CELL MECHANICS

When tissues collide

Quantitative analysis of colliding cell monolayers reveals surprising wave phenomena involving contractility, jamming and activation of epithelial cells.

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Developing a multicellular organism from a single fertilized egg certainly is one of the greatest achievements of nature. Anyone observing this feat, either live under the microscope or in a recorded movie, has to be stunned by the precision and purpose by which this intricate process unfolds itself in time and space. Not only do dividing cells have to differentiate and form distinct types of tissue, they also have to define and maintain the boundaries and geometrical shapes of these tissues[1]. Writing in *Nature Materials*, Xavier Trepat and colleagues[2] now show that newly formed boundaries between two epithelial monolayers are not only static barriers, but also the source of mechanical waves that propagate into the surrounding tissues.

From the viewpoint of a physicist or materials scientist, the formation of tissue boundaries during development might be described by the concepts of phase transitions and spontaneous symmetry breaking. As two different cell types develop, differential interactions between them lead to their segregation in space, very much so as two sufficiently different kinds of molecules phase separate. Following these concepts, cell segregation was initially described in terms of the adhesive tension that results from cellular surface proteins (mainly cadherins), as proposed in the differential adhesion hypothesis (DAH)[3]. More recently, this view was complemented by the differential interfacial tension hypothesis (DITH), which states that mechanical tension at the cell surface has at least an equally important role as does adhesive tension[4].

Rather than mixing different cell populations and watching them sort themselves out, Trepat and colleagues followed a different approach to study the formation of tissue boundaries. They applied a cell monolayer collision assay — often used to study wound healing and collective cell migration — where two epithelial cell monolayers are separated by an inert physical barrier[5] (Fig. 1a). Removal of the barrier creates an empty space into which cells migrate collectively, eventually leading to tissue collision (Fig. 1b). If the same cells are used for both populations, the two monolayers fused into one (Fig. 1c). In contrast to what is typically explored in this classical approach, Trepat and colleagues used two different cell populations, one with the receptor EphB2 and the other with the ligand ephrinB1. On collision the two monolayers formed a fluid-filled cleft stabilized by supramolecular cables (Fig. 1d), which can later be filled with extracellular material and thus form a permanent tissue barrier.

