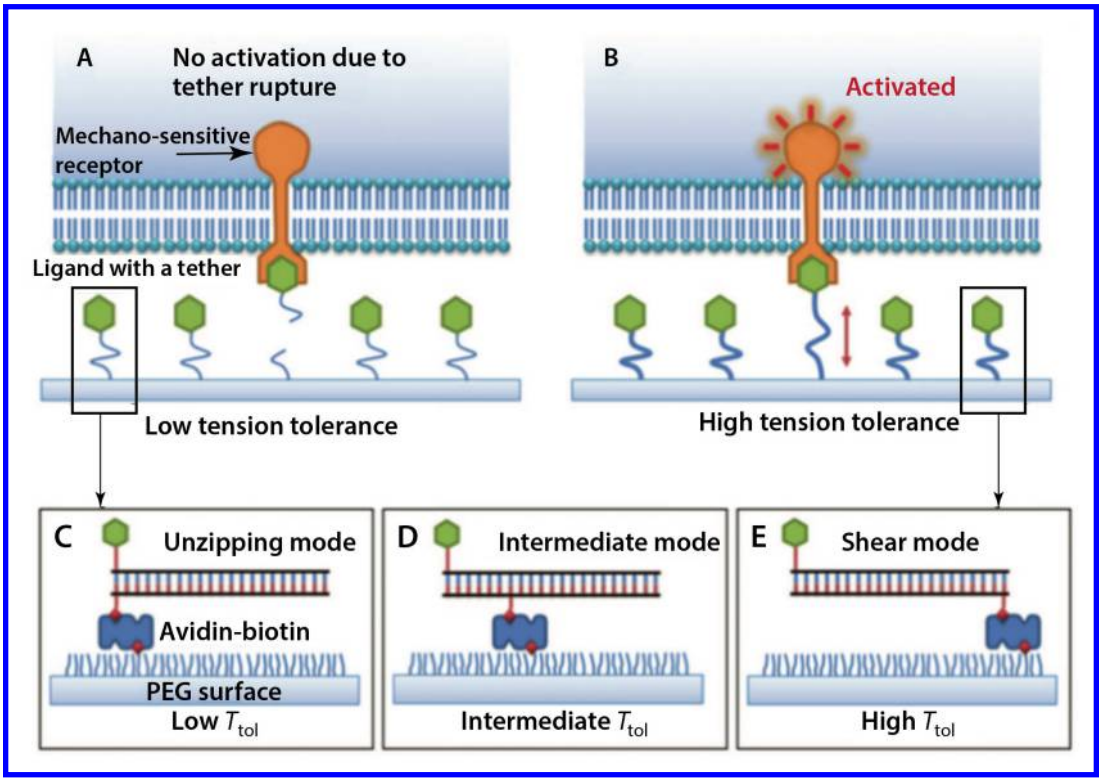


FIGURE 2.5: TGTs take advantage of the different force required to unzip vs. shear double-stranded DNA. By modulating the DNA geometry Wang and Ha were able to create a series of tethers of various strength to probe Notch receptor signaling and integrin binding during cell spreading. Adapted from Wang and Ha (2013).

CHAPTER 3

Mechanical Properties of Cells

The mechanical properties and interactions of the cell with its external environment are the product of the coordinated interaction of many thousands of molecules, and thus can differ drastically from the molecular behavior described in the previous chapter. While an individual myosin motor generates force on the scale of a few pN (Finer et al., 1994; Veigel et al., 2003), collections of myosin motors in the cytoskeleton combine to generate several nN of force at focal adhesions (Stricker et al., 2011). Similarly, while actin filaments assemble and disassemble on second timescales (Pollard and Borisy, 2003), remodeling of the entire cytoskeleton occurs on the timescale of tens of minutes (Aratyn-Schaus et al., 2011; Hotulainen and Lappalainen, 2006). When we measure force generation and adhesion strength, we are measuring the collective behavior of thousands of molecules working together.

Addressing cellular scale measurements requires a different set of tools that are sensitive to forces ranging from 10 pN to 100 nN, timescales ranging from s to hrs, and length scales of many μm. Here we focus on techniques that measure mechanical properties of cellular systems. For some approaches the definition and the units of the measurements are clear and universally agreed upon. For instance, in one dimension stiffness is measured as an object's resistance to deformation, and as such has units of [force]/[length]. Other measurements, such as contractile strength or adhesion, can be more ambiguous and confusing. For each approach we discuss we will attempt to clarify and define the quantity, the magnitude and the units of the measurements. Similar to the caveat offered at the beginning of the previous chapter, it is important to note that these techniques measure properties of collections of proteins and thus do not expressly inform us about individual molecular activities.

3.1 FORCES GENERATED BY CELLS: TRACTION FORCE MICROSCOPY

Traction Force Microscopy (TFM) is a broad term used to describe techniques that measure stress applied by a cell to an external substrate. The glass and plastic dishes that are typically used to culture cells in the lab are incredibly rigid, making it impossible for the cell to deform them. It was only when Harris et al. (1980) developed a system of soft elastic substrates that it became possible to directly investigate contractile forces generated by single cells. Since then, the use of flexible substrates has helped to establish the importance of mechanical signaling in a broad array

of biological systems (Discher et al., 2005; Dufort et al., 2011; Gardel et al., 2010; Hoffman et al., 2011; Lecuit et al., 2011).

TFM requires a deformable elastic substrate to which a cell can adhere. The substrate must be soft enough to deform measurably, but stiff enough that the deformations remain small and thus in the linear regime of the elastic behavior of the material. If the deformations become large enough, the substrate can exhibit non-linear responses that are difficult to interpret. Approaches to TFM can be divided into two categories: soft elastic films and micropillar arrays.

3.1.1 SOFT ELASTIC FILMS

Soft elastic films can be constructed from a number of different polymers, but the most common are polyacrylamide (PAA) (Aratyn-Schaus et al., 2010; Plotnikov et al., 2014) and polydimethylsiloxane silicone (PDMS) (Style et al., 2014). Once polymerized, the surface of the gel is coated with extracellular matrix (ECM) protein to enable the cell to bind sufficiently. In the case of PAA, proteins must be covalently linked to the surface (Aratyn-Schaus et al., 2010; Kandow et al., 2007) while on PDMS ECM proteins can simply be adsorbed to the surface. The mechanical properties of the gel are tuned by varying the ratio of polymer to crosslinker, and can be measured directly using a variety of macroscopic approaches, including rheology and AFM (Frey et al., 2007; Pelham and Wang, 1997; Tse and Engler, 2010; Yeung et al., 2005). Both PAA (Frey et al., 2007; Yeung et al., 2005) and PDMS (Cesa et al., 2007; Chen et al., 2011) are able to access a broad range of physiological stiffness. While both of these materials are optically transparent, and thus amenable to high-resolution imaging, only specific formulations of PDMS are compatible with techniques like Total Internal Fluorescence (TIRF) Microscopy (Gutierrez et al., 2011). PAA cannot be used for TIRF because as a hydrogel its refractive index is the same as water.

Deformations of the gel are typically directly measured by tracking fluorescent microspheres which act as fiducial markers (Pelham and Wang, 1999; Sabass et al., 2008; Style et al., 2014) (Figure 3.1). The strain in the substrate is determined by comparing images of the strained gel (i.e., with a contractile cell attached) with a reference image taken after the cell has been detached. Using particle tracking or techniques like particle image velocimetry (PIV), a 2D strain field can be measured (Martiel et al., 2015; Plotnikov et al., 2014; Sabass et al., 2008). To calculate the traction stresses the problem of a point force applied to an elastic half space (Landau and Lifshitz, 1986) must be inverted and solved. This can either be done directly (Dembo et al., 1996) or in Fourier space (Butler et al., 2002; Sabass et al., 2008) to produce a 2D stress map. Under optimal setups the spatial resolution is ~ 0.5 μm (Sabass et al., 2008; Stricker et al., 2010), and depending on the elastic modulus of the substrate, stresses on the order of 10-10^5 Pa can be reliably measured. While these formulations implicitly assume that the applied stress is a shear stress (i.e., in the plane of the gel), there is in actuality also a normal component. The contributions of the normal direction can

be measured by tracking displacements of beads in 3D (Franck et al., 2011; Legant et al., 2013; Toyjanova et al., 2014). While the contributions of the out of plane stresses can be significant, the 2D approximations are sufficient for well spread cells. A recent review offers a more thorough discussion of the various force reconstruction approaches (Schwarz and Soiné, 2015).

