Pulling towards the void

TOPOLOGICAL INSULATORS
Currents on the edge

LITHIUM-ION BATTERIES
A reversible redox process

PHOTOCATALYSIS
Energetic diamond
Doping electron-acceptors such as In\(^{3+}\) into rutile has little or no effect on the permittivity but results in lower dielectric losses at higher frequencies.

Hu and co-workers\(^2\) have shown that co-doping In\(^{3+}\) and Nb\(^{5+}\) into rutile produces local lattice defects that are highly correlated, a conclusion supported by density functional modelling. The complex stoichiometry of \((\text{In}^{3+} \text{Nb}^{5+})_x \text{Ti}^{4+}_{3-x} \text{O}_2\) gives rise to defect clusters where the electrons created by both Nb\(^{5+}\) doping and the reduction of Ti\(^{4+}\) to Ti\(^{3+}\) are contained by the presence of In\(^{3+}\) close by. Without the latter, electrons would delocalize and lead to high dielectric losses. These intrinsic defect complexes formed by co-doping give rise to strong dipoles that are responsible for the extraordinarily high intrinsic values of \(\varepsilon \geq 10^9\) without having to rely on, for instance, textured ceramic materials or other extrinsic effects that achieve colossal permittivity through the internal barrier layer capacitance effect\(^7\). More significantly, this material displays exceptionally low dielectric losses over most of the radiofrequency range with excellent thermal stability.

This remarkable new approach of chemically balancing the competing materials interactions with ambipolar co-doping is precisely the advance needed to allow the use of colossal permittivity materials in high-performance capacitors and permit further scaling advances in electronic devices.

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References

**CELL MIGRATION**

**Towards the void**

Cells at the edges of migrating epithelial sheets pull themselves towards unfilled space regardless of their direction of motion.

Eric R. Dufresne and Martin A. Schwartz

Wound healing, gastrulation and migration of carcinomas all involve coherent movements by large groups of interconnected cells. Although in vitro studies of migrating single cells have clarified many of the critical regulatory mechanisms that govern individual cell migration, we know much less about how ensembles of cells coordinate their movements. The current view is that soluble molecules, signals from adhesion receptors (such as integrins and cadherins) and mechanical stresses all mediate cell–cell communication in these systems. The challenges are to identify the specific cell-autonomous regulatory mechanisms and to explain how they lead to emergent behaviour in collective migration. In this regard, Jeffrey Fredberg and colleagues report in *Nature Materials* an analysis of the collective motion of a migrating epithelial sheet past an area where the cells could not adhere. They found a surprising disconnect between the motion of the cells located near the non-adhesive area and the forces they exerted.

Fredberg and co-workers examined the sheet of cells at an intriguing intermediate length scale, where they could resolve individual cells while still capturing large-scale flow. The motion of the cell sheet resembled a fluid flowing past an obstacle: the sheet of cells neatly split into two just upstream of the non-adhesive island and reconnected in a disorganized wake. Far from the non-adhesive island, the directions

![Figure 1](https://www.nature.com/naturematerials)

**Figure 1** Colour maps of traction forces exerted on an underlying elastic substrate by a migrating cell sheet approaching and surrounding a non-adhesive island (values are averages of six identical cell sheets). \(a, b\), As the cell sheet (which moves from west to east) approaches the non-adhesive island (white outline), the directions of traction forces and velocities align (\(T_x\) indicates the x-component of the traction forces, where x is a horizontal axis running from west to east). \(c, d\), The y- (vertical, \(c\)) and x- (horizontal, \(d\)) components of the traction forces for cells north \((c)\) and east \((d)\) of the island point towards it. The inset in \(c\) shows that north of the island there is no predominant direction for \(T_x\). Scale bar, 100 \(\mu\)m.

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of motion of the cells were well aligned with the forces they applied to the substrate (traction stresses). This correlation, however, broke down for cells located near the island. In this region, traction stresses were systematically oriented perpendicular to the edge of the sheet (Fig. 1). Although perpendicular orientation of traction stresses near the edge of clusters of epithelial cells have been reported for both freely advancing and stationary cell clusters, Fredberg and collaborators found that the cells’ pull towards the empty space was unexpectedly independent of their direction of motion.

