Durotaxis

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For an embryo to develop, for a tumor to metastasize, or for the immune system to eliminate pathogens, living cells must be able to undergo directed cell migration. Directed cell migration is often guided by spatial gradients in a physicochemical property of the cell microenvironment, such as chemical concentration (chemotaxis), electrical fields (galvanotaxis) or light (phototaxis). More than one century after the discovery of the first forms of gradient-guided migration, we came to learn that cells are also able to direct their migration following gradients in the stiffness of their extracellular matrix (ECM), a phenomenon called durotaxis, after Latin *durus* (hard) and Greek *taxis* (regular arrangement). Durotaxis was first reported in the year 2000 as the observation that isolated fibroblasts tend to move from the soft to the stiff region of an ECM-coated substrate (Fig. 1A). Since then, several laboratories have reproduced findings of durotaxis using a diversity of cell types including fibroblasts, mesenchymal stem cells, human cancer cell lines and immune cells. Although in most of the experiments durotaxis is positive (towards stiff regions), some studies have reported that durotaxis can also be negative (towards soft regions). This occurs during the development of the *Xenopus* optic pathway, when retinal ganglion cell axons grow towards softer tissue. These experiments suggests that durotaxis displays a much more intricated phenomenology than initially thought. In this primer we will review the strategies and main challenges in studying durotaxis *in vivo* and *in vitro*, the current understanding of the underlying mechanisms, and how these mechanisms can be generalized to explain other forms of directed cell migration. Finally, we will focus on collective durotaxis, a new mode of migration in which multicellular clusters exhibit efficient durotaxis even if their isolated constituent cells do not.

Studying durotaxis *in vitro*

The main challenge in studying durotaxis *in vitro* is to fabricate substrates with precise and reproducible stiffness gradients. The most common strategy to obtain such gradients has been to polymerize a polyacrylamide gel with a spatial distribution of crosslinking. Early studies generated this distribution by rudimentarily polymerizing two adjacent drops containing different concentrations of acrylamide and its crosslinker (Fig. 2A). A more precise implementation of this method involves mixing different polyacrylamide solutions with a microfluidic device (Fig. 2B). Even though this technique is able to produce substrates with well-defined rigidity gradients, the achievable range is limited. Moreover, this technique is costly, labor intensive and time consuming.

These limitations are overcome by a straightforward method that generates a stiffness gradient by taking advantage of the porosity of polyacrylamide gels to create a differential diffusive pattern of monomers and crosslinkers (Fig. 2C). In this method, a first polyacrylamide gel with varying thickness is prepolymerized. Later, a second polyacrylamide gel is polymerized on top of the first one. The differential diffusion of acrylamide and its crosslinker across the varying thickness of the prepolymerized gel creates a stiffness gradient. Although the resulting stiffness range is relatively small (1-8 kPa/mm) and the thickness of the gel (up to 1 mm) limits the observation of cells with high magnification objectives, the simplicity of this method makes it suitable for many laboratories that do not have access to clean room facilities. To obtain more abrupt gradients, a variation of this method can be implemented by introducing sharp thickness variations in the prepolymerized gel using microfabricated molds (Fig. 2D). This technique provides variations of up to 300 kPa/mm. However, the difference of swelling will produce a variation of several microns in height, potentially mixing durotaxis with other physical sensing mechanisms.

The study of durotaxis requires the analysis of cell migration in gradients of different stiffness slope, from shallow (1-10 kPa/mm) to very steep (50-400 kPa/mm). One of the most flexible methods to obtain this wide stiffness range is to photopolymerize an acrylamide solution with a photoinitiator. The stiffness of the resulting polyacrylamide gel will depend on the amount of light received. The most versatile method to obtain a graded exposure is to cover a polyacrylamide mix with an opaque mask, which is displaced at controlled pace to obtain progressive UV exposure (Fig. 2E). While this approach provides a high control of the stiffness slope, it requires specialized equipment such as a stepper motor. The photopolymerization method can also be combined with chrome masks to obtain very sharp gradients (>400 kPa/mm). However, as in other approaches, differential hydrogel swelling will result in variations in thickness across the sample.