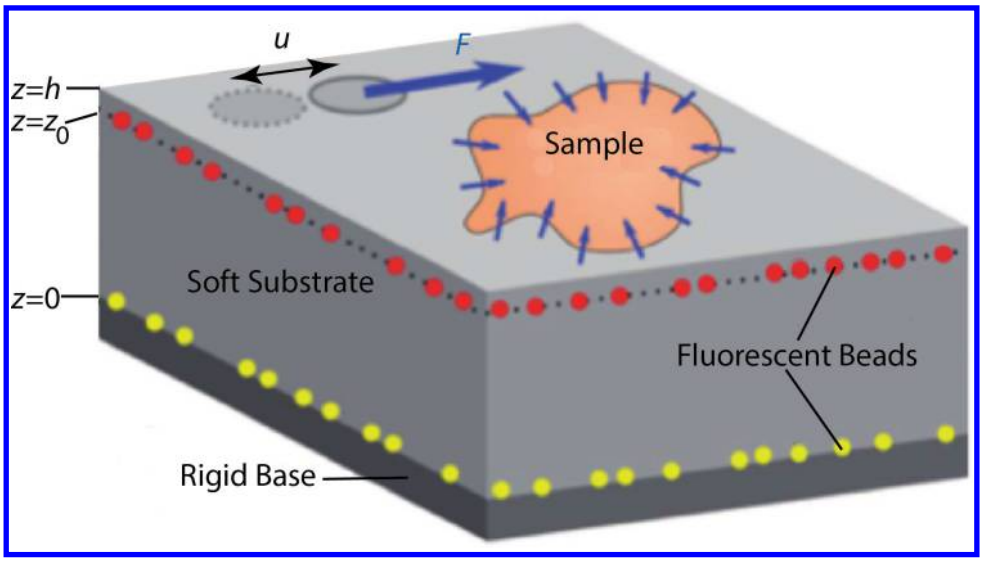


FIGURE 3.1: The typical setup for TFM on a soft elastic substrate. A thick film, typically polyacrylamide or PDMS, is impregnated with fluorescent microspheres. Deformations produced in the substrate by the cell generating stress are measured by tracking the displacements of the fluorescent beads. Beads lying deep in the substrate, or well outside the strain field produced by the cell, are used to align the images. Image adapted from Style et al. (2014).

3.1.2 MICROPILLAR ARRAYS

Micropillar arrays consist of a series of periodically spaced posts, with dimensions ~ 1 μm in diameter and ~1–15 μm in height. Typically, made using PDMS, these arrays are fabricated from molds created via photolithography (Gupta et al., 2015; Roure et al., 2005; Tan et al., 2003). Liquid PDMS is poured into the mold and cured to produce an array of uniform pillars (Figure 3.2). The array is then detached from the mold and coated with ECM protein by bringing the tips of the pillars into contact with a surface coated with ECM. The effective spring constant of the pillars is tuned by altering both the height and the dimensions of the posts (Fu et al., 2010; Han et al., 2012; Rodriguez et al., 2011; Saez et al., 2005; Trichet et al., 2012). Forces applied by the cell are deter-

mined by measuring the displacement of the pillar centroid from the undeflected state. According to work from Fu et al. (2010) the bending modulus of pillar lengths greater than 5 μm is well described by the classical Euler-Bernoulli beam theory approximation $K = 3\pi Ed^4 / (64L^3)$, while for lengths shorter than 5 μm the spring constant can be extracted from Finite Element Modeling analysis. Forces measured on pillars typically range from 1-50 nN (Ghassemi et al., 2012; Han et al., 2012; Roure et al., 2005; Tan et al., 2003).

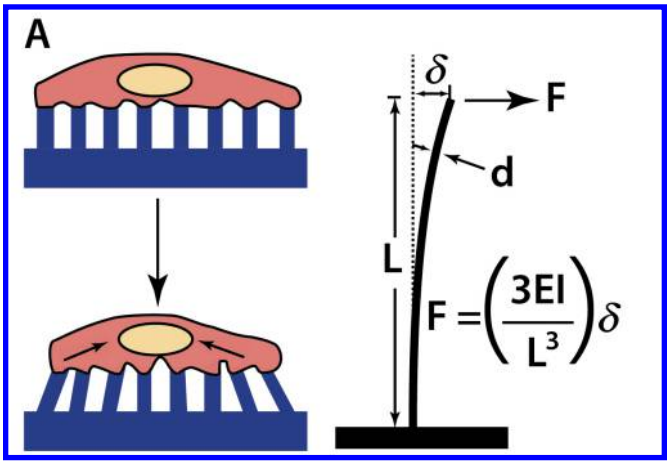


FIGURE 3.2: TFM performed on arrays of PDMS micropillars. Each pillar is treated as an isolated elastic spring, which when pulled by the cell, bends. The apparent stiffness of the array can be tuned by changing the pillar height and geometry. Strain fields are determined by measuring the deviation of each pillar centroid from its rest position. Adapted from Tan et al. (2003).

At the subcellular level, TFM has primarily been used to study focal adhesion proteins which link the contractile elements in the cytoskeleton to the elastic substrate. It has been shown that initial engagement of an adhesion with the substrate results in a change in cytoskeletal dynamics (Gardel et al., 2008; Thievessen et al., 2013), suggesting that adhesions behave like a clutch (Aratyn-Schaus and Gardel, 2010; Chan and Odde, 2008; Lee et al., 1994). Such a mechanism, in conjunction with variations in contractile behavior, could potentially be used to probe the local rigidity of the substrate (Ghassemi et al., 2012; Plotnikov et al., 2012). Finally, while a number of works have investigated the role of adhesion size in regulating traction stress (Balaban et al., 2001; Beningo et al., 2001; Goffin et al., 2006; Riveline et al., 2001; Tan et al., 2003), it has been recently shown that a strong correlation only exists during the initial growth phase of the adhesion (Stricker et al., 2011).

Interpreting traction stress data at the cellular scale has proved challenging. Traction stresses have been shown to correlate with spread area (Califano and Reinhart-King, 2010; Lo et al., 2000; Mertz et al., 2012a; Reinhart-King et al., 2003, 2005; Wang et al., 2002), the number of focal adhesions and their area (Dumbauld et al., 2013; Fu et al., 2010; Han et al., 2012; Weng and Fu, 2011), substrate stiffness (Califano and Reinhart-King, 2010; Fu et al., 2010; Ghibaudo et al., 2008; Han et al., 2012; Lo et al., 2000; Paszek et al., 2005; Weng and Fu, 2011), and cell geometry (Rape et al., 2011; Roca-Cusachs et al., 2008). The main difficulty in understanding these results has been that a number of these parameters are inherently coupled. For example, a bigger cell will almost always have more focal adhesions. Making matters more complicated, the field has used a number of different metrics (including but not limited to: average stress, max stress, total force, and strain energy) to quantify TFM data. To overcome this obstacle, TFM in combination with micropatterning of the ECM was used to isolate single regulatory parameters governing traction stress generation, and found that strain energy per unit area is an invariant measure of cellular contractility (Oakes et al., 2014).

TFM has also been extended to include analysis of multiple cells. Under limiting conditions of two cells it is possible to use first principles of force balance to reconstruct the amount of tension that is transmitted across cell-cell junctions, as the total stress in the system must balance (Figure 3.3) (Liu et al., 2010; Maruthamuthu et al., 2011; Sim et al., 2015; Tseng et al., 2012). Efforts have been made to expand these relationships to more complex geometries (Mertz et al., 2012b; Ng et al., 2014), although it has been suggested that small colonies (< 30 cells) of cells behave like a single cell of equivalent area (Mertz et al., 2012a). At larger length scales that include entire monolayers this relationship breaks down and are replaced by interesting dynamics of internal stresses and pulsatile flows as cells pull on both the substrate and each other (Brugués et al., 2014; Kim et al., 2013; Reffay et al., 2014; Roure et al., 2005; Trepat and Fredberg, 2011; Trepat et al., 2009; Vedula et al., 2012). By making some basic assumptions about the mechanical properties of the system, Tambe et al. devised a way to interpret local stresses within the monolayer in a technique called monolayer stress microscopy (Figure 3.4) (Serra-Picamal et al., 2015; Tambe et al., 2013, 2011). These techniques have been used to study the propagation of stresses within the monolayer (Serra-Picamal et al., 2012) and regulatory roles of cadherin in intercellular force buildup (Bazellieres et al., 2015).

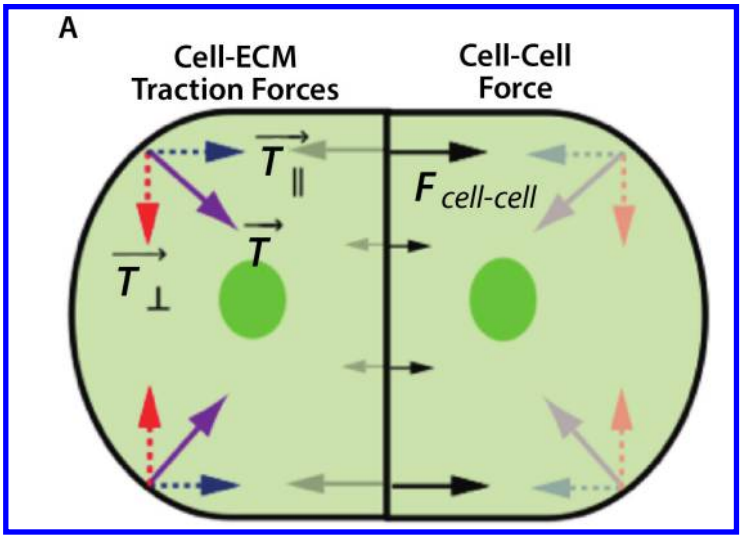


FIGURE 3.3: For the simple case of two cells it is possible to infer the stress transmitted across the cell-cell junction as it has to balance the stress being applied to the substrate by each cell. The representative force diagram is shown here for two cells. In this example, the blue arrows in the cell on the left represent the residual stress that must be balanced across the cell membrane by tension generated by the cell on the right. Adapted from Maruthamuthu et al. (2011).