In the absence of a robust correlation between traction stresses and movement, the researchers looked deeper for a mechanical basis for the motion. They inferred internal stresses from the measured traction stresses by balancing forces on cells within the sheet and making some simple assumptions about their mechanical properties. For cells located far from the non-adhesive island, they found a tight connection between movement and stress anisotropy. Specifically, the cells tended to move in the direction of the maximum principal stress, especially when the internal state of stress was highly anisotropic.

However, this connection broke down as the epithelial sheet split and recombined past the island.

If cell motion is not robustly correlated with traction or internal stresses, then what governs the direction of movement? The structure of the cell sheet provides some clues. Downstream of the non-adhesive area, individual cells exhibited a wider distribution of sizes, orientations and eccentricities (Fig. 2). The researchers suggest that this heterogeneity in shape may arise from the mismatch between cell movement and the orientation of traction and internal stresses. Such an upstream–downstream asymmetry echoes the turbulent wake that often appears in fluid flow downstream of an obstacle (as for mountain water past a boulder). Still, whereas in fluids inertia provides ‘memory’ of the upstream flow, for cells inertia is negligible yet memory can be provided by cytoskeletal reorganization and intracellular signalling pathways.

Fredberg and colleagues’ data provide a rich view of the mechanics of migrating cell sheets that will inform the development of theories of coordinated cell behaviour. One promising direction in this regard comes from the field of active matter, which seeks to understand how energy sources embedded within materials couple their structure, properties and emergent behaviour. Although this approach has had some notable successes, one must not lose sight of the fact that the cells’ internal regulatory mechanisms are also critical for the coordination of movement among large groups of cells. Here, adhesion-receptor signalling and mechanotransduction are prime suspects. For example, recent work showed that cadherins initiate two distinct signals that govern migratory cell polarity (one through P120ctn, which determines the protrusive front of the cell, and one through beta-catenin, which determines the contractile rear of the cell). During gastrulation in frog embryos, cadherins mediate tension-dependent signals that determine the direction of migration. Integrins also initiate signals that determine migratory cell polarity, with dynamic integrins at the front and so-called sliding or breaking adhesions at the rear. Interestingly, both integrin- and cadherin-dependent adhesions strengthen under force in some circumstances yet weaken or break under force in others, with yet-to-be-identified pathways controlling this switch. Ultimately, an integrated approach that enables feedback between cellular signalling networks and material properties will be needed to fill the current void in the understanding of a broad class of emergent phenomena in groups of cells and tissues.

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References
Propulsion and navigation within the advancing monolayer sheet

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As a wound heals, or a body plan forms, or a tumour invades, observed cellular motions within the advancing cell swarm are thought to stem from yet to be observed physical stresses that act in some direct and causal mechanical fashion. Here we show that such a relationship between motion and stress is far from direct. Using monolayer stress microscopy, we probed migration velocities, cellular tractions and intercellular stresses in an epithelial cell sheet advancing towards an island on which cells cannot adhere. We found that cells located near the island exert tractions that pull systematically towards this island regardless of whether the cells approach the island, migrate tangentially along its edge, or paradoxically, recede from it. This unanticipated cell-patterning motif, which we call kenotaxis, represents the robust and systematic mechanical drive of the cellular collective to fill unfilled space.