In most hydrogel materials, stiffness and pore size are coupled, thus raising the question of whether cells respond to the former or the latter. To address this question, durotaxis assays have been developed based on the idea that varying the thickness of a soft substrate is mechanically equivalent to varying its stiffness. By polymerizing acrylamide onto topographically defined glass substrates, it is possible to obtain gels with different thickness but uniform pore size (Fig. 2F). Although it is difficult to calculate the effective stiffness that cells are experiencing, these assays confirmed that cells are able to follow stiffness gradients independently of other constitutive properties of the ECM.

Challenges in studying durotaxis *in vivo*

Whereas durotaxis is a well-established phenomenon *in vitro*, its role *in vivo* remains poorly studied. The main challenge has been to measure tissue rigidity with sufficient resolution to characterize stiffness gradients and their time evolution in living animals. This challenge is now beginning to be addressed thanks to the development of new techniques such as Brillouin microscopy, *in vivo* atomic force microscopy or multipole magnetic tweezers. This latter technique unveiled a mesodermal stiffness gradient across the early mouse limb bud. Cells migrated collectively along this gradient, suggesting that durotaxis could be implicated in bud development. Similarly, atomic force microscopy revealed that the embryonic *Xenopus* brain builds up a gradient in stiffness over the course of hours. Axons responded to this gradient by turning towards the soft side. With the exception of these recent studies, the potential role of durotaxis *in vivo* is estimated from *in vitro* experiments. For example, microglia cells, key players in immune response of the central nervous system, display durotaxis when seeded on stiffness gradients mimicking physiological conditions, suggesting that durotaxis could have a role in the immune response. Durotaxis has also been implicated in different diseases. For example, it has been proposed to be involved in positive feedback loops driving tumor growth and fibrosis. Similarly, targeting durotaxis of pancreatic stellate cells has been proposed as a treatment in pancreatic ductal adenocarcinoma. As technological advances bring new tools to probe ECM stiffness in living tissues, new roles for durotaxis *in vivo* are likely to emerge.

Mechanisms of durotaxis

How cells durotax remains incompletely understood. Early studies pointed to molecular players at the cell-ECM interface, such as integrins and focal adhesion kinase (FAK). More recently, non-muscle myosin II in its various isoforms (myosin II-A and B) have been added to this list. In mesenchymal stem cells, a small reduction in myosin II-B was sufficient to impair durotaxis, whereas only a large knockdown of myosin II-A produced the same effect. Interestingly, the composition of the extracellular matrix seems to be critical as well. For example, smooth muscle cells durotax on fibronectin but not on laminin. Negative durotaxis has been attributed to the capacity of the cell membrane to transduce mechanical signals. Indeed, blocking the mechanosensitive ion channel Piezo1 resulted in aberrant axonal growth of retinal ganglion cells. These results suggest that different modes of durotaxis (positive and negative) may operate on different pathways.

The search for molecular mechanisms has been paralleled by the development of different types of theoretical models. One simple model is based on a random-walk process that links persistence of movement to substrate stiffness. It is well documented that cell speed and persistence increase with substrate rigidity. This fact, without further assumptions, leads to universal soft-to-stiff motion on gradient substrates. Other models

emphasize that focal adhesions reduce their chemical potential when force is applied. Using a stability criterion, they obtain that cytoskeleton stability increases as cells move from soft to stiff regions.