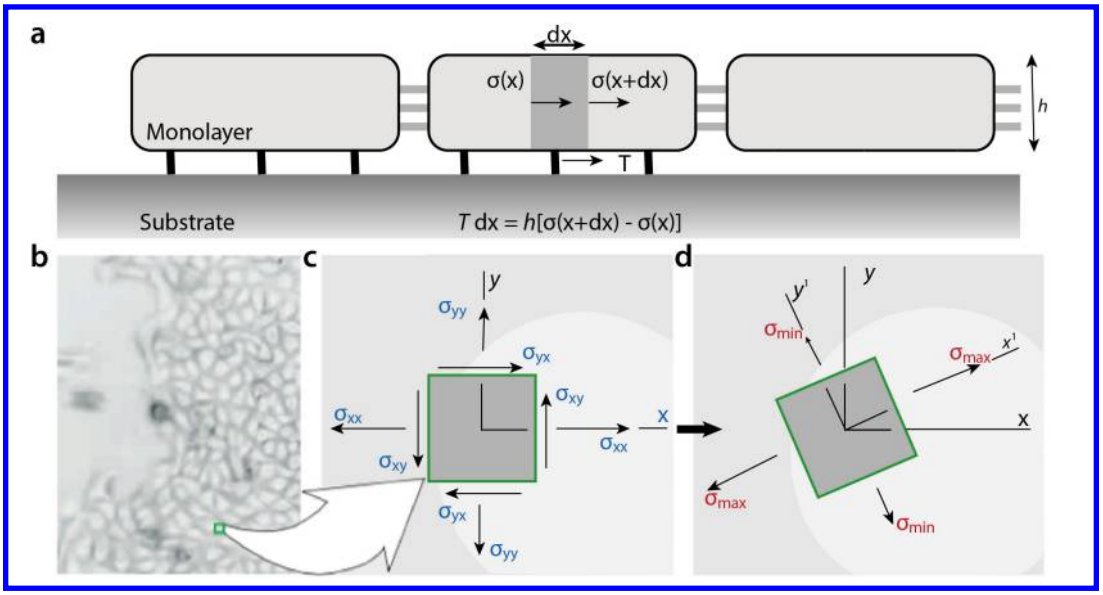


FIGURE 3.4: A technique called Monolayer Stress Microscopy has been used to estimate intercellular stresses in large collections of cells. This technique uses the traction stresses from traditional TFM to estimate the shear stress between cells by determining the rotation necessary to make shear stresses vanish locally. The orientations of this rotation, marked as x' and y', indicate the principle maximum and minimum internal stresses within the monolayer. Adapted from Tambe et al. (2011).

3.2 CELLULAR ADHESION FORCES: SPINNING DISK ADHESION

While TFM can measure the forces that cells generate while pulling on the ECM, they can only set a lower bound on the adhesive forces of cells. The adhesive force is the amount of force required to detach cells from the ECM, and is often at least an order of magnitude greater than the forces that cells generate (Coyer et al., 2012). There are many ways to measure adhesive forces that keep cells bound to a substrate when subjected to an external force (Zhou and García, 2015). The most simple of these is merely washing a surface of adhered cells with physiological buffers and counting the number of cells per unit area that remain. While the appeal of this approach lies in its accessibility, it offers little in regards to reproducibility and gives no information about the magnitude of applied force. In order to measure these parameters we must be able to control both the flow profile and the flow velocity. While this can be accomplished using microfluidic setups (Truskey and Pirone, 1990; Zhou and García, 2015), an easier approach makes use of a spinning disk (Engler et al., 2009; Gallant and Garcia, 2007; García et al., 1997).

Cells are plated on a circular coverslip and allowed to attach and spread for a given amount of time. The coverslip is then mounted on a disk and spun at a constant speed for a fixed amount of time (Gallant and Garcia, 2007). As the disk spins, the cells are subjected to a shear force that is determined by their radial position from the axis of rotation (r), the rotation speed (ω), and the fluid density (ρ) and viscosity (μ) (García et al., 1997):

$$\tau = 0.8\ r\ (\rho\mu\omega^3)^{1/2}.$$

Once spun, the cells are fixed, stained, and counted at different radial distances. When the number of cells is normalized by the center region where no stress was applied, the resulting detachment curve as a function of radial distance is sigmoidal in shape (Figure 3.5) (Gallant et al., 2005). The adhesion strength is then defined as the shear stress required to detach 50% of the population (Gallant and Garcia, 2007; Gallant et al., 2005). This approach has the benefit of being able to apply a large range of forces that vary linearly, with a typical max shear stress of ~250 Pa.

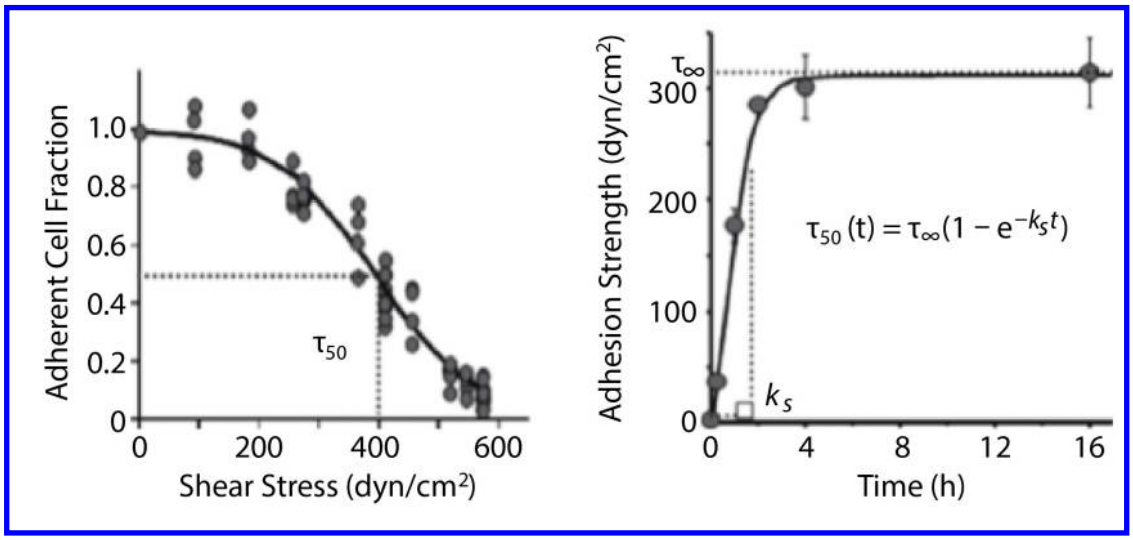


FIGURE 3.5: A representative detachment curve for cells during a spinning disk adhesion assay. The data is fit with a sigmoidal curve and the shear stress at which 50% of the cells remained attached is considered the average adhesion strength. This value can then be plotted as a function of the time cells are allowed to spread to determine the steady state adhesion strength. Adapted from Michael and Garcia (2007).

This assay has been used to measure changes in adhesion strength regulated by focal adhesion proteins (Dumbauld et al., 2010; Michael et al., 2009) and integrins (Friedland et al., 2009; García et al., 1998). The magnitude of the adhesion strength is impacted by both the size of the cell and

the number of adhesive ligands. These effects can be controlled for by combining the spinning disk adhesion assay with micropatterned coverslips, thereby tightly controlling the adhesive area and geometry (Coyer et al., 2012; Dumbauld et al., 2010; Gallant et al., 2005). This setup has also been used to help select for a human pluripotent stem cells from a mixed population, isolating them based on a difference in adhesion strength (Singh et al., 2013). While this technique measures the adhesion strength from a population, the mechanism of detachment is not always the same. Engler et al. (2009) showed that detachment of cells from fibronectin coated glass occurred at ECM-integrin interface, while cells cultured on crosslinked fibronectin matrices instead ruptured by breaking fibronectin filaments. Thus, care must be taken in interpreting where in the adhesion linkage rupture is taking place.