Perhaps the most basic fact of monolayer biology is that neither epithelium nor endothelium tolerates unfilled space. Confronted by a cell-free gap, the monolayer ordinarily advances its free edge until the available space is covered1. To explain such behaviour, gradients of morphogen or chemokine can be important but are not sufficient2, and resulting cellular motions must be mediated through the agency of some mechanical force acting over some defined area, the ratio of which is a mechanical stress. Patterning motifs and underlying physical principles that might explain this basic process more fully remain obscure. Although the innate complexity of biological systems may be partly to blame, perhaps the greater obstacle to understanding has been that mechanical stresses at work within the monolayer itself have remained almost invisible. Indeed, to explain collective cellular migration, the notion of intercellular stresses has been postulated in mathematical models3, inferred from structure4 and approximated from tissue recoil following laser microsurgery5,6, but until the past few years they have not been precisely defined or experimentally measured7–11. Random eddy-like swirling motions and associated stress fluctuations are now known to comprise 10–50% of cellular stresses in relationship to the systematic migration velocities that they might cause. To make hidden forces visible we used monolayer stress microscopy6,11, and to perturb migration dynamics we placed in the path of an advancing epithelial cell sheet an island on which cells could not adhere. The advancing monolayer encounters this island but is unable to fill the available space and is therefore said to become frustrated. Using this approach, we examine whether a causal mechanistic formulation linking motion and stress might be discerned, or, short of that, whether new patterning motifs might be identified.

Perturbing the advancing cellular sheet

To perturb the advancing monolayer sheet we deposited a circular pillar of polydimethyl siloxane (diameter = 1 mm) on a polyacrylamide gel (Young’s modulus = 1.2 kPa, thickness = 100 μm). After coating the gel with collagen I, the pillar was carefully removed to leave a circular island of bare gel on which cells could not adhere. We then seeded Madin-Darby canine kidney (MDCK) epithelial cells 3 mm from the island and allowed them to adhere and grow. After about 3 days, the advancing monolayer encounters (Fig. 1a) and ultimately surrounds this island (Fig. 1b,c,d and Supplementary Fig. S1). Local migration velocity was measured using particle imaging velocimetry12 (PIV; Methods). For velocities as well as the other variables reported below, spontaneous fluctuations tend to be as large as, or larger than, corresponding local mean values. To probe the relationship among the mean values of these variables, we smoothed these fluctuations by averaging each field across an ensemble of six identical monolayer systems, and denote such ensemble averages by brackets (…) (Methods).

Gradients of cellular velocity and substrate traction

Local velocity vectors (Fig. 1e,i) show that the speed of movement is highest at the free edge of the advancing front and decreases with distance from the island. To test whether this relationship between motion and stress is far from direct, we used a strain microscopy approach13 to assess the magnitude of intercellular stresses acting on the cells in the cell sheet against which the advancing monolayer encounters an island on which cells could not adhere. The advancing monolayer encounters this island but is unable to fill the available space and is therefore said to become frustrated. Using this approach, we examine whether a causal mechanistic formulation linking motion and stress might be discerned, or, short of that, whether new patterning motifs might be identified.

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edge (Fig. 1f,g,j,k and Supplementary Movie SM1), and eventually envelops the island (Supplementary Fig. S1). At the rear of the island another stagnation point develops, at which point the divided streams merge, stagnate and then turn sharply to rejoin the bulk migratory downstream flow (Fig. 1h,l).

Local tractions exerted between the cell and its substrate were measured using Fourier-transform traction microscopy\(^{10}\) (Methods). At each point the local traction exerted by the cell on the substrate is necessarily equal and opposite to the traction exerted by the substrate on the cell (Supplementary Fig. S2); it is helpful to depict the latter of these here in order that maps of migratory motions versus those of associated tractions would be closely similar if the motions roughly follow substrate-to-cell tractions. Even after averaging across the ensemble, tractions demonstrate strong fluctuations in magnitude and even fluctuations in sign (Fig. 2a–d); such dynamic heterogeneity is also a characteristic feature of collective cellular migration\(^{9,10,13}\). Upstream of the island the \(x\)-component of the traction vector, \((T_x)\), shows a preponderance of blue, indicating that average tractions upstream of the island tend to pull the monolayer eastward towards the frustrated edge (Fig. 2b). However, downstream of the island the \(x\)-component of the traction vector shows a preponderance of red, indicating that average tractions downstream of the island tend to pull westward, again towards the frustrated edge (Fig. 2d). Finally, near the north pole, the \(y\)-tractions, \((T_y)\), pull predominantly southward, yet again towards the frustrated edge (Fig. 2c). Importantly, regardless of cellular position along the frustrated edge, cells close to the frustrated edge exert tractions that tend to pull the monolayer towards that edge.