A different model is based on the idea that cells probe the rigidity of their substrate through a molecular clutch that links the actin cytoskeleton and the ECM in a dynamic manner. The term "molecular clutch" was introduced in 1988 by Mitchison and Kirschner, who proposed that the dynamic link between actin and the ECM can be understood in analogy with a clutch engaging and disengaging the transmission of power between a shaft and an engine. Later on, Odde and co-workers applied the clutch model to understand how cells sense and respond to the stiffness of their environment. In a further study, the same authors proposed that this model could also be used to explain why cell migration depends on ECM stiffness. In the clutch model, cells exert forces on their ECM largely through contraction of the actin cytoskeleton by myosin molecular motors (Fig. 3A, *left*). Force is transmitted from the actin cytoskeleton to the ECM through integrins and through a series of adaptor proteins that link integrins to actin. The dynamics of the linkage between actin and the ECM (the clutch) is controlled by a key parameter, the loading rate, which is defined as the speed at which force in the clutches builds-up when they are engaged. The loading rate depends on myosin motors pulling on actin, on substrate stiffness, and on the number, arrangement and molecular properties of ECM ligands, integrins, and accessory proteins. The myosin-powered contractility produces a flow of actin, often termed "retrograde flow", that moves from the cell edge (where cell–ECM adhesions form) towards the cell center. The direction of this retrograde flow opposes that of actin polymerization, which pushes the cell membrane outwards. At the leading edge of a migrating cell, the speed of polymerization exceeds that of the retrograde flow, thus favoring membrane expansion. Conversely, at the trailing edge of the same cell, the polymerization speed will fall behind the retrograde flow, thus favoring membrane retraction.

The simplest application of the clutch model to durotaxis describes a migrating cell as a contractile continuum adhered to the substrate through two sets of identical clutches, located at the stiff and soft edges (Fig. 3B). Force generated by the contractile cytoskeleton is transmitted from one clutch to the other. Since migration takes place at low Reynolds number, we can neglect inertial forces and assume that both clutches are submitted to the same force and, consequently, the same loading rate. Under these loading conditions, the dynamics at both edges is identical but the substrate displacement is larger on the soft edge than on the stiff one. As such, contraction of the cell systematically shifts the cell center towards the stiff side, thereby resulting in durotaxis (Fig. 3B). A mechanical analogy that captures the main concepts of this mechanism is depicted in Fig. 3C. A (microscopic) skater pulls on two springs, one stiff and one soft. Because of force balance, the soft spring will deform more than the stiff one, resulting in net motion of the skater towards the stiff spring. It is important to note that this mechanism does not require components of the clutch be sensitive to local stiffness. Rather, it simply requires force transmission between opposing clutches, which naturally leads to asymmetric substrate deformation.

Towards a generalized clutch model for directed cell migration

The application of the clutch model to durotaxis might be a particular case of a more general mechanism that uses the cellular mechanosensing machinery to guide cell migration. This general mechanism would rely on three ingredients: (1) a set of clutches at both sides of the cell, (2) force transmission across the cell, and (3) an asymmetry between edges that perturbs clutch dynamics. Examples of such an asymmetry could include a difference in stiffness (durotaxis), in ligand density (haptotaxis) or even in geometrical constraints (curvotaxis, rachetotaxis). In each of these scenarios, force transmission across the cell would result in an asymmetric difference between actin polymerization and retrograde flow, thus biasing migration. An attractive feature of such a mechanism is that sensors and actuators are mechanically coupled in the cytoskeleton, rather than distributed in different subcellular compartements as in chemotaxis. This hypothetical mechanism would provide a simple, robust, and potentially general framework for distinct types of directed migration.

Collective durotaxis: better together

Some cell types do not display significant durotaxis when migrating in isolation, but they durotax efficiently as cohesive clusters (Fig. 1B). Collective durotaxis is an example of an emergent phenomenon in which a

group displays a property that its individual components lack. Other examples of emergent guidance in nature include the directed migration of leukemia cell clusters during chemotaxis or that of fish schools during phototaxis. When compared with isolated cells, clusters have access to a broader range of strategies to direct their migration. One of these strategies simply stems from the fact that multicellular clusters cover a broader area than single cells and, therefore, they can probe a larger difference in rigidity between their edges. Given that cell clusters transmit forces from edge to edge through cell-cell junctions, the same clutch model described above can be extended from single cells to clusters (Fig. 3A, *right*); long range force transmission enables a cell cluster to behave as a giant "supracell", increasing its sensitivity to mechanical gradients.