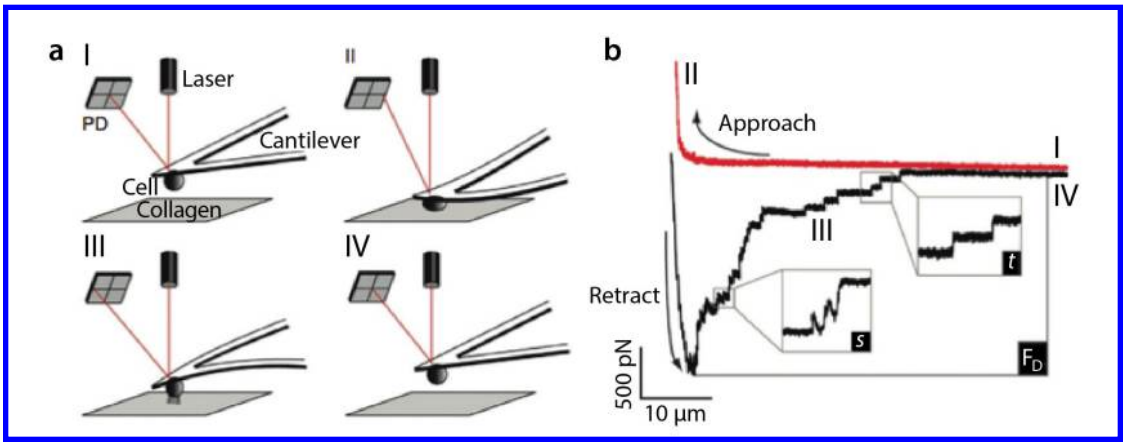
3.3 CELLULAR ADHESION AND FORCE GENERATION: ATOMIC FORCE MICROSCOPY

The Atomic Force Microscope (AFM) was first developed as an outgrowth of scanning tunneling microscopy and was based upon the principle of bringing a very fine tip into physical contact with a non-conducting surface (Binnig et al., 1986). By watching the deflection of the tip, and knowing the spring constant of the cantilever, it was possible to both map the surface topography and measure the stiffness of the material. Although originally conceived as an imaging technique, the AFM was soon harnessed in a variety of ways to directly measure interactions with molecules. With its high sensitivity and ability to move small distances controllably, the AFM has been an incredibly useful tool to measure adhesion and unfolding of both single molecules and cells (Benoit et al., 2000; Dufrêne and Pelling, 2013; Evans and Calderwood, 2007; Heinisch et al., 2012; Taubenberger et al., 2014).

The basic principle of an AFM experiment relies on a cantilever of known stiffness being brought into contact with a cell via a piezo driven motor (Figure 3.6). The position of the cantilever is monitored via a laser that is reflected off the back of the tip of the cantilever and onto a photodiode. As the tip makes contact with the cell, the cantilever is deflected, and thus changes the reflection of the laser on the diode. In its simplest form it is possible to use Hooke's law, $F = kx$, and convert the deflection directly into a measure of force. The response of the probe is determined by a combination of tip geometry and the material of which the cantilever is composed. Depending on these factors, the AFM can be made to apply a wide range of forces ranging from 10 pN to 100 nN, over distances of 1 nm to 100 μm and time resolutions of 0.1 s to 10 min (Friedrichs et al., 2010, 2013; Helenius et al., 2008).

In the case of Single Cell Force Spectroscopy (SCFS) experiments, the cell is attached to either the substrate or the cantilever, and an adhesion molecule is attached to the other surface (Figure 3.6) (Friedrichs et al., 2013; Helenius et al., 2008; Taubenberger et al., 2014). The cell is then

compressed between the cantilever and the substrate for a short period of time before retracting the cantilever. The amount of time the cell is held between the two surfaces is directly related to the number of bonds that the cell will form, with longer waiting times resulting in more bonds being established. As the cantilever is retracted it will be bent towards the surface due to the adhesion bonds formed during compression. Each bond that ruptures will reduce the deflection of the cantilever and thus be measured as a change in the force applied to the cantilever (Figure 3.6). Thus, by monitoring the deflection of the cantilever during the entire retraction, it's possible to measure both the force of single bonds breaking, and the total adhesive force of a cell (Benoit et al., 2000; Li et al., 2003; Puech et al., 2006).



FIGUR 3.6: (a) The general principle of Atomic Force Microscopy is illustrated on the left. A laser is reflected off the back of a cantilever and onto a photodiode. Changes in the position of the laser on the diode reflect deflections of the cantilever. (b) A representative trace of a cell adhesion experiment where the cell is attached to the cantilever tip, and pressed into the substrate. As the cantilever is retracted away from the surface, the force applied to the cantilever is measured. Individual bonds breaking can be seen as jumps in the insets. When the cell fully detaches the cantilever returns to its zero force deflection. This technique is sometimes referred to as Single Cell Force Spectroscopy (SCFS). Adapted from Friedrichs et al. (2010).

In addition to adhesion experiments, the AFM microscope is also perfectly suited to measure contractile behaviors of cells. Instead of monitoring the deflection of the cantilever during retraction, the deflection is monitored instead once contact is made with the cell. As the cell contracts, it pulls on the cantilever causing it to deflect (Liu et al., 2012). The power of this approach is magnified when combined with optical imaging techniques to visualize changes in the cell morphology

(Figure 3.7) (Chaudhuri et al., 2009). Using this approach it was possible to measure the total contractile force of individual platelets, which had previously been difficult to measure due to their propensity to aggregate upon activation (Lam et al., 2011).

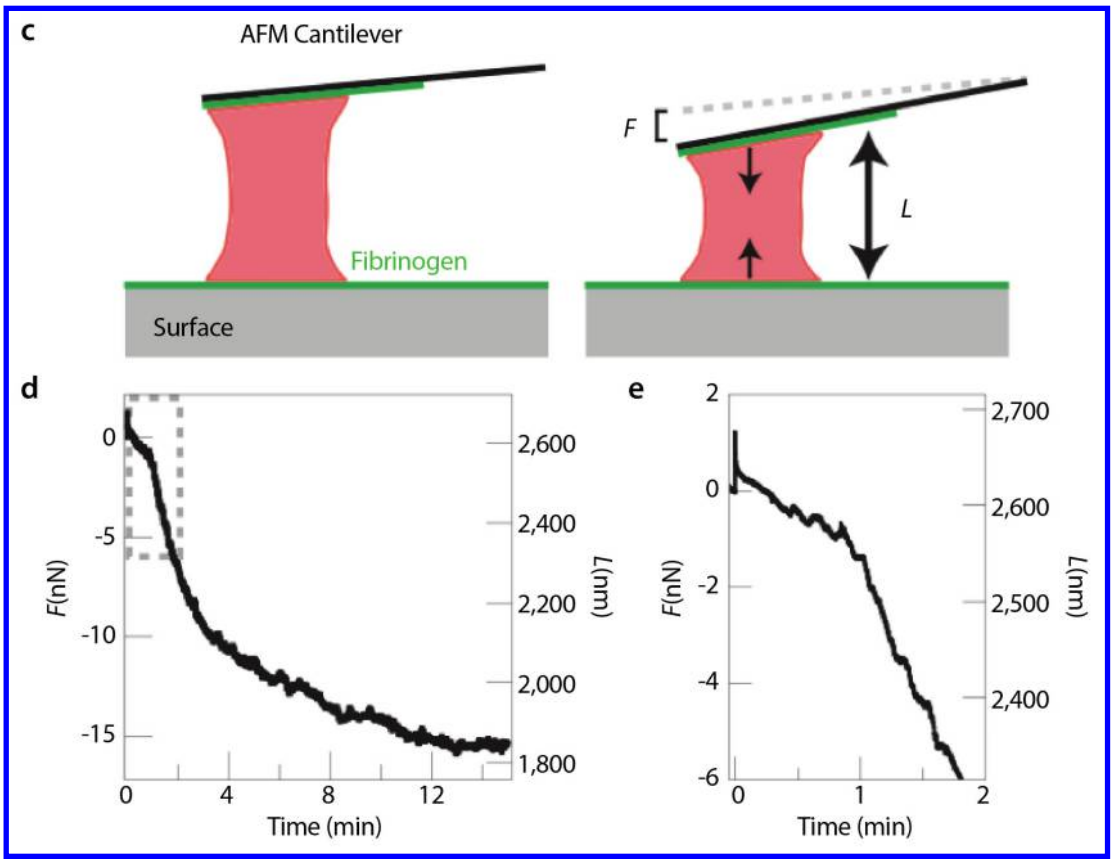


FIGURE 3.7: The AFM can also be used to measure the force generating capabilities of individual cells. (c) A platelet is brought into contact with an ECM coated cantilever and surface. (d) As the platelet contracts, it pulls on the cantilever causing a deflection. (e) A zoomed in region of the first two minutes of contraction. The initial increase in height (L) is the small compression force indicating contact between the platelet and the surface. This is followed immediately by contraction of the platelet. Adapted from Lam et al. (2011).

By incorporating feedback controls to the piezo motor, it is also possible to hold samples under constant tension or at a constant height, thus probing their response to constant applied stresses and strains. By modifying the feedback loop a constant stiffness can be maintained (Web-

ster et al., 2011), which makes it possible to investigate how cells respond to changes in stiffness in real time (Crow et al., 2012; Webster et al., 2014). They found that cells responded to the change in apparent stiffness and attempted to mimic the stiffness of the clamp. A very similar approach applying the same principles but relying on calibrated microplates instead of a cantilever achieved similar results (Bufi et al., 2015; Mitrossilis et al., 2009, 2010). These experiments highlight the many ways that the AFM can be used to probe strain, stress and stiffness of cells.

3.4 CORTICAL STIFFNESS AND MEMBRANE TENSION: PIPETTE ASPIRATION

Before the advent of the AFM and optical tweezers, calibrated glass micropipettes were often used as probes to push and pull on cells. Although today they have mostly been replaced in this regard with more precise instruments, the micropipette remains incredibly useful as a tool to measure cortical and membrane tension (Hochmuth, 2000).

