

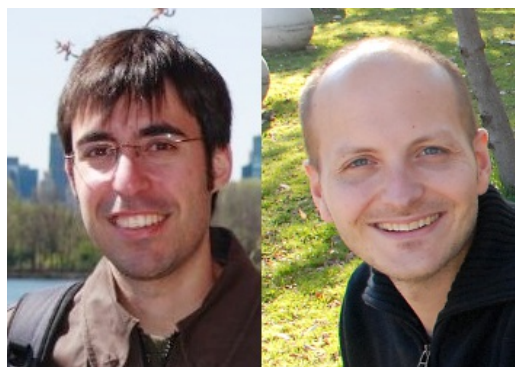
COOL BIOPHYSICS

Mechanobiology of collective cell systems

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The concept of *physical force* is deeply integrated in our daily life. We experience it routinely with every heartbeat, walking step, or deep breath. As such, it may seem inconceivable to think that forces are not an integral part of the mechanisms that drive biological function. However, for a long time, modern biology attempted to explain life solely on the basis of the biochemistry of genes and proteins, ignoring any potential role that physical forces could play in biological processes. Yet, it becomes increasingly clear that physical cues are not only as important

as biochemical ones, but also that they could help us understand and treat diseases such as atherosclerosis, acute inflammation, fibrosis and cancer.

Mechanobiology is the emergent discipline that explores the role of mechanical forces in cell development, physiology and disease. As a multidisciplinary field, it combines concepts from biology, biochemistry and physics. Challenges in mechanobiology cover from the specific mechanisms by which single cells sense and respond to forces (mechanotransduction) to how a tissue monolayer folds into a 3 dimensional structure during organogenesis. Still being a relatively young field, mechanobiology is starting to provide evidence showing that major biological processes are fundamentally ruled by forces. For example, a class of mesenchymal stem cells tends to differentiate into distinct cell types depending on the stiffness of their surroundings [Engler, et al. 2006]. Other examples include force modulation of apoptosis (programed cell death) and cell division [Slattum & Rosenblatt 2014].

Perhaps the collective migration of epithelial monolayers is one of the areas into which mechanobiology has shed more light in the recent years. Cells often move in groups with coordinated polarity without completely disrupting their cell-cell contacts. This harmonic migration is responsible for closing gaps when a monolayer is wounded or determining organ shape during morphogenesis. To fully understand such processes, it is necessary to have access to one fundamental parameter that has been elusive for decades: the physical force.

Making forces visible at the cellular scale

The notion that physical forces could explain important biological processes has been around for about 100 years [Thompson 1942; the first edition is from 1917]. Nevertheless, until 1980 the direct evidence that forces exist at cellular scale and were measurable was missing. In a seminal work, Harris *et al.* observed that adherent cells generated wrinkles when seeded on soft elastomeric substrates [Harris, Wild, & Stopak 1980] (Figure 1A). Two decades later, Dembo and Wang developed an experimental and mathematical framework able to quantify those forces [Dembo & Wang 1999]. Their approach was simple and only applicable to single adherent cells. First, cells were seeded on an elastic substrate with embedded fiducial fluorescent markers. While cells exerted force on the substrate, the fiducial markers were displaced from their relaxed position (Figure 1B). Then, the relaxed position was determined after removing the cell by the addition of trypsin (a protein that catalyzes the hydrolysis of peptide bonds) (Figure 1C). Finally, by comparing the two images of the fiducial markers –deformed and relaxed– and taking into account the mechanical properties of the substrate, a precise map of the traction forces was computed (Figure 1D–G). This technique was named *Traction Force Microscopy* and its appearance was the tipping point that triggered a succession of studies refining [Butler *et al.* 2002], improving [Sabass *et al.* 2008] and extending this technique to cell monolayers [Treppe *et al.* 2009] and three-dimensional environments [Legant *et al.* 2010].

