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Hidden in the mist no more: physical force in cell biology

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Abstract

To drive its migration through a fibrillar matrix—and thus to spread, invade or metastasize—a cancer cell must exert physical forces. The first visualization of these forces in three dimensions reveals surprising migration dynamics.

How does a cancer cell invade and migrate through surrounding tissue? In this issue, Steinwachs *et al.*¹ help us to understand this process by mapping for the first time the physical forces exerted by a cell migrating in a three-dimensional (3D) fibrillar collagen matrix. Moreover, they report the surprising finding that in the case of breast cancer cell lines, cellular forces within such a matrix are insensitive to changes in collagen concentration and bulk material properties. This finding stands in contrast to cellular forces measured previously in less physiological systems, including cell migration on a two-dimensional (2D) linearly elastic material such as polyacrylamide gel or PDMS (polydimethylsiloxane), or even within a 3D linearly elastic hydrogel such as PEG (polyethylene glycol). In the case of collagen matrices, by contrast, material viscoelasticity and nonlinearity severely complicate the problem of force recovery, but these complications represent merely the tip of the iceberg. This is because some collagen fibers straighten, extend and stiffen in response to an imposed force, whereas other fibers in the very same region simultaneously compress and buckle. All can change local orientation and spacing. As a result, local deformations on the fiber scale do not follow deformations on the bulk scale, and are therefore said to be non-affine. Steinwachs *et al.*¹ now take these factors into account for a single cell migrating through a fibrillar matrix.

Physical forces at the cellular scale have remained unmeasurable for a long time, even though for at least 100 years we have known that these forces have a central role in

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biological growth, form, adaptation, wound healing and remodeling. For example, the classical work of Thompson² emphasizes the relationship between form and function, Wolff's law³ describes the adaptation of bone structure to the load that the bone must support, and Murray's law⁴ describes how fluid shear stress exerted on the endothelial wall regulates the adaptation of vessel diameter to the blood flow through the vessel. McMahon's principle of elastic similarity⁵ explains how variations in energy metabolism, muscle mass and bone size scale across species as a function of body mass. Much more recently we learned that physical forces can direct stem cell fate⁶.

To infer the presence of physical forces at the cellular scale, Harris *et al.*⁷ used wrinkles generated by a single adherent cell contracting upon a thin silicone rubber sheet, but it was Dembo and Wang⁸ who first formalized the problem of traction microscopy, making such forces visible, quantifiable and mappable (Fig. 1a). Their breakthrough was followed in rapid succession by computational streamlining⁹, refining¹⁰, extension to multicellular clusters^{11,12} and sensing by fluorescence resonance energy transfer. To extend traction microscopy to 3D systems, Legant *et al.*¹³ reconstructed cellular force fields in PEG hydrogel matrices, but deformations in such matrices are linearly elastic and affine, whereas those in fibrillar collagen matrices are not.

In their efforts to overcome the conceptual obstacles presented by traction microscopy in 3D fibrillar collagen matrices, Steinwachs *et al.*¹ recognized that the central problem was not so much the nonlinear elastic behavior of such matrices as how that nonlinear behavior could be understood in terms of non-affine fiber deformations. With a continuum description capturing that behavior in hand, they went on to use confocal reflectance microscopy to quantify local matrix deformations and then to compute the distribution of forces exerted by an MDA-MB-231 breast carcinoma cell migrating through a fibrillar collagen matrix (Fig. 1b).

In the case of a cell migrating upon a flat, linearly elastic 2D matrix, physical forces increase with increasing matrix stiffness, but it has now been shown that for a cell migrating within a 3D fibrillar collagen matrix, this sort of relationship does not hold. Rather, a cell migrating in 3D generates roughly similar forces regardless of matrix stiffness. The reasons for this surprising behavior, at least for now, remain a matter of speculation. Steinwachs *et al.*¹ did not independently control for matrix stiffness, ligand density or pore size, so a simple explanation might be that a cell in a stiff matrix with small pores is unable to spread and extend enough to generate the amount of force it would have in a similarly stiff but more porous matrix. Another open question now concerns the manner by which a cell seems to glide within the 3D collagen matrix, in contrast to the 'inchworm' migration commonly observed on a 2D substrate. Nor do we understand how the cell perceives and responds to the difference between 2D and 3D microenvironments, or how cell navigation is altered by associated differences in cell-matrix adhesion and cytoskeletal dynamics.

Where does traction microscopy go from here? Shortcomings of the present approach are clear. Difficult but nevertheless important issues not yet taken on include improving spatial resolution and accuracy and better defining the roles of time-dependent mechanical

properties of the matrix such as viscoelastic relaxation processes, cross-linking dynamics, interstitial fluid flows and active matrix remodeling¹⁴.

The greater challenge, however, will be to devise a strategy for measuring physical forces exerted by a cell embedded in a 3D cell cluster or organoid *in vitro*, or even in a living tissue (Fig. 1c). For a cell in a 2D cellular collective, such as an endothelial or epithelial layer *in vitro*, this problem has already been solved^{11,12}. This success derives in part from the fact that the properties of the substrate material upon which such a layer migrates are controlled by the experimentalist; the substrate is typically homogeneous and elastic, as well as passive and well characterized. But for a cell in a 3D cellular collective, the surrounding material comprises mainly neighboring cells, and thus the material properties are defined not by the experimentalist but by the cells themselves. Accordingly, these properties tend to be not only heterogeneous and inelastic but also active and poorly characterized. For a cell in a 3D cellular collective, therefore, the traction microscopy strategies described above are fundamentally inapplicable; they cannot be made to work through either refinement or extension. Thus no strategy for force mapping in a 3D cell cluster is yet in sight.

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Figure 1.

Traction stresses generated by a single cell. **(a–c)** Arrows denote stresses for a cell on a 2D matrix **(a)**, within a 3D fibrillar collagen matrix **(b)** and in a multicellular 3D cellular cluster or tissue **(c)**.