Micropipettes are pulled from glass capillaries to diameters on the order a few μm and brought into contact with the cell membrane (Kee and Robinson, 2013; Oh et al., 2012). Suction is applied through the micropipette at pressures ranging from 10 Pa–100 kPa at the cell surface, causing the cell to partially extend into the pipette (Figure 3.8). The force applied to the cell is the pressure multiplied by the cross sectional area of the micropipette, and thus micropipette aspiration can apply forces on the order of 10 pN–100 nN. While this technique has been used extensively to probe membrane mechanics and red blood cells (Evans and Yeung, 1989), direct interpretation of the mechanical properties of other cell types is complicated by their viscoelastic nature (Diz-Muñoz et al., 2013; Hochmuth, 2000). Depending on the relative contributions of the elastic and viscous components of the system (i.e., whether the cell behaves more like a liquid or a solid), different mechanical models are required (Henriksen and Ipsen, 2004; Hochmuth, 2000).

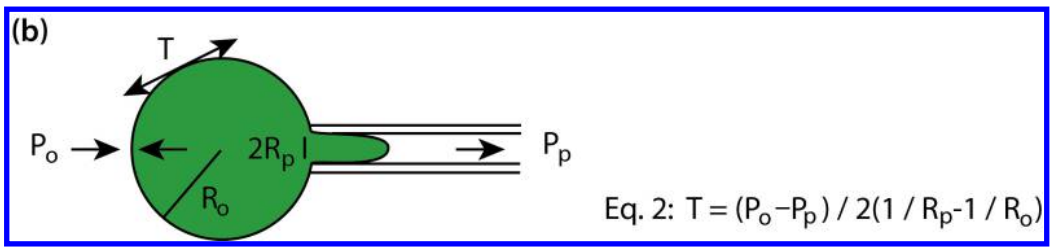


FIGURE 3.8: Micropipette aspiration is a technique used to measure the stiffness of the cellular cortex. A pipette with a diameter of a few μm is brought into contact with a cell and a negative pressure is applied. The surface tension can be calculated from the geometry of the cell and the magnitudes of applied pressure. Differences in cortical stiffness can be determined by comparing how far the cell aspirates into the pipette. Adapted from Diz-Muñoz et al. (2013)

Even in the light of the difficulty with direct absolute measurements, micropipette aspiration is incredibly valuable as a tool to probe relative changes in stiffness (Sun et al., 2014; Tinevez et al., 2009). It has been used to illustrate the importance of lamin-A in regulating nuclear stiffness (Shin et al., 2013), in Arp2/3 coupling the cortex to the plasma membrane (Beckham et al., 2014), and in RhoA in regulating cortical stiffness (Suzuki et al., 2013). Because micropipette aspiration applies a local force to the cell surface, it is perfectly suited to see both recruitment of proteins in response to applied tension such as myosin II (Effler et al., 2006; Kim et al., 2015; Ren et al., 2009) and α-actinin (Luo et al., 2013), or reduction of proteins such as Scar/Wave and Rac (Houk et al., 2012). Finally, by using two pipettes Maitre et al. were able to probe the differences between cell-cell adhesion and cortex tension in regulating sorting during zebrafish gastrulation (Biro and Maître, 2015; Maître et al., 2012).

3.5 MEMBRANE TETHERS: OPTICAL TWEEZERS

Optical tweezers have become one of the standard tools for probing biomechanical interactions (Fazal and Block, 2011; Neuman and Block, 2004). First reported by Ashkin et al. in 1986, optical tweezers rely on the basic physical principle of light transferring momentum to particles as it is refracted and reflected (Figure 3.9) (Ashkin et al., 1986; Nieminen et al., 2007; Svoboda and Block, 1994). In this case the light comes from a tightly focused laser beam that is usually passed through a high numerical aperture microscope objective and brought to a focus at the focal plane of the objective. This allows for high-resolution imagining simultaneously with trapping. While in principle an optical tweezers can be made from any wavelength of light, setups typically take advantage of near-infrared light which is transparent to most biological samples (Svoboda and Block, 1994). The amount of force the particle feels is proportional to the displacement from the focal point of the trap. The exact position of the particle in the trap can be monitored using either high-speed cameras or quandrant photodetectors. With these approaches it is possible to measure displacements with nm precision at up to kHz frequencies. The position of the trap relative to the sample can be controlled by either moving the sample stage, or steering the trap with acousto- or electro-optic deflectors. Multiple traps can be created simultaneously in the same field of view by using time sharing of a single beam or by adding diffraction elements into the optical train (Dufresne et al., 2001; Visscher et al., 1996). While the exact magnitude of the stiffness of the trap is dependent on a number of factors, the typical range of forces being applied range from 1–100 pN.

The list of materials, both living and non-living, that have been trapped is incredibly broad, spanning plastic beads to bacteria, viruses, sub-cellular organelles and even whole cells (Difato et al., 2013; Lang and Block, 2003). One of the most useful objects to trap has been the plastic bead. In addition to being simple to trap, they are easy to conjugate making them perfect handles to probe biological systems. Dai and Sheetz (1995, 1999) used this method to probe the mechanical

properties of the cell membrane and the interaction between the membrane and the cytoskeletal cortex (Figure 3.10). They brought trapped IgG coated beads into contact with the cell membrane for a short period of time, before withdrawing the bead from the cell. If the cell bound the IgG, a membrane tether would form during retraction, resulting in a force being applied to the bead. By comparing tethers pulled along the cortex to tethers pulled at regions of blebbing, they were able to show that pure membrane tension is very weak compared to the interaction between the cortex and the membrane (Dai and Sheetz, 1999). It is typically believed that during tether pulling that the tether is composed of pure membrane (Raucher et al., 2000), but evidence suggests that cytoskeletal components can be incorporated under certain conditions (Diz-Muñoz et al., 2010; Pontes et al., 2011). It should be noted that converting the force required to pull a membrane tether to a measurement of membrane tension is not straightforward and relies on a number of assumptions (Diz-Muñoz et al., 2013). Somewhat confusingly, the term membrane tension in the literature is often used to describe contributions from the membrane, the underlying cortex and the interaction between the two (Diz-Muñoz et al., 2013). This approach is thus best suited for showing relative differences between conditions instead of exact magnitudes. For instance, it has been shown that proteins like myosin I can mediate the interaction between the plasma membrane and the cortex (Nambiar et al., 2009), while cytoskeletal dynamics such as the filament assembly driving protrusion can also increase the internal pressure on the membrane (Batchelder et al., 2011; Houk et al., 2012; Lieber et al., 2013).

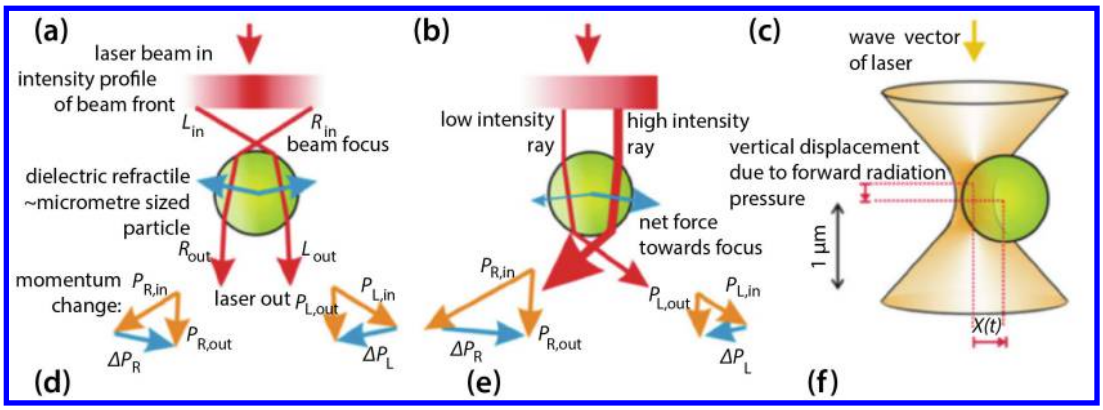


FIGURE 3.9: Optical tweezers rely on the momentum transferred between tightly focused laser light and a particle to create a trap. (a) Shows the force balance due to the refraction of light through a bead centered in the trap. (b) Shows the same system with the bead off-center from the trap and the corresponding restoring force, which pushes the bead back towards the focus of the trap. (c) Shows the general shape of the trap. Adapted from Leake (2012).

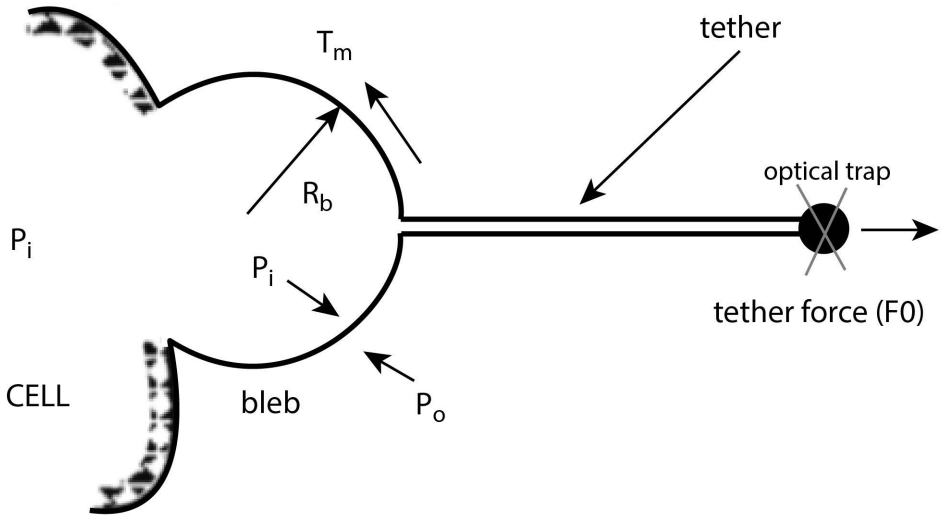


FIGURE 3.10: A diagram showing the force balance for a membrane tether pulled from a bleb in the cell. Here P_o is the pressure outside the cell, P_i is the pressure inside the cell, R_b is the blebb radius and T_m is the membrane tension. Adapted from Dai and Sheetz (1999).