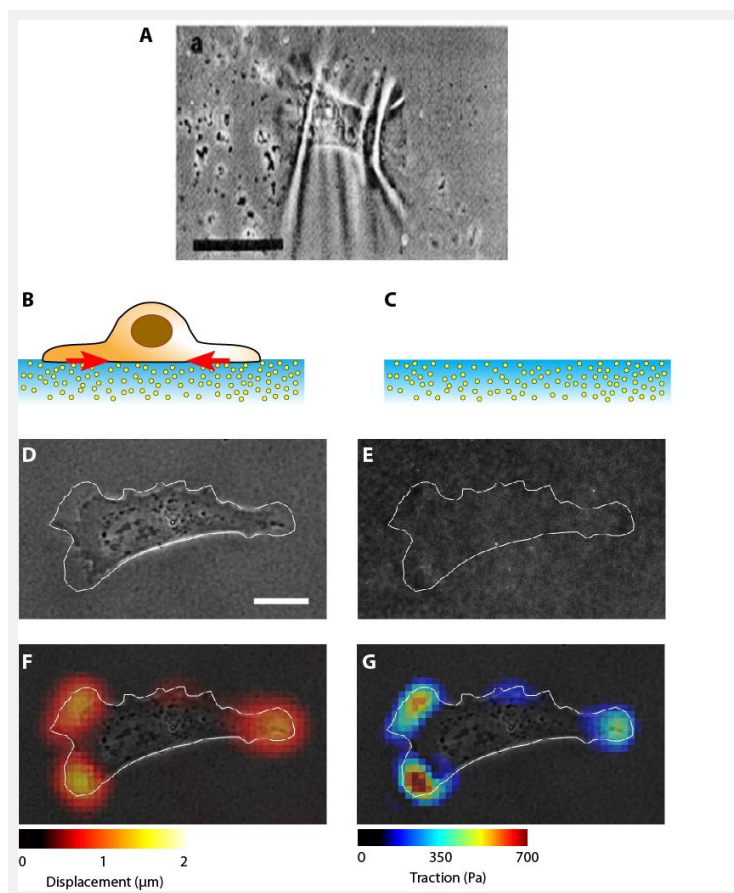


Figure 1. Overview of Traction Force Microscopy. (A) First attempts to observe forces at a cellular level showed how adherent cells generated wrinkles when seeded on soft elastomeric substrates, from Harris *et al.* *Science* 1980, 208: 177-179. Reprinted with permission from AAAS. (B–C) Sketch of Traction Force Microscopy. (B) Adherent cells are seeded on an elastic substrate with embedded fiducial fluorescent markers. As cells exert force (red arrows), the substrate and the fiducial markers are displaced from their relaxed position. (C) After the addition of trypsin, the cell is detached and the gel returns to its original position. By comparing the two images of the fiducial markers –deformed and relaxed– and taking into account the mechanical properties of the substrate, a precise map of the traction forces is computed. (D–G) Example of Traction Force Microscopy. (D) Single human bone osteosarcoma epithelial cell cultured on a soft polyacrylamide gel (5 kPa) and imaged with phase contrast microscopy. The line drawn is the contour of the cell. (E) Image of the fiducial markers embedded into the gel. (F) Displacement map generated by the cell. (G) Traction map computed from the displacement map. The scale bar in D corresponds to 20 μm .

How cell crowds play the tug of war

For a long time, it was unknown whether the global motion of cell monolayers was driven by the action of leader cells at the front of the monolayer, pulling the cells behind [Poujade et al. 2007], or by internal pressure, due to cell division that pushed the leading cells forward (Figure 2A and B, respectively). Recent improvements brought by Traction Force Microscopy have provided some evidence to elucidate which of these mechanisms was more plausible [Treat et al. 2009]. First, the detailed mapping of traction forces normal and parallel to the cell edge of the monolayer showed that traction forces were exerted many rows behind the leader cells and propagated over long distances (Figure 2C–E). These data suggested that the idea of leader cells dragging the passive followers could not fully explain the collective migration. Moreover, force propagation over long distances established that collective migration not only involved interactions with the substrate but also interactions with neighboring cells. Consistent with this view, the average traction force in the monolayer was not concentrated at the edges but decayed slowly keeping the values larger than zero (Figure 2F).

These findings implied that cell sheets play a global *tug of war* that requires cell-cell junctions (Figure 2G). Interestingly, by applying Newton's 2nd law, the cell stress within the monolayer can be calculated [Tambe 2011]. The stress transmitted through cell-cell junctions increased as a function of the distance to the monolayer edge. Such a tensile stress ruled out the idea that cell division and proliferation