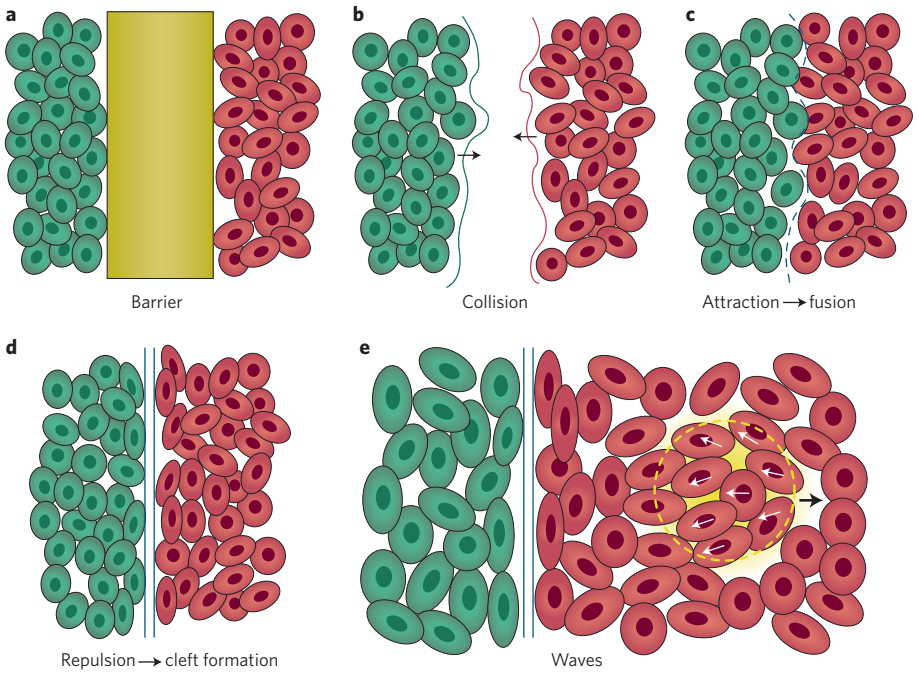

Figure 1 | Collision assay for epithelial cell monolayers. **a**, The removable barrier assay is usually employed to study wound healing and collective cell migration. The green and red colours represent epithelial monolayers starting at the left and the right, respectively. **b**, Removal of the barrier leads to collision of the two cell monolayers. **c**, If monolayers of the same cell type are used, the two monolayers fuse because the colliding cells interact adhesively with each other. **d**, When the heterotypic Eph–ephrin system is active, the two monolayers repel each other on contact and a stable cleft (double blue lines) forms at the boundary. **e**, Close to the cleft, small groups of cells are activated to migrate together and waves are emitted into the bulk. In this example, the group migrates to the left (as indicated by the white arrows) while the activated region (yellow-filled dashed circle) propagates to the right. Similar waves are observed for monolayer collisions with rigid walls and homotypic monolayers.

By itself, this phenomenon is not surprising, considering that the Eph–ephrin system is known to supress cadherin-mediated adhesion, leading to repulsion between adjacent tissues[6]. Nonetheless, when quantitatively analysed by Trepat and colleagues, this experiment revealed unexpected features of tissue collision. The authors first reconstructed the cellular traction forces measured from the deformation of the soft elastic substrate underlying the cell layers and found that it spatially oscillates perpendicular to the cleft in a Turing-like pattern. They also measured the monolayer velocity fields using particle-imaging velocimetry and found that groups

of cells move together as if pulling themselves towards the boundary. At the same time, however, the regions of cell migration shifted away from the boundary, as if a localized activation propagated into the bulk (Fig. 1e). These wave-like phenomena weakened when the cadherin-based junctions in the monolayer were disrupted, contractility inhibited or cell-division supressed. Together, these data indicate that these waves require mechanical tension to propagate across the whole system and that they are based on jamming effects in the dense monolayers.

The authors also investigated the case of a cell monolayer colliding with a wall and that of two colliding homotypic monolayers (both expressing the same Eph or ephrin). Although no specific biochemical signal was present in the first setting and no cleft formed in the second, waves emanating from the collision zone were detected in both situations. These findings suggest that these oscillations are essentially physical consequences of the force balance that arises when a monolayer collides with an obstacle. Given that this behaviour was also observed during collision of two homotypic monolayers further suggests that long-time memory of the earlier collision persisted even after fusion has occurred.

From the conceptual point of view, the results from Trepat and co-workers add another degree of complexity to the field of tissue boundaries. Rather than being determined only by local properties of the boundary, as suggested by DAH and DITH, the process of tissue-boundary formation also seems to involve oscillations and waves that spread across the whole system and that could in principle act as a feedback mechanism from far-away regions of the tissue. These results nicely tie in with recent publications showing that long-ranged force transmission has an important role in cell and tissue function, namely in stress fibres mechanics[7] and durotaxing[8]. While the mechanisms underlying the reported phenomena have not been revealed yet, this study demonstrates that they require long-ranged propagation of mechanical stress, high cell density and propagation of cell activation. In the future, the complex interplay between these different factors might be tested best in mathematical models that can represent all of them in one common framework. Different approaches of this kind have been developed over the past few years to describe collective cell migration, including particle-based simulations[9], phase field models[10] and cellular Potts models[11]. These methods, when applied to this system, should be able to explain, for instance, why groups of cells move in one direction while their activation spreads in the other. One possible explanation is that these processes result from the activation of specific signals in neighbouring cells by mechanical stress.

Once a systematic understanding of the underlying mechanisms of cell collisions and tissue boundary formation has been reached, this knowledge could be applied in the framework of synthetic biology, where artificial receptor–ligand interactions affecting tissue organization have already been employed[12]. In the long run, it might be possible to capitalize on this quantitative understanding to design novel tissue-like materials with programmable functions, which is a very challenging task using traditional man-made materials. ❐

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