3.6 PUSHING AND PULLING: MAGNETIC TWEEZERS

By directly applying forces to cells we can also extract information about their mechanical properties (Goldmann, 2014). Cells are viscoelastic and thus exhibit different behaviors based on the timescale of measurement. It is thus possible to determine elastic and viscous behaviors by applying forces and measuring both the magnitude and periods of deformation and relaxation. While we have covered previous techniques such as AFM, micropipette aspiration, and optical tweezers already, magnetic probes offer another approach to directly interact with the viscoelastic properties of cells.

Magnetic probes are one of the oldest methods of applying forces to cells (Crick and Hughes, 1950; Hiramoto, 1969). Magnetic beads can be coated with a variety of materials and introduced to the media. When coated with ligands for cell receptors, the beads will bind to the plasma membrane. If allowed to incubate for a long enough period of time, cells with often internalize the beads via phagocytosis making it possible to probe the internal structures of the cell. While the specific geometry of the setup can vary depending on the application, typically a single electromagnetic needle whose position can be manipulated via a micromanipulator is used (Figure 3.11) (Kollmannsberger and Fabry, 2007). The strength of the magnetic field produced in such a

setup depends on the core material of the needle, the size of the solenoid (i.e., the material and the number of turns) and the current applied to the solenoid. The magnitude of forces that can be applied via this setup also depend on the distance between the probe and the magnetic particle, but magnitudes of up to 100 nN at ranges of 10 μm are feasible (Kollmannsberger and Fabry, 2007), although typically forces applied by magnetic tweezers are in the single nN range. One drawback of this method is that it is almost impossible to determine how well the bead is attached to the cell or organelle. While this is less of a concern in situations of a constant applied force, when looking at bead twisting in response to a changing field it can have strong effects (Irmscher et al., 2012).

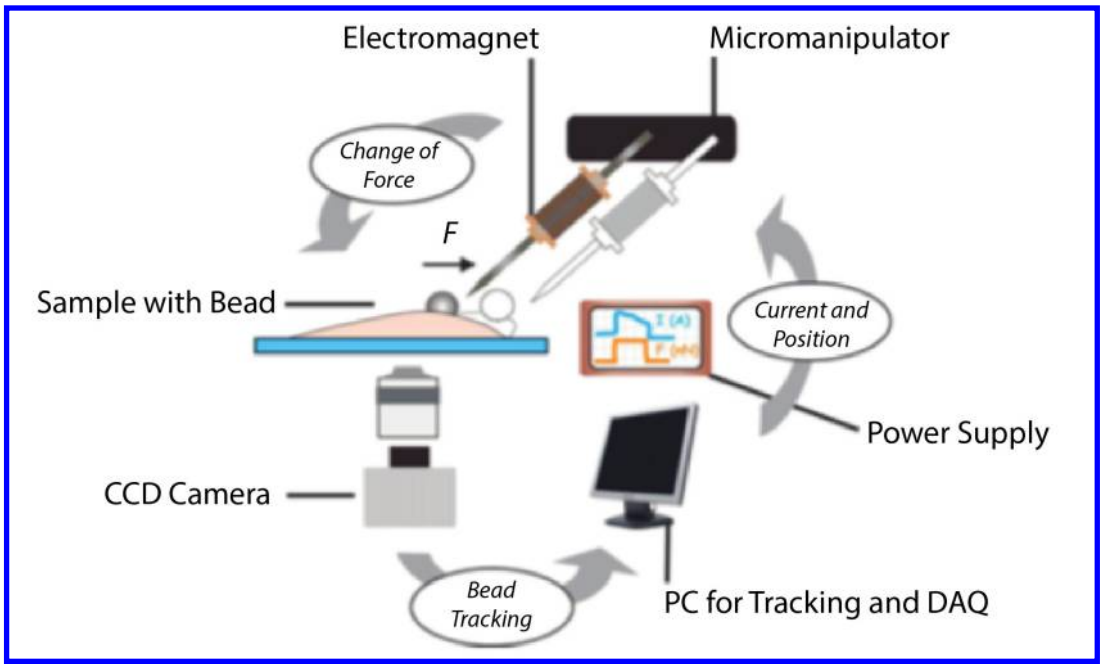


FIGURE 3.11: The typical setup of a magnetic tweezers. Magnetic particles are introduced into the media and allowed to bind to the cell. An electromagnetic needle attached to a micromanipulator is used to apply a magnetic field. The displacements of the magnetic particles in response to the applied field are tracked using high-resolution cameras attached to the microscope. Adapted from Kollmannsberger and Fabry (2007).

Magnetic tweezers have been mainly used to probe the rheological properties of cells (Kollmannsberger et al., 2011). Using knockdown cell lines, roles for FAK (Fabry et al., 2011) and vinculin (Mierke et al., 2008) have been established in mediating the mechanical response of the cytoskeleton to applied forces. Anisotropy in cell stiffness has also been probed studying the twisting of beads in response to more complex magnetic fields (Hu et al., 2004; Massiera et al.,

2007; Park et al., 2010). By combining this approach with microfabrication techniques it is even possible to probe tissue length scale responses to applied forces (Zhao et al., 2014a, 2014b). Finally, by conjugating the beads with different ligands it has been possible to study the response to applied forces on specific proteins, including integrins (Batra et al., 2012), cadherins (Weber et al., 2012), nesprins (Guilluy et al., 2014), and actin stress fibers (Sugita et al., 2011).

CHAPTER 4

Perturbing Cellular Mechanics

The approaches covered so far have mostly examined the cell at a steady state, attempting to measure mechanical properties and interactions without greatly disturbing the system as a whole. Primarily, these measurements have come from monitoring the material response of the probe and not the cell. For instance, turning the FRET measurements in Chapter 2 into a force requires knowledge of the spring constant of the linker. In Chapter 3, TFM relies on knowing the elastic properties of the substrate, while the AFM relies on knowing the mechanical properties of the cantilever. We can, however, gain additional insight by turning our attention from the probes to the material response of the cell. By directly perturbing cells, we can watch how they react and thereby determine the cell's material properties. In this chapter we will focus on those techniques that alter the cell steady state at both subcellular and cellular length scales, and the information that can be gathered from these approaches.

4.1 COMPRESSION AND EXTENSION: STRETCHING CELLS

The advent of soft elastic substrates dramatically changed our understanding of how the mechanical properties of the extracellular environment can impact cellular physiology (Discher et al., 2005). Simply by modifying the stiffness of the substrate, we can modulate a variety of cellular behaviors (Engler et al., 2006; Oakes et al., 2014; Paszek et al., 2005; Pelham and Wang, 1997). These reports, however, have all relied upon static experiments. They compare cells plated in identical circumstances but on different substrates. By taking advantage of the fact that these substrates are inherently elastic, we can actively apply strains to the substrate and track cellular responses in real time.

Strains can be applied in either a constant or cyclic fashion. Constant strains are most easily achieved by attaching a PDMS membrane between two parallel plates, one of which is fixed, and the other of which can be translated by turning a screw (Chen et al., 2013; Goffin et al., 2006; Ives et al., 1986; Wipff et al., 2007). This applies a uniaxial strain across the entire membrane which can be held indefinitely. Alternatively, strains can be applied by mechanically pressing the substrate over an indenter (Figure 4.1) (Huang et al., 2010; Krishnan et al., 2009; Lam et al., 2012; Shao et al., 2014). Depending on the geometry of the indenter, which can be a ring or two parallel plates, biaxial or uniaxial strains, respectively, can be achieved. Finally, stretching can also be achieved by using air pressure to push on the substrate and stretch it like the surface of a balloon (Aragona et al., 2013). Cyclic strains are achieved using similar approaches, but are oscillated at frequencies

typically ranging from .01–10 Hz (Carisey et al., 2013; Faust et al., 2011; Goldyn et al., 2010, 2009; Greiner et al., 2013, 2015; Livne et al., 2014; Matsuda et al., 2005; Shao et al., 2013; Shirinsky et al., 1989; Ueda et al., 2015; Zeller et al., 2013). Strains ranging from ~2–30% can be readily maintained through these methods.