**Build-up of intercellular stress and its gradients**

Local stresses exerted between each cell and its immediate neighbours across cell–cell junctions were computed using monolayer stress microscopy\(^{9,11,14}\) (Supplementary Fig. S2; Methods). This method rests on the assumption of a local balance of forces in which inertial effects are taken as being negligible; inertial effects scale roughly as tissue density times the square of tissue velocity, and are smaller than measured elastic stresses, frictional stresses and traction stresses by roughly 14 orders of magnitude. Local tension on average builds from zero at the advancing free edge to the highest tension at the centre of the monolayer (colour scale, Fig. 2e); this build-up of tension is known to occur as a result of a cellular tug-of-war that is characteristic of collective cellular systems, each cell pulling not only on the substrate but also on the one behind, thereby causing tension to build progressively with distance\(^{10,15}\) (Supplementary Fig. S2). When the free edge

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**Figure 1** | Advancing monolayer of MDCK cells encounters and envelops a non-adherent island. **a–d**, MDCK cells in phase contrast at a sequence of times. In each of these panels, the inset depicts the whole island at the corresponding time point. **e–h**, Corresponding vectors of instantaneous migration velocities (obtained from PIV; Methods). **i–l**, Migration velocities, \(\langle V \rangle\), averaged over an ensemble of 6 such islands. Three findings are of note. First, fluctuations of velocity are comparable to or exceed local mean values. Second, two points of zero velocity, called stagnation points (red arrows), are evident; the positions of these stagnation points fluctuate in time but reside on average at the equator. Third, as a result, the flow of cells divides into two streams at the upstream stagnation point, and these merge at the downstream stagnation point. Scale bar in **a**, 100 \(\mu\)m. Velocity scale bars in **e**, **i** apply to **f**–**h** and **j**–**l**, respectively.
of the advancing front encounters the island, small regions of compressive stress are occasionally observed, but the state of stress is overwhelmingly tensile; the stagnation point corresponds to the region of minimal tension, and systematic components of the tension gradient are readily apparent (Fig. 2f).

Tension alone is an incomplete description of the state of mechanical stress, however. Stress borne within the monolayer itself by the cytoskeleton and cell–cell junctions is a tensorial property possessing multiple tensor components, with the familiar tension as described above being the scalar corresponding to the trace of the stress tensor (Supplementary Fig. S2). Being a tensorial quantity, the intercellular stress need not be isotropic and, as shown below, the intercellular stress is in fact strongly and systematically anisotropic. We show the local state of stress using an elliptical representation, wherein the major axis of each ellipse corresponds to the maximum principal stress, and the minor axis corresponds to the minimal principal stress.

Far from the frustrated edge, local traction vectors (blue arrows, Fig. 2i) act uniformly to pull the monolayer from west to east, and the direction of tractions corresponds closely to that of cellular motions (black arrows, Fig. 2i); velocity and traction directions are roughly coincident with average angular differences near 0°. Such an alignment between tractions and resulting motions might seem intuitive in so far as cells might move mainly along the direction of the tractions that they exert. Moreover, far from a frustrated edge these results would also be consistent with the notion of tension-induced cadherin-dependent cell polarization16, but this has never before been demonstrated experimentally in monolayer sheets. Far from the frustrated edge, local orientations of maximal principal stress (major ellipse axes) versus local migration velocity (black arrows) coincide as well (Fig. 2i), and this coincidence is consistent with plithotaxis, defined as the tendency for each individual cell within a monolayer to migrate along the local orientation of the maximal principal stress, or equivalently, minimal intercellular shear stress9,11,15,17. Approaching the frustrated edge, however, local velocity vectors veer systematically away from orientations of principal stress and away from orientations of local
tractions by angles approaching 90° (Fig. 2j). This extreme and systematic misalignment is neither intuitive nor consistent with tension-induced cell polarization. Regardless of cellular position or motion along the frustrated edge, tractions pull nearly perpendicular to that edge as if trying but failing to extend the monolayer into adjacent unfilled space (Fig. 2j,k). Moreover, local velocity vectors departing the frustrated edge, near the downstream stagnation point, veer away from local tractions by 180°, in an anti-parallel fashion (Fig. 2i). In this neighbourhood, the anti-parallel nature of tractions versus velocities is counter-intuitive and paradoxical.