Collective durotaxis has not yet been demonstrated in vivo. However, mechanical gradients are ubiquitous in the animal body, raising the possibility that collective durotaxis directs cell migration in physiology and disease. During development, cell clusters migrate long distances from the location where they are specified to that where they will carry out their biological function. This is the case, for example, of the neural crest cluster in *Xenopus*, the lateral line primordium in zebrafish or the border cell cluster in *Drosophila*. During homeostasis, the directed movement of cell sheets contributes to tissue self-renewal, as illustrated by the intestinal epithelium, which continuously migrates as a monolayered sheet from the bottom of the crypt to the tip of the villus. These are just a few examples of a variety of physiological processes that could be mediated by collective durotaxis, but this hypothesis remains to be tested.

Tissue rigidity is a dynamic variable, as shown during the morphogenesis of *Xenopus*, when the head mesoderm underlying the cephalic neural crest stiffens due to an accumulation of cells. Stiffening initiates an epithelial-to-mesenchymal transition in neural crest cells and triggers their collective migration. In this context, it is plausible that dynamic regulation of tissue stiffness causes stiffness gradients large enough to trigger collective durotaxis. Similarly, ECM matrix deposition during wound healing could also coordinate the migration of epithelial cells and accelerate the healing process through collective durotaxis. Finally, collective durotaxis could guide collective migration in pathological processes involving local changes in tissue stiffness. Solid tumors are widely known to be stiffer than surrounding tissue, which may favor or prevent collective invasion.

Outlook

Durotaxis is emerging as a robust mechanism to drive directed migration of single cells and clusters. However, we remain far from having a comprehensive understanding of underlying mechanisms. Key ingredients include cell-ECM adhesion through molecular clutches and long-ranged force transmission across the cytoskeleton and cell-cell junctions. However, how the different layers of regulation of these complex processes explains different phenomenological outcomes is unknown. To address this challenge we need to combine better theoretical models with controlled molecular perturbations and tools to visualize the adhesive/motile machinery of the cells in high resolution. We also need improved methods to produce and characterize robust stiffness gradients. One key unknown in the field is what will be the relevance of durotaxis in development, homeostasis and disease. Given the growing body of evidence obtained *in vitro* and the increasingly well-documented existence of stiffness gradients in healthy and diseased organs, we expect durotaxis to explain a number of migratory movements *in vivo* that are currently unaccounted for.