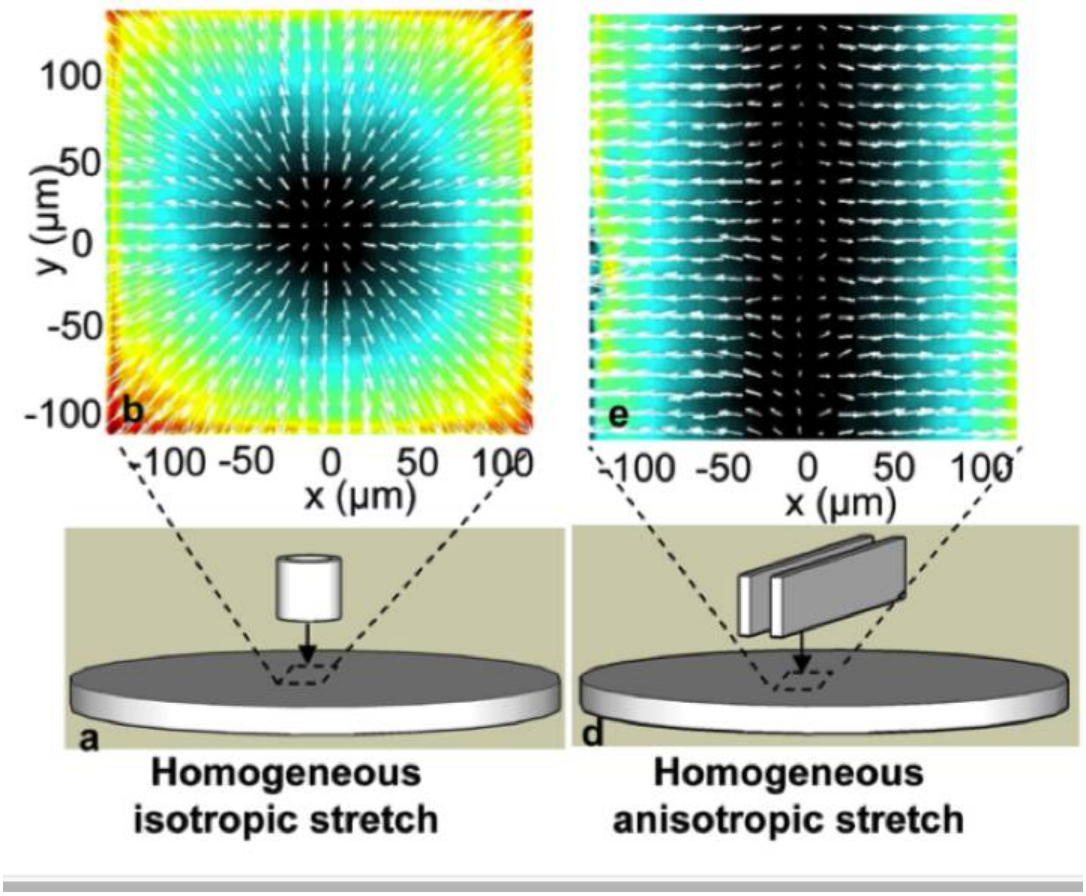


FIGURE 4.1: A constant strain can be applied by indenting a soft elastic substrate. By using either an annulus or parallel plates it is possible to generate homogenous isotropic biaxial (top left) or anisotropic uniaxial strains (top right), respectively. These strains can be calculated by tracking the deformation of the substrate using fluorescent beads as fiducial markers. Adapted from Krishnan et al. (2009).

Mechanical stretching of the substrate has been shown to cause endothelial cells to change shape and enforce alignment (Ives et al., 1986). Cells typically reorient their cytoskeleton so that the stress fibers are perpendicular to the direction of cyclic stretch (Faust et al., 2011; Goldyn et al.,

2009, 2010; Ives et al., 1986; Livne et al., 2014), although there is evidence that the response is frequency and cell type specific (Greiner et al., 2015). The reorganization of the cytoskeleton also leads to a reorganization of focal adhesions (Carisey et al., 2013) and causes changes in signaling from adhesion proteins (Chen et al., 2013; Matsuda et al., 2005; Ueda et al., 2015; Zeller et al., 2013) and the YAP/TAZ pathway (Aragona et al., 2013). By combining flexible substrates with TFM it is also possible to infer effects of strain on the contractile behavior of cells (Krishnan et al., 2009; Lam et al., 2012; Shao et al., 2014), and the propagation of stress in monolayers (Casares et al., 2015).

4.2 CUTTING THE CYTOSKELETON: LASER ABLATION

While plating cells on substrates of different stiffness can alter the internal tension in the cytoskeleton, it does not allow for acute changes in tension and structure. Cell spreading and reorganization of the cytoskeleton occurs on timescales of 10 min (Aratyn-Schaus et al., 2011; Giannone et al., 2004). Similarly, drug treatments and genetic perturbations require significant amounts of time to affect changes in the mechanical properties of cells. In order to affect changes at the rapid timescale of a few seconds, it is necessary to directly disrupt tension in the system. Tightly focused, high intensity lasers are one such platform for perturbing the mechanical equilibrium of the cell. They combine the requisite speed (~s timescales) and spatial resolution (~100 nm to 10s of μm) to directly perturb cytoskeletal structures (Berns et al., 1981; Colombelli and Solon, 2013; Colombelli et al., 2007; Wu et al., 2012).

A number of commercial instruments are available to perform laser ablation, but they all function on the same basic principles. Laser light ranging from short UV to the infrared is focused to a point in the sample, inducing photon absorption and ionization. As the energy density is increased, molecular bonds begin to break and a plasma forms (Vogel and Venugopalan, 2003). The exact geometry of the plasma and the extent of the damage done to the local region is a product of beam shape, wavelength, pulse width and beam intensity (Colombelli et al., 2004; Heisterkamp et al., 2005). For example using an infrared laser centered at 790 nm with 100 fs pulses, Heisterkamp et al. (2005) were able to deliver nJ energies per pulse to diffraction limited spots within the cell causing depolymerization of microtubules. Similarly, Colombelli et al. used a 355 nm UV laser with 470 ps pulses to deliver 50 nJ per pulse to dissect actin stress fibers in the cytoskeleton (Colombelli et al., 2009). To aid in tracking the dynamic response to ablation and severing, often fiducial marks are first created in the sample by photobleaching specific regions (Figure 4.2) (Colombelli et al., 2009; Kumar et al., 2006). This makes it possible to measure the material response at different regions in the sample.

As a widely available tool, the uses of laser ablation have been broad and varied. In the context of cellular mechanics, laser ablation can give us specific insights into the structural arrangement of microtubules as severing also induces rapid deployerization. It is thus possible to identify the

plus and minus ends of individual microtubules in structures such as mitotic spindles (Colombelli et al., 2005; Decker and Brugues, 2015; Heisterkamp et al., 2005). Similarly, structural information about spindle organization can be determined by ablating part of the centrosome and watching the subsequent motion of the fragments (Grill et al., 2003). A more popular approach, however, aims to modulate tension within the actin cytoskeleton, both subcellularly and at the tissue scale. In particular, it is possible to measure the contribution of individual stress fibers to the transmission of stress across the length of the cell by measuring the retraction dynamics following stress fiber severing (Chang and Kumar, 2013; Colombelli et al., 2009; Kumar et al., 2006). Similarly, by disrupting tension along cell-cell boundaries it has been possible to probe the roles of tension in E-cadherin localization (Cavey et al., 2008), myosin phosphorylation (Kasza et al., 2014), and cell rearrangement and intercalation in cell monolayers (Fernandez-Gonzalez et al., 2009; Heller et al., 2014; Rauzi et al., 2008). It has also been used to study the role of tension in physiological processes like wound healing (Brugués et al., 2014), cortical flow during development (Mayer et al., 2010), dorsal closure during development (Hashimoto et al., 2015; Hutson et al., 2003), and cytokinesis (Herszterg et al., 2013). Finally, laser dissection has also be used to alter membrane tension in the pseudopods of migrating neutrophils (Houk et al., 2012), and tension in the cortex of suspended cells (Tinevez et al., 2009).