Contours of constant tension are denoted by dashed lines (Fig. 2i,j). One might have reasonably imagined that migrating cells progressively build the local tension gradient through a tug-of-war mechanism9,11,15,17, and therefore migrate down that local gradient, as demonstrated previously when no island is present22. However, near a frustrated edge this is not true (Fig. 2j). Velocity vectors do align with the tension gradient far from a frustrated edge (Fig. 2i), but align nearly perpendicular to the tension gradient approaching a frustrated edge (Fig. 2i).

**Kenotaxis**

Whether approaching a frustrated edge, migrating tangentially along it, or receding from it, the cell, even several rows back from the edge, is therefore seen to exert tractions tending to pull itself systematically towards that edge. This unanticipated but robust tendency of the cellular collective to generate local tractions pulling systematically and cooperatively towards unfilled space we call kenotaxis, from the Greek κένος meaning vacuum and ταχύς meaning arrangement, and is not to be confused with the random manner in which cells migrating stochastically and independently might also fill an unfilled space18. Kenotactic tractions are fully revealed at a frustrated edge because motions into unfilled space that would have occurred otherwise have been stalled. Also revealed is the paradoxical uncoupling of these kenotactic tractions from local cellular velocities and from local intercellular stresses. Near an advancing free edge, of course, these multiple physical factors and their range of possible effects are not so readily discerned or separated. It is perhaps not surprising that traction forces at a free edge should align with the direction of local cellular velocities19, but it is not at all intuitive, at least to us, that traction forces at a frustrated edge should continue to pull towards that edge, and thus nearly perpendicular to local cellular velocities, or even contrarywise. Although these counter-intuitive behaviours seem to prevail only when the monolayer encounters an obstacle, kenotaxis is seen to be at work along any edge separating filled from unfilled space.

At a migrating free edge20 and throughout narrow strips with frustrated edges21 extracellular signal-regulated kinase (ERK) 1/2 is known to be activated. Using immunofluorescence staining, we observed that ERK 1/2 is activated, but not preferentially at or near frustrated or free edges (Supplementary Fig. S3). This discrepancy with previous reports may be attributable to different timescales; previous experiments spanned timescales of hours to one day20,21 whereas ours spanned several days. When we inhibited ERK 1/2 using the inhibitor U0126 (10 µM), kenotactic tractions and migration velocities were little changed (data not shown). When we inhibited Src family tyrosine kinases using pyrazolopyrimidine (PP1, 10 µM; ref. 20), kenotactic tractions decreased but cellular migration speed decreased markedly (Supplementary Fig. S4D,H). These findings represent another example of the surprising decoupling of migration velocities from tractions. This decoupling suggests redundant mechanisms by which edges are sensed.

One possible mechanism is mechanical. At the cell–cell junction, as well as within the cytoskeleton itself, the stress field back from the frustrated edge is predominantly tense; our experiments show that tensile stress persists all the way to the cells encountering a frustrated edge. It follows that the traction exerted by the cell on the substrate must polarize towards any adjacent unoccupied space to satisfy force balance locally. However, this is merely a description of the stress fields, and is not to be confused with a causal explanation of why cells cause tractions to polarize as they do. Do tractions polarize because the cell at the frustrated edge senses and responds to tension at its cell–cell junctions? Or instead do tractions polarize and tensions build because the cell senses and responds to unoccupied space? Either interpretation is consistent with the local force balance. However, which is the cause and which the effect remains unknown. Moreover, neither interpretation explains the relationship of the intercellular stress and the traction to the velocity vector. These represent important unanswered questions.

