From static to animated: Measuring mechanical forces in tissues

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Cells are physical objects that exert mechanical forces on their surroundings as they migrate and take their places within tissues. New techniques are now poised to enable the measurement of cell-generated mechanical forces in intact tissues in vivo, which will illuminate the secret dynamic lives of cells and change our current perception of cell biology.

One morning over breakfast, my seven-year-old son suddenly asked, "What is ER?" Confused and searching for context, I walked over to where he was sitting at the kitchen table and realized that he was looking through a recently acquired cell biology coloring book, and was indeed asking about endoplasmic reticulum. (Imagine my delight!) The coloring book was full of beautifully rendered drawings of the inner anatomy of cells, featuring organelles that all cell biologists come to appreciate deeply as we study how cells accomplish their many functions, from DNA synthesis to oxidative phosphorylation to protein trafficking. In many ways, the pictures in the coloring book were no different from the schematics that we use to illustrate our textbooks and review articles: The drawings were idealized, boxy, and (most significantly) static.

This, of course, is where our two-dimensional artistry fails us. Cells are most certainly not static beings. Some, like macrophages, are sentinels and spend their lives exploring their host body, moving dynamically through tissues and organs. Because cells are physical objects and subject to Newton's laws, this motility requires the macrophage to transiently adhere to a substratum and exert force to propel itself forward. Even a relatively stationary cell, like an epithelial cell lining an airway in the lung, exerts force as its cilia wobble like whips and experiences force from the movements of other cells and fluids in its local microenvironment. The ability to both exert and experience force is proving to be critical for most cellular functions. Although we cannot yet draw a cartoon of cellular force, per se (the concept of mechanics was sadly absent from my son's coloring book), new technologies are inching us closer to being able to measure the forces that cells exert to sculpt themselves into functional tissues.

As the macrophage explores its territory, it pulls on its surroundings to exert sufficient traction to generate a velocity. In cell culture experiments, the tractions exerted on the microenvironment can be quantified in terms of traction stress (force per unit area) to define roughly how much force it takes for the macrophage to crawl. Because force itself is invisible, quantifying traction stress relies on techniques that monitor the tiny motions made by the substratum in response to force (again, Newton's laws). Early approaches cultured cells on very thin elastic membranes and inferred stress from the wrinkles that the cells made in the membranes as they moved; later techniques embedded micrometer-diameter fluorescent beads within the substratum and quantified stress by measuring the movements of the beads. These traction force microscopy techniques are simple, both conceptually and experimentally. The challenge is in the algorithms required to accurately convert the bead movements that are measured into stresses, and several strategies have been described recently that both simplify this process and increase its accuracy (Brask et al., 2015; Schwarz and Soiné, 2015).

Early studies that measured traction stresses generated by a variety of cells gave us a glimpse into the tiny athletes that occupy our tissues and organs. These glimpses are gross simplifications, of course, because they relied on a reductionist approach in which individual cells were isolated from their native context and monitored as they moved on a planar surface. In the body, most cells inhabit a dense 3D jungle of proteins and other cells. Recent advances have made it possible to monitor traction stresses exerted by cells in 3D culture models. Most of these approaches embed cells within synthetic polymers that have idealized mechanical properties, which makes it easier to convert bead movement into stress (Legant et al., 2010), or within fibrous extracellular matrix–like type I collagen, which behaves as a linearly elastic material when subjected to small enough strains (Gjorevski and Nelson, 2012).

It remains unclear whether these measurements accurately reflect the forces exerted by cells in vivo. Tissues are not composed of idealized polymers nor are they static; developing tissues show significant alterations in geometry and composition as they are sculpted in the embryo. Accurately quantifying traction force or stress exerted by cells within native tissues requires an understanding of the material properties of the tissue itself (that is, how fluid- or solid-like is the tissue) as well as a method for tracking deformations in real time. For the latter, 3D traction force microscopy was recently adapted to measure the mechanical stresses exerted during the convergence and extension process that drives *Xenopus laevis* gastrulation (Zhou et al., 2015). This technique was able to provide information about traction stresses at the tissue level, albeit in an explanted system. Finer spatial resolution has been achieved by injecting micrometer-scale oil droplets into embryonic tissues (Campàs

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et al., 2014; Lucio et al., 2015). Coating the surface of the oil droplets with ligands that bind to cell-surface receptors enables cells to exert forces on the droplets. Deformation of a droplet changes its shape, and because the material properties of the oil are known, this change in shape can be used to quantify the mechanical stresses that are exerted locally on the droplet by the surrounding cells. First used in *Drosophila melanogaster* embryos, it will be exciting to adapt the oil-droplet mechanosensors to a wider range of tissues and organisms.

All of the aforementioned force-reporting techniques are disruptive: They require the investigator to isolate cells, tissues, or embryos and surround or embed them with nonbiological markers that allow the invisible (force) to become visible (displacement or deformation). This places serious limitations on the types of questions that we can ask, and forces us (pun intended) to assume that the markers have no effect on the system that we are measuring. Ideally, one would like a force sensor that is integrated seamlessly into the tissue, and better yet that the cells within the tissue manufacture themselves. One possibility is to engineer a molecular force sensor that could report the traction stresses exerted by cells locally and in real time. Such an approach could build off of some of the exciting recent efforts to engineer intracellular proteins that stretch in response to an applied force, with the amount of stretch causing an increase or a decrease in förster resonance energy transfer (FRET) of fluorescent peptides appended on the protein (Grashoff et al., 2010; Borghi et al., 2012; Cai et al., 2014; Yamashita et al., 2016). Many of these FRET-based force sensors take advantage of our understanding of the composition of focal adhesions and adherens junctions, using proteins such as vinculin, α-actinin, and E-cadherin to localize the forces exerted on these molecules in the cytoplasm.

These are elegant systems that are beginning to unlock how actomyosin contraction impacts individual proteins within the spot welds that form cell–cell and cell–matrix adhesions, and there are already efforts to express these intracellular force sensors in living embryos and mature animals (Kelley et al., 2015). In the not-so-distant future, we might know precisely how much force that macrophage exerts as it migrates through different tissues in vivo, which would give insight into the drivetrain of the cell (does the macrophage have a single gear like a tricycle or multiple gears like a high-end mountain bike?). For questions about mechanical force at the tissue scale, however, these particular sensors might not be the most useful (do we need to know how much force is exerted at a specific cell–matrix attachment site in one epithelial cell among the millions that line an airway?). But one can imagine similar FRET-based force sensors that use extracellular matrix proteins as their anchor, and thus function akin to the fluorescent beads or oil microdroplets described above. Combining these approaches with the latest advances in imaging would reveal the tensile and compressive forces that are exerted by entire tissues during early embryonic development, organogenesis, wound healing, and disease progression.

As cell biologists, so much of what we understand is communicated in schematics. We have mapped out much of the inner anatomy of cells and carry these maps with us as mental snapshots. These pictures form the foundation of our hypotheses, inform the design of our experiments, and limit the conclusions that we draw from our data. The ability to measure force in real time in real tissues will by necessity alter these snapshots and give us a deeper understanding of the rich and dynamic lives of cells. Now, how do we draw these invisible forces in our cartoons?

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