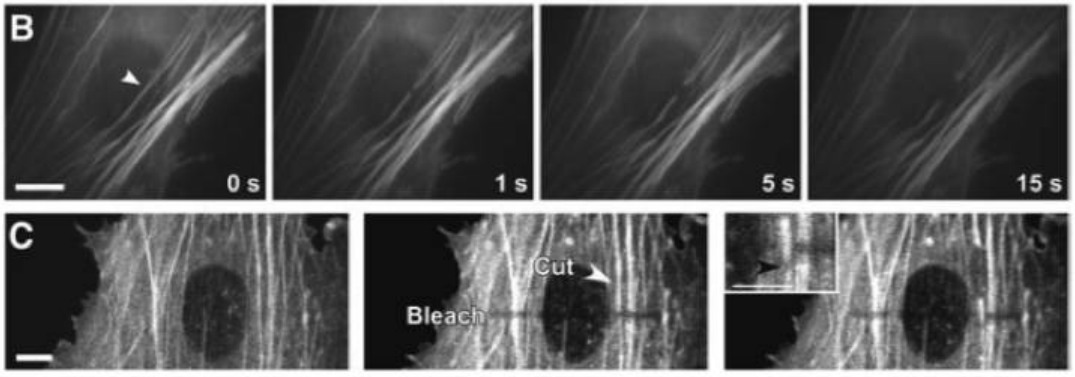


FIGURE 4.2: A stress fiber is ablated by focusing high intensity pulsed light from a laser on a small volume. The point of ablation is indicated by the white arrow head. By bleaching regions around the cut to create fiducial marks, the local response compared to the surrounding network can be determined. Adapted from Kumar et al. (2006)

4.3 COMPRESSING AND SWELLING: OSMOTIC PRESSURE

Tension in cells is typically considered in terms of the active processes that promote contractility and shape change. They are not, however, the only sources of tension in the cell. While greatly reduced in magnitude compared to the stresses generated by motors in the cytoskeleton and the cell cortex, the plasma membrane is constantly applying a uniform pressure to the cell. Indeed, this membrane tension is sufficient to generate contractile forces on its own (Murrell et al., 2014). Unlike an empty vesicle, however, the cell actively regulates this pressure via a number of channels and pumps which facilitate the transport of ions across the membrane and maintain cells at a physiological pressure (Burg et al., 2007; Hoffmann et al., 2009; Lang et al., 1998). The regulation of this intracellular pressure, in conjunction with the interaction with the underlying actin cortex, is thought to play an important role in regulating various cellular processes (Gauthier et al., 2012; Pedersen et al., 2013). Specifically, membrane tension is thought to play a role in maintaining the shape of migrating keratocytes (Keren et al., 2008) and could be important in adhesion independent migration (Bergert et al., 2015).

Changing the osmotic pressure is as simple as changing the osmotic concentration of the surrounding fluid. Because the response of the cell to changes in osmotic concentration is actively regulated, it is important to consider the relevant timescales (~ mins) when interpreting the effects of acute osmotic shocks. Introduction of hypotonic media results in an immediate increase in pressure difference and a corresponding increase in cell volume as water rushes into the cell (Figure 4.3) (Lang et al., 1998). Hypotonic media is typically made by diluting culture media with sterile water. Following the exposure, the cell attempts to correct the pressure imbalance via mechanisms like releasing more membrane (Gauthier et al., 2011; Spagnoli et al., 2008). The regulation of membrane tension is mediated by the actin cortex and can respond to changes in osmotic concentration on the scale of minutes (Bottier et al., 2011; Gauthier et al., 2011; Gómez-Martínez et al., 2013). The initial increase in membrane tension is sufficient to restrict protrusion of the membrane at the leading edge (Gauthier et al., 2011; Tsujita et al., 2015). Following the initial swelling, however, cells will actually undergo a volume decrease as they adjust to the change in osmotic conditions (Wang et al., 2015).

Conversely, introduction of a hypertonic media results in a shrinkage of cell volume and an immediate reduction in pressure difference, which is ameliorated as water effluxes from the cell (Figure 4.3) (Burg et al., 2007). Hypertonic media is typically made my adding molecules like sucrose or sorbitol to standard culture media (Burg et al., 2007). Stewart et al. (2011) have shown that regulation of the hydrostatic pressure regulates the ability of the cell to round up during mitosis. Similar to hypotonic shocks, cells respond on the scale of minutes to hypertonic shocks. In contrast to hypotonic exposure which can halt protrusion, hypertonic exposure can stimulate Rac and Cdc42 activity at the membrane (Di Ciano et al., 2002) and FAK at focal adhesions (Rasmussen et al., 2015).

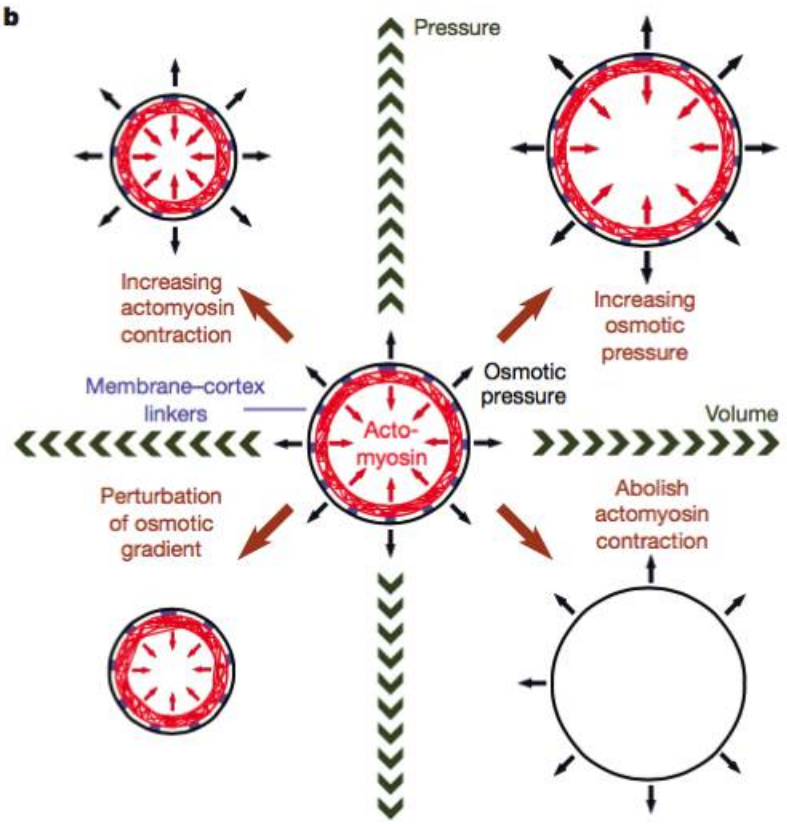


FIGURE 4.3: A phase diagram of pressure and volume in a cell comparing the effects of osmotic pressure in comparison to actomyosin contractility in the cell cortex. A hypotonic treatment causes water to enter the cell, swelling the volume and thus increasing pressure on the cortex. Conversely, during hypertonic treatment water leaves the cell causing a decrease in volume and a concomitant decrease in pressure. Adapted from Stewart et al. (2011)

CHAPTER 5

Concluding Remarks

The increase in quantitative approaches in cell biology has shined a new light on the role of mechanical interactions in regulating an abundance of physiological processes. These interactions extend from the molecular to the tissue scale, and span a number of orders in magnitude. While the techniques that we have covered here are by no means exhaustive, our aim is to provide a solid foundation in methods to probe the mechanical properties and interactions in cells. Specifically, we want to highlight the practical magnitudes of measurements, and the relevant range of length and time scales that can be probed (Table 5.1).

The future of cell biology lies in being able to quantitatively describe the complex interactions that drive the cell. These measurements not only quantify mechanical properties, but also provide the foundation for building physical models (Bergert et al., 2015; Brugues and Needleman, 2014; Guthardt Torres et al., 2012; Mogilner et al., 2012; Oakes et al., 2014; Prost et al., 2015; Ronan et al., 2014; Tee et al., 2015). We can use these models to gain insight into the mechanisms that drive these processes, and make further testable predictions. It is thus important that we are able to make precise and accurate measurements of the physical properties of the systems. While many of these techniques are best suited to working on flat adherent cells, the next generation of approaches should focus on the role of mechanical signaling in three dimensions (Elliott et al., 2015; Legant et al., 2010). These endeavors should only be aided by the recent advances in microscopy (Chen et al., 2014; Liu et al., 2015). In conclusion, the combinations of these approaches have laid the groundwork for an exciting and bright future for cell mechanics.

TABLE 5.1: A summary of the techniques discussed. Magnitudes of the measurements are representative of typical experimental setups. Timescales reflect the actual time required for the measurement to be made or applied

Technique	Measurement	Typical Magnitude of Measurement	Typical Timescale of Measurement
Förster Resonance Energy Transfer (FRET)	Distance between fluorescent probes Tension across linker	1–10 nm 1–6 pN	~ 0.01–1 s
Molecular Tension-based Fluorescence Microscopy (MTFM)	Distance between fluorescent probe and quenching molecule Tension across linker	5–25 nm 0–25 pN	~ 0.01–1 s
Tension Gauge Tethers	Force required to rupture DNA thethers	12–56 pN	~ 0.01–1 s
Traction Force Microscopy: Soft Elastic Films	Stress applied to the elastic substrate	10–105 Pa	~ 01–1 s
Traction Force Microscopy: Micropillar Arrays	Force to displace pillar tips	1–50 nN	~ 0.1–1 s
Spinning Disk Adhesion	Adhesion strength: shear stress required to detach 50% of cells	1–250 Pa	~ 5 min
Atomic Force Microscopy	Adhesion strength/ Force generation	10 pN – 100 nN 1 nm – 100 μm	~ 0.1 s – 10 min
Pipette Aspiration	Force applied to the cell cortex	10 pN – 100 nN	~ 1 s – 10 min
Optical Tweezers	Force to pull membrane tethers	1–100 pN	~ 1 s – 10 min
Magnetic Tweezers	Force applied to magnetic particles	1–100 nN	~ 1 s – 10 min
Cell Stretching	Apply a strain to a cell	2–30%	0–10 Hz
Laser Ablation	Severing filaments in the cytoskeleton	1–50 nJ applied energy	< 1 s
Osmotic Pressure Changes	Pressure difference between cell and surrounding media	10–1000 Pa	~1 s – 10 min

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