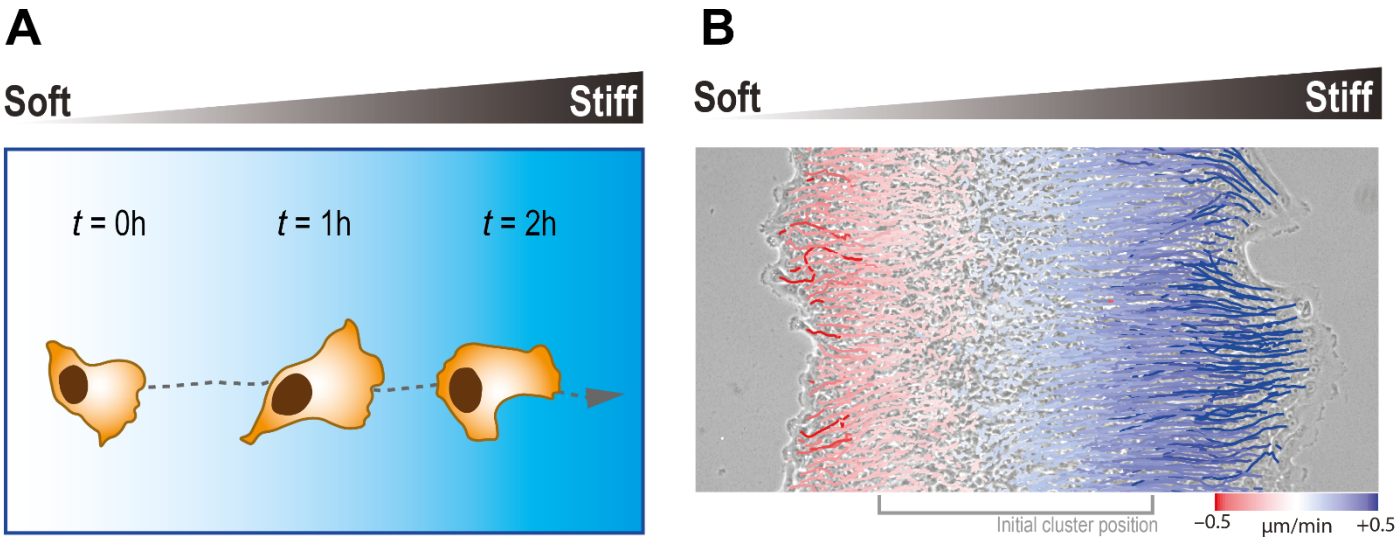

Figure 1. Durotaxis is the tendency of single cells to follow stiffness gradients. (A) Representation of the first experiment that reported durotaxis in the year 2000. Yu Li Wang and co-workers observed that NIH 3T3 fibroblasts tended to migrate from soft to stiff regions when seeded on the softer side of a polyacrylamide gel of graded stiffness. Conversely, when fibroblasts were seeded on the stiff part of the gradient, they changed their direction as they entered the gradient and moved along the boundary. (B) Phase contrast image of a cluster of human mammary epithelial cells (MCF-10A) after migrating for 10h on a stiffness gradient. Initial cluster position is indicated at the bottom. Lines indicate individual cell trajectories, illustrating the asymmetric expansion. Color code indicates individual cell speed.

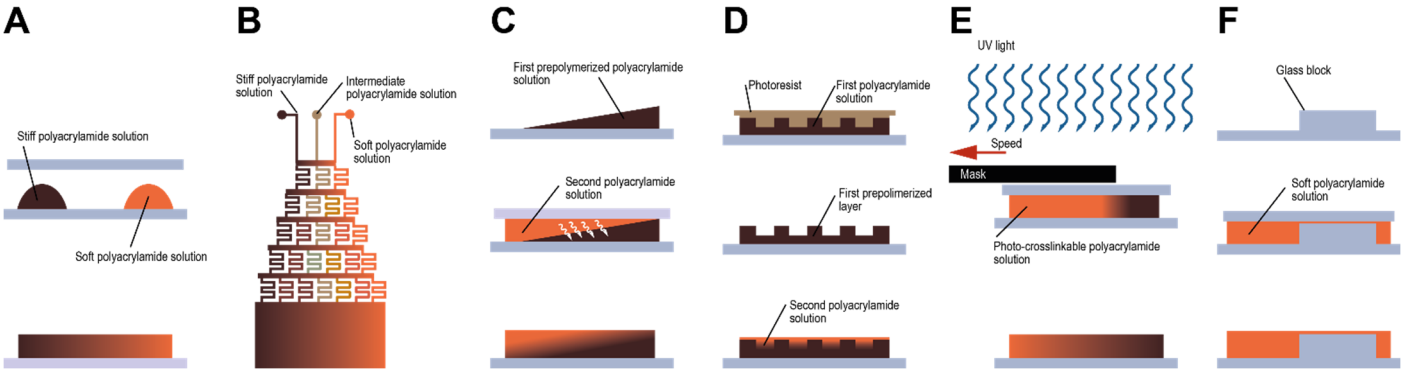

Figure 2. Common fabrication methods to create stiffness gradients on polyacrylamide gels. (A) A rudimentary stiffness gradient can be obtained by polymerizing two adjacent drops containing different concentrations of polyacrylamide and its crosslinker. (B) A relatively steep stiffness gradient can be generated by mixing different acrylamide solutions using microfluidics. (C) A straightforward method consists on polymerizing a first gel with varying thickness. Then, a second polyacrylamide gel is polymerized on top. The differential diffusion across the first gel will create a stiffness gradient. (D) A variation of the method in panel C can be used to fabricate sharp stiffness gradients. A first polyacrylamide gel with varying thickness is polymerized using a microfabricated photoresist mold. Then, a second gel is polymerized on top. The difference in diffusion will create abrupt variations in the stiffness across the gel. (E) Gels with different stiffness slopes can be created by uncovering a photopolymerizable acrylamide mix with an opaque mask, which is displaced at controlled pace while being illuminated with a conventional UV lamp. (F) To uncouple stiffness from other constitutive properties of the matrix such as pore size, polyacrylamide is polymerized on a glass with defined topology. The resulting polyacrylamide gel will have thick and thin regions. This thickness change is mechanically equivalent to varying stiffness.