**Kenotactic instabilities**

Of some special interest in this regard is the motion of cells in the vicinity of the downstream stagnation point. In the wake of the island, such cells find themselves exerting traction antiparallel, not parallel, to their local migration velocities, and migrating up, not down, the local gradient in tissue tension. Compared with every other constituent cell anywhere else within the monolayer, such an alignment in both regards is topsy-turvy. Farther downstream, the orientation of local cellular tractions relative to that of local cellular velocities realigns to the more usual parallel alignment. This realignment requires either clockwise or anticlockwise rotations of the traction vectors. The existence of both modes of reorientation with equal probability implies the possibility of unstable patterns, (as with a fair coin balanced on its edge, which may tip with equal probability either to the left or to the right). This leads us to the surprising prediction that the mechanics of cellular migration within the wake must be not only complex but also unstable.

To test this prediction we looked for anomalies in downstream monolayer structure that are not evident elsewhere in the cellular migratory flow (Fig. 3). To demark cell boundaries, cell shape and cell size, we imaged the tight junction protein ZO-1, and to demark cytoskeletal structure we imaged F-actin. In the vicinity of the upstream stagnation point, the distributions of ZO-1 (Fig. 3g) and corresponding cell boundaries (Fig. 3b) were unremarkable. Cells approaching the frustrated edge showed some slight tendency towards modest eccentricity and alignment (Fig. 3c,d) but no tendency to become larger (Fig. 3e) or longer (Fig. 3f). Near the downstream stagnation point (arrow), in contrast, strong perturbation of nearly every structural metric was evident. Cells closest to the stagnation point were not any larger but were highly eccentric, aligned and elongated (Fig. 3c–f), as if pulled from the frustrated edge like taffy candy. Indeed, for these cells local tractions exerted at the cell base pull westward, whereas intercellular forces exerted at cell–cell junctions pull eastward, implying that shear forces in this special region go hand-in-hand with the observed cellular axial extension (Supplementary Fig. S5). Just surrounding this region of cellular extension, cells were appreciably eccentric, lengthened and enlarged (Fig. 3c–f) in a manner reminiscent of foreign-body epithelioid-cell granulomas as observed near sutures and micro-implants. Actin structure, similarly, was unremarkable except in the vicinity of the downstream stagnation point (Fig. 3h,i). Although it remains unclear from these experiments whether the scale of these anomalous structural perturbations is set by the size of the island or rather by some feature of unstable inter-cellular dynamics, it is clear that these perturbations are strongest in the immediate vicinity of the downstream stagnation point, and that they ramify over a scale of distance much greater than cellular dimensions.

Just behind an advancing free edge, structures known as cryptic lamellipodia extend in the direction of sheet flow4, and tractions exerted by these cells are substantially larger than those generated in the rows far behind10. It has been argued that cryptic lamellipodia drive sheet flow4, although that interpretation has been disputed because leader cells contribute only insignificantly to the global
Figure 3 | Cellular morphology, tight junction structure, and actin structure near the island. **a**, ZO-1 immunofluorescence micrograph at $t = 24$ h when a monolayer of MDCK cells fully enclosed the island. **b**, Cell boundaries retrieved from the ZO-1 micrograph in a. Segmentation was performed using a watershed algorithm. **c–f**, Eccentricity (**c**), orientation (**d**), cell area (**e**) and major axis length (**f**) determined from cell boundaries in **b**. Red arrows depict downstream stagnation points. Scale bar in **a**, 60 µm. **g**, ZO-1 immunofluorescence micrographs at $t = 24$ h when a monolayer of MDCK cells fully enclosed the island at the west (left), at the northern pole (middle) and at the east (right) of it. **h,i**, The projected actin (**h**) and basal actin (**i**) immunofluorescence micrographs correspond to the locations shown in **g**. Scale bars in **g,h,i**, 30 µm.