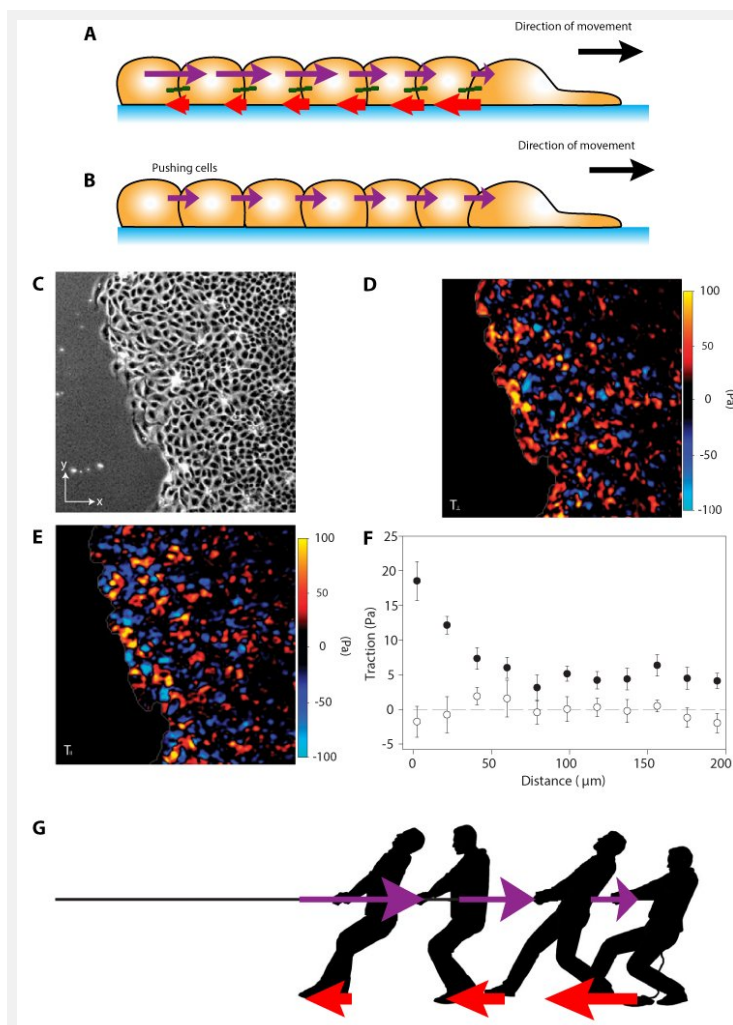


Figure 2. Physical forces during collective cell migration. (A–B)

Migration of cell monolayers can be governed by different mechanisms.

(A) Leader cells at the edge pull forward the cells inside the monolayer.

Forces that cells exert on the substrate are depicted in red whereas forces acting on cells are purple. (B) Alternatively, cell division in the interior of the monolayer push neighboring cells forward.

(C–E) Traction forces during collective cell migration. (C) Phase contrast image of a MDCK monolayer cultured in a soft polyacrylamide gel. Tractions normal (D) and parallel (E) to the edge of the monolayer.

(F) The average normal traction decays slowly with distance from the edge (filled symbols), whereas the average parallel traction was negligible and independent of the distance from the edge (open symbols). Error bars indicate standard errors. C–F are reprinted by permission from Macmillan Publishers Ltd: Nature Physics (Treat et al. Nat Phys 5: 426 – 430), copyright 2009. (G) The *tug of war* illustrates the mechanisms by which a migrating cell monolayer integrates local tractions (red) into long-ranged gradients of intra- and inter-cellular tension (purple).

pushed the monolayer forward. This kind of *tug of war* motion has been observed in other contexts, such as wound healing [Brugués et al. 2014, Vedula et al. 2014] and cancer progression [Wagstaff, Kolahgar & Piddini 2013].

Moving together towards stiff: how the “tug of war” guides cell groups

A remaining question in epithelial monolayer migration is how the *tug of war* is modulated by external cues, such as the stiffness of the environment. In single cells, it is well established that cells tend to move from soft to stiff regions when seeded on a rigidity gradient matrix [Lo et al. 2000]. This migration was termed *durotaxis*, after de Latin *durus* (hard) and the Greek *taxis* (regular arrangement). Such a guided motion, however, is very small and only appreciable when averaged over many cells or in the presence of very steep gradients (Figure 3A and B). Surprisingly, when cell clusters were seeded on matrices with graded stiffness, durotaxis was far more efficient than in single cells (Figure 3C–F). Again, the measure of traction force turns out to be the key to understand this phenomenon.