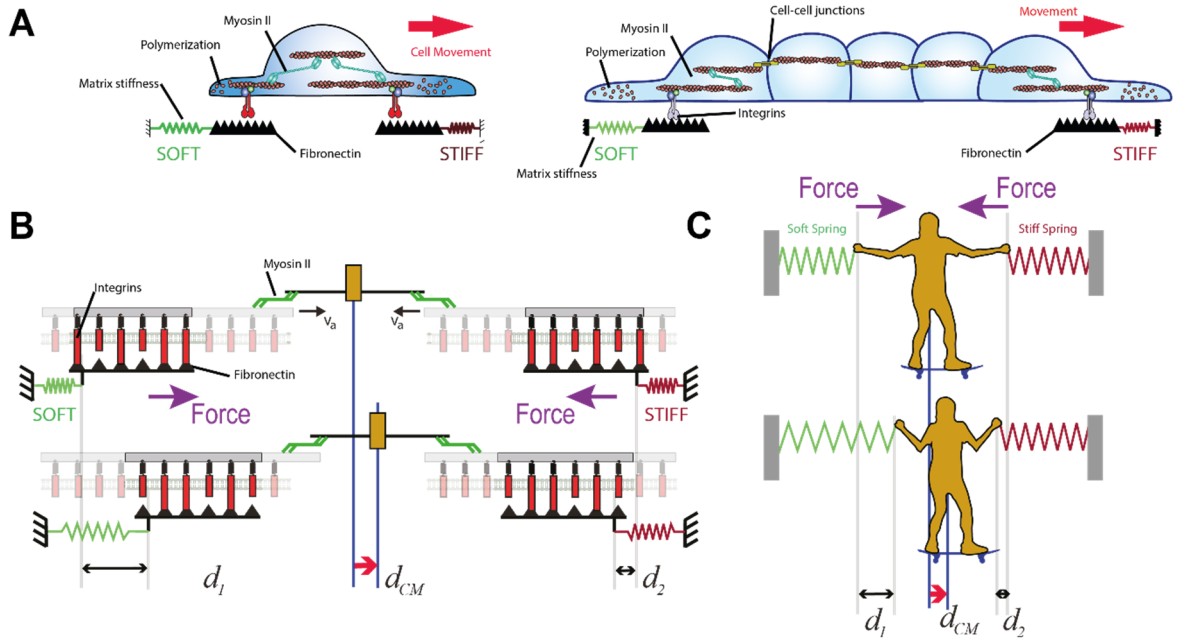

Figure 3. Molecular clutch model proposed to explain durotaxis. (A) The model has been applied to both single cells (*left*) and monolayers (*right*). Three elements are critical: (1) force generation through myosin motors, (2) force transmission across the cell (through the cytoskeleton) or monolayer (through cell-cell junctions), and (3) cell-ECM adhesion. Substrate deformation is represented as a spring. Myosin driven contraction creates a retrograde flow from the cell edge towards the cell center. The direction of this retrograde flow opposes to that of actin polymerization, which pushes the cell membrane outwards. (B) The integrins/accessory proteins at each edge of the cell/cluster are modelled as clutches with given binding and unbinding rates. As a result of force balance across the monolayer, after each myosin-driven contraction step the substrate is pulled by a larger amount on the soft side (d_1) than on the stiff side (d_2), thereby driving overall expansion (d_{CM}) toward the stiff side ($d_{CM} = d_1 - d_2$). (C) Mechanical analogy that captures the main concepts of this model. By pulling with both hands the springs, the (micro-scale) skater will move towards the stiffer spring. For simplicity, the skate friction is not included in the force balance and inertial effects are neglected.

Further reading

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