Build-up of tension gradients far behind the free edge. Near a frustrated edge, in contrast, might cryptic lamellipodia exist, and, if so, in what direction might they extend? We found cryptic lamellipodia but found no consistent relationship between the direction of lamellipodium extension and that of the local velocity vector, the local traction vector or the local principal stress orientation (Supplementary Movie SM2).

**Patterning motifs of the cellular collective**

These findings seem not to be restricted to our particular choice of experimental system. For example, when using rat pulmonary microvascular endothelial cells, which are spindle-shaped, the same kenotactic motif was evident (Supplementary Fig. S6). When using MCF10A mammary epithelial cells, and when overexpressing empty vector in those cells (Fig. 4a,c,e,g), the same motif was again evident, although overexpressing the oncogene product 14-3-3ζ (Fig. 4b,d,f), which disrupts adherens junctions, caused tractions near the frustrated edge to become not only smaller (Fig. 4i) but also less well aligned towards the frustrated edge (Fig. 4j). When we inhibited myosin II using blebbistatin (25 µM), tractions far from the island decreased markedly and the bare island, which comprises elastic gel, recoiled centripetally, thus indicating a release of monolayer tension; tractions near the frustrated edge were attenuated but remained well aligned towards the frustrated edge (Supplementary Fig. S7). Finally, using a crescent-like island shape, traction vectors were seen to align towards the frustrated edge in a manner that was indifferent to the sign of edge curvature (Supplementary Fig. S8).

Accordingly, kenotaxis is not to be confused with any mechanism of wound collapse that is driven by hoop tension acting through the law of Laplace over some positive (convex) local radius of curvature, as in the purse-string mechanism.

Complete fields of cellular velocity, traction and intercellular stress are now laid bare. To link these factors, discovery of a mechanistic equation of motion based on Newton's laws together...
Kenotactic tractions are evident in human mammary epithelial cells MCF10A vector, but are attenuated in MCF10A 14-3-3ζ, which disrupts adherens junctions. a,b. Phase-contrast images of non-transformed human mammary epithelial cell line, MCF10A, vector control (a) and cells overexpressing 14-3-3ζ, which have decreased expression of cell–cell junctional markers (b; ref. 23). c,d. Traction vectors, \( \langle T \rangle \), averaged over an ensemble of 4 monolayers corresponding to cell types in a, b (Methods). e,f. Colour maps of the x-component of tractions, \( \langle T_x \rangle \). g,h. Colour maps of tractions normal to the frustrated edge, \( \langle T_n \rangle \). In the case of non-transformed MCF10A vector cells, tractions near the frustrated edge are largest and oriented towards the edge (c,e,g). In the case of MCF10A 14-3-3ζ cells, however, both the magnitude and alignment of tractions near the edge are attenuated (d,f,h). i. Normal component of tractions at the frustrated edge normalized by the root-mean-square (r.m.s.) traction across the entire maps, \( \frac{T_{n,\text{edge}}}{T_{\text{r.m.s.}}} \) for three cell types, MDCK (black), MCF10A vector (blue) and MCF10A 14-3-3ζ (red; see Methods). *: \( T_{n,\text{edge}}/T_{\text{r.m.s.}} \) of 14-3-3ζ-transfected MCF10A cells is smaller than that of vector-transfected MCF10A cells or that of MDCK cells (mean ± s.e.m.; \( P < 0.05 \) by Kruskal–Wallis test). j. The alignment angle, \( \phi \), between traction vectors at the frustrated edge and normal vectors to the edge for three cell types in i. MDCK and MCF10A vector cells are seen to exert tractions highly oriented towards the frustrated edge, which are largest at that edge (i,j). In contrast, MCF10A 14-3-3ζ cells exert tractions of a smaller extent towards the edge, the alignment angle of which is widely distributed, as if they are not frustrated by the edge (i,j). Scale bars in a, e, 100 μm. Each bar in i includes observations from 6 monolayers of MDCK cells and 4 monolayers for each MCF10A cell type. Distributions in j have more than 7,000 observations.