Measuring traction forces in matrices of non-uniform stiffness is not straightforward. Conventional Traction Force Microscopy algorithms are designed for uniform stiffness substrates in which the mechanical properties are constant along all coordinates. Conversely, on matrices of graded stiffness the application of a given force will produce different displacements depending on whether this force is applied to a soft or a stiff region. To extend Traction Force Microscopy to arbitrary stiffness profiles, the use of finite element methods (FEM) is

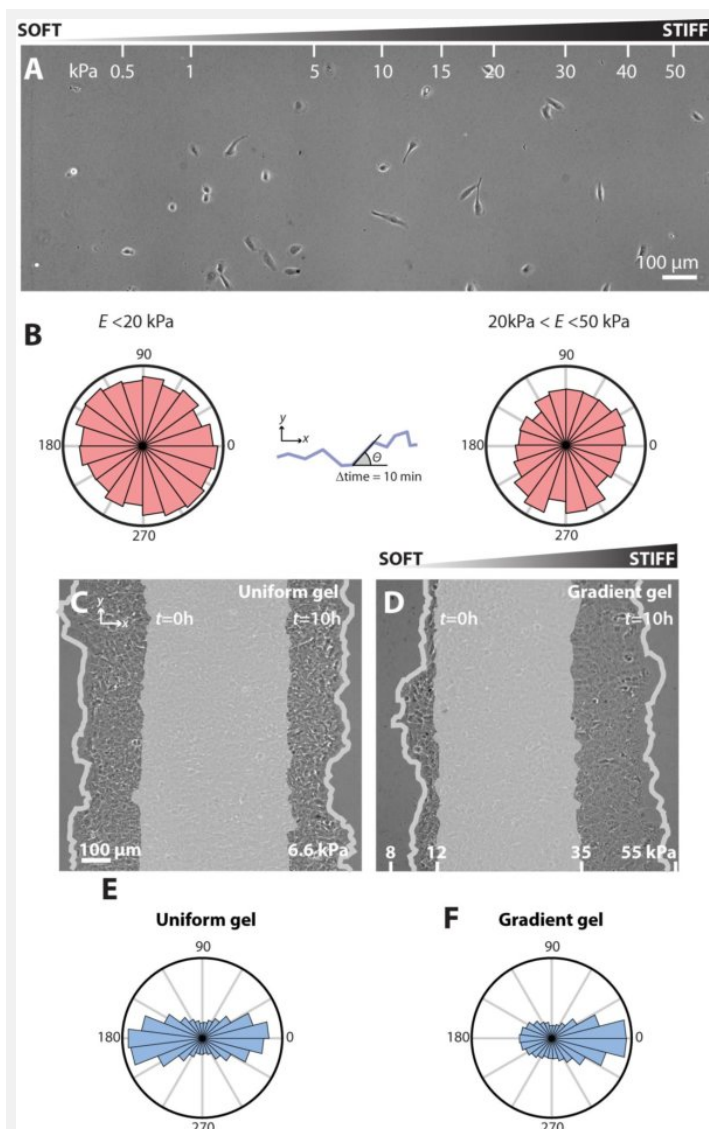
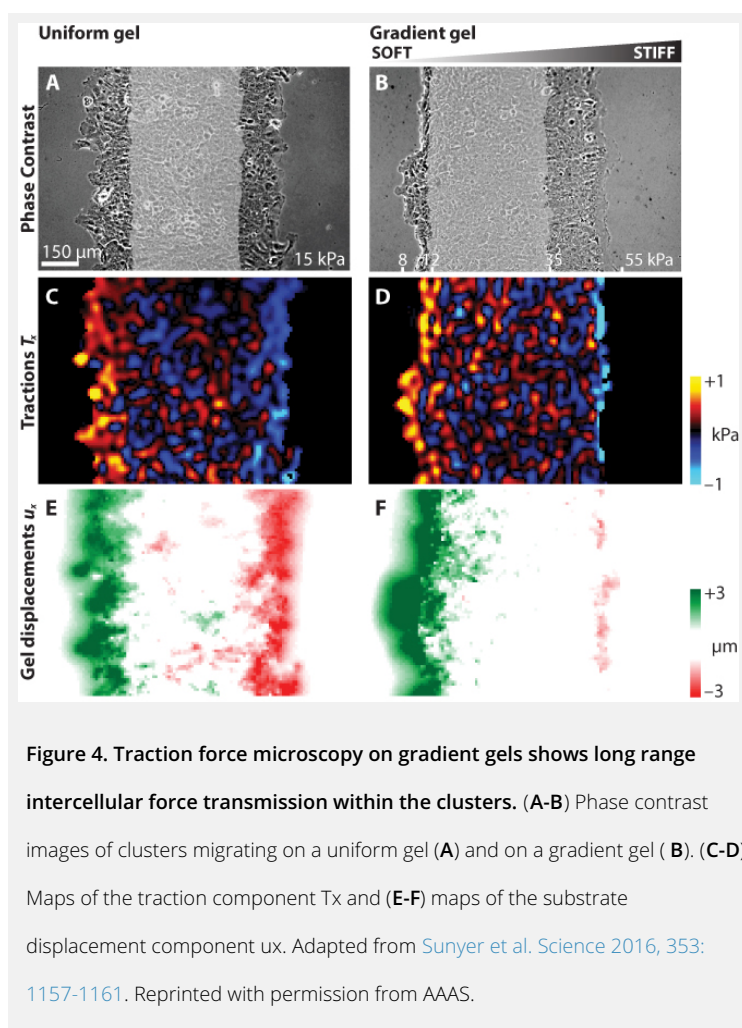


