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Pulling it together in three dimensions

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Abstract

The most abundant proteins in our cells are there to generate mechanical forces, and measurement of these forces has just become possible.

The study of mechanobiology is not at all new, and we have known for quite a long time that physical force is central to biological form and function¹. Throughout the vasculature, for example, the local diameters of blood vessels roughly match the local blood flows so as to keep fluid shear stresses at the vessel wall nearly constant². Across species of widely varying body mass, from shrew to elephant, long bones thicken³ and overall energy metabolism increases⁴ in a manner that is attributable to avoidance of buckling under mechanical load⁵. Much more recently, we have come to learn that physical forces at the cellular level play pivotal roles in cancer biology⁶, monolayer barrier disruption⁷ and stem cell differentiation⁸. In this issue of *Nature Methods*, Legant *et al.*⁹ use mathematical analysis of matrix deformations to make visible the physical forces at the interface between a cell and its three-dimensional microenvironment.

If some of the over-riding principles of mechanobiology at the organ level are now known, then our understanding of underlying mechanisms at the cellular level still remains unclear. For example, how does the individual cell manage to sense physical forces locally in its three-dimensional microenvironment, respond to those forces or even generate those forces? More specifically, how do physical forces in three dimensions modulate cell adhesion, cytoskeletal tension, the rate of cytoskeletal remodeling as well as chemotaxis, durotaxis and cellular responses to tissue stretch? Do local physical forces guide cellular migrations, or are forces a by-product of those migrations?

Except in highly unphysiological cell culture systems with a flat two-dimensional substrate, understanding of these basic questions has been held back because no method existed by which local physical forces themselves were accessible to quantitative measurement or direct visualization. We have come to learn that cell forces are inherently three dimensional and will cause a flat surface to wrinkle, ruffle and bulge¹⁰ (Fig. 1a). But in regard to

experimental investigation of physical forces in which the cell is fully embedded in a three-dimensional environment, it is fair to say that we have been navigating in the dark.

With the methodological advance of Legant *et al.*⁹, however, these forces are now open to experimental attack. A transparent polyethylene glycol hydrogel with biodegradable domains and adhesive ligands serves as a three-dimensional matrix that cells can invade. Embedded in that matrix, the cell then spreads, adopts physiologically relevant morphologies and develops traction forces. Resulting matrix deformations are visualized by tracking fluorescent tracers with a confocal microscope. The traction forces that give rise to those deformations are then inferred using the laws of elasticity.

We are still far from our ultimate goal of studying the regulation of cell function by physical forces in the cell's native microenvironment, however. One central obstacle is the complexity of the native extracellular matrix, which is heterogeneous, viscoelastic and strongly nonlinear. Moreover, matrix varies with time: cells continuously degrade and synthesize matrix, which restricts force measurements to scales of time that are short compared with many important biological events. Therefore, obtaining reliable traction maps will require substantial improvements in experimental or modeling strategies.

Finally, traction forces reported by Legant *et al.*⁹ represent only those forces generated by one cell acting on a passive matrix (Fig. 1b), thus leaving active forces of mutual cell-cell interactions (Fig. 1c) not only as a major unknown, but also, in all likelihood, a dominant one. Without this information, mechanisms of force regulation during processes involving collective cellular behavior—as in development, collective cell migration or cancer cell invasion—will remain inaccessible experimentally. Despite these limitations, the method presented by Legant *et al.*⁹ constitutes a dramatic advance on our way to understanding the interaction between a cell and its physical microenvironment in experimental models of increasing physiological relevance.

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Figure 1.

Cell traction forces. **(a)** Traditional traction microscopy measures forces at the interface between the cell and the two-dimensional substrate. **(b)** Measurement of cell tractions across the entire three-dimensional cell-matrix interface. **(c)** Although cell-matrix forces (red) are now accessible⁹, forces at the cell-cell interface (green) remain inaccessible to experimental observation.