Figure 3. Durotaxis in single cells and multicellular clusters.(A) Phase contrast image of human mammary epithelial cells (MCF-10A) cultured in isolation on a gel with graded stiffness. Numbers at the top indicate Young's modulus values measured with AFM. (B) Distribution of the angle θ between the instantaneous velocity vector and the x-axis for isolated cells. The inset shows a cell trajectory (blue) and the definition of the angle θ . (C) A representative cell cluster expanding on a soft uniform gel of 6.6 kPa. The gray transparent area indicates initial the cluster position ($t = 0h$) and the phase contrast image shows the cluster after 10 h. Gray lines indicate cluster edges at 10 h. (D) Example of a cell cluster expanding on a graded stiffness gel. The gel stiffness increases towards the right of the panel. Numbers at the bottom indicate Young's modulus values measured with AFM. (E–F) Distribution of the angle θ between the instantaneous velocity vector and the x-axis (see inset) for the experiments displayed in panels C and D, respectively. Figure adapted from Sunyer et al. *Science* 2016, 353: 1157-1161. Reprinted with permission from AAAS.

required [Sunyer et al. 2016]. Using such methods, it was found that multicellular clusters migrating on gels of graded stiffness exhibit traction force maps with highest tractions localized at the edges and pointing towards the midline of the cluster, whereas relatively lower tractions in the bulk showed no particular orientation (Figure 4A–D). Unlike traction forces, substrate displacements on gradient gels are larger on the soft edge than on the stiff one (Figure 4E–F). These force measurements establish that the monolayer expands by generating contractile traction forces of equal magnitude at both edges, and that these forces are transmitted across the cluster. The net advance of the monolayer is therefore a consequence of the difference between the soft and the stiff deformation.

These results can also explain why durotaxis is less efficient in single cells when compared to multicellular clusters. In single cells, the difference in stiffness across a cell length is not large enough to trigger durotaxis. Multicellular clusters, however, act like a *super-cell*: cells within it are connected through cell-cell junctions and capable to transmit forces. Due to the large size of clusters, the variation in stiffness across its length will be much larger than in single cells. Consequently, durotaxis will be stronger. The fact that collectives are more efficient at responding to environmental gradients than their isolated constituents is often referred as *collective intelligence*. This phenomenon has been observed in cell clusters during chemotaxis [Camley et al. 2016, Mayor & Etienne-Manneville 2016], fish schools during phototaxis [Berdahl et al. 2013], and human groups during online gaming [Krafft et al. 2015].



Conclusion: moving forward in cell mechanobiology

The idea that living cells sense and exert physical forces has been around for a long time. However, until the last two decades the measurement of those forces has been elusive. Today it is widely accepted that mechanical cues are fundamental to fully explain biological processes in health and disease. The migration of epithelial monolayers is just an example of how a simple mechanical concept –the *tug of war*– can help us to understand a universal migratory mechanism present in complex biological processes such as morphogenesis or wound healing, as well as in diseases such as fibrosis

and cancer. As the field of mechanobiology continues to grow, it also faces new challenges. One of the most exciting ones is to translate the basic findings of mechanobiology to clinical applications. The first promising attempts to diagnose pathologies, such as malignant transformations with mechanical phenotypes are already on the way [Tse et al. 2013, Otto et al. 2015].

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