with constitutive cellular properties might now seem an attainable objective, but challenges remain formidable. For example, no theory in the literature has anticipated, or can explain, the cooperative patterning motifs implied either by plithotaxis or by kenotaxis. Nor does any theory predict or explain the downstream kenotactic effect seems clear. Simply put, kenotaxis drives non-random filling of unfilled space. Random motion of cells would eventually fill space in the plane, of course, as can directed motion of cells guided by diffusible morphogens or physical cues including durotaxis or haptotaxis in special situations. Kenotaxis would be far more general, acting even in the absence of specific cues. In the practical matters of tissue engineering and regenerative medicine, central to any tissue engineering design are the polymers, nanomaterials or de-cellularized connective tissues that comprise the extracellular scaffold, and cells seeded within such a scaffold must migrate collectively while navigating particles, posts and intercellular stress reveal dynamics that are mechanically richer, more intricate and counter-intuitive. Compared with other mechanisms of patterning and guidance, including gradients of morphogens and phase-gradient encoding of gene oscillations, kenotaxis is likely to be more primitive, but its ultimate physiologic effect seems clear. Simply put, kenotaxis drives non-random filling of unfilled space. Random motion of cells would eventually fill space in the plane, of course, as can directed motion of cells guided by diffusible morphogens or physical cues including durotaxis or haptotaxis in special situations. Kenotaxis would be far more general, acting even in the absence of specific cues. In the practical matters of tissue engineering and regenerative medicine, central to any tissue engineering design are the polymers, nanomaterials or de-cellularized connective tissues that comprise the extracellular scaffold, and cells seeded within such a scaffold must migrate collectively while navigating particles, posts and
pores. To achieve desired attributes of cell colonization, the patterning motif expressed by cellular collectives, as reported here, is likely to provide an array of unanticipated considerations for rational engineering design.

On the basis of the evidence provided above, we conclude that keratixons comprises a systematic patterning motif, if not a dominant one, that provides the migrating cellular collective with redundant strategies to achieve robust and coordinated filling of space over distances as might occur during tissue engineering, wound healing, development or invasion.

Methods

Cell culture. MDCK cells (strain II), rat pulmonary microvascular endothelial cells and human mammary epithelial cell lines (MCF10A vector and MCF10A overexpressing 14-3-3) were cultured following published protocols\(^{12,28}\) and incubated at 37° and 5% CO\(_2\).

Monolayer preparation. Polyacrylamide gels (Young’s modulus = 1.2 kPa, thickness = 100 µm) were prepared using a protocol described previously\(^{26}\). Polydimethyl siloxane (Sylgard 184 kit, Dow Corning) membranes were fabricated using a previously described protocol\(^{29}\). A circular pillar (diameter = 1 mm) or a crescent-like pillar (diameter = 1.5 mm, concave arc curvature = 1 mm\(^{-1}\)) was punched from a membrane and deposited on the gel. After coating the gel with collagen I (BD Biosciences), the pillar was carefully removed to leave a circular island of bare gel. Cells were gently seeded\(^{3}\) mm from the island and incubated at 37° and 5% CO\(_2\) for 48 h.

Measurement of local migration velocities and gel displacements. All experiments were conducted in a culture environment on an inverted optical microscope (Leica, DMI 6000B). Fluorescence and phase-contrast images were acquired at 5 min intervals for 2 h before an expanding edge of a monolayer encountered the island and for 10–22 h after the encounter. Local migration velocities and gel displacements were obtained by the PIV method\(^{12,14}\). In this method, the cross-correlation window size was 32 × 32 pixels, and the window overlap was 28 pixels. Local migration velocities were quantified from phase-contrast images with a time interval of 5 min. Gel displacements were quantified from an image of embedded fluorescent markers at any experimental time point and a reference image obtained after trypanblue.

Recovery of substrate tractions and monolayer stresses. To obtain substrate tractions, we used the numerical procedure from monolayer stress microscopy\(^{12,14}\). Briefly, we computed a map of the tractions, \(T\), exerted by the substrate on the cells using gel displacements. From these tractions, we obtained the distribution of intercellular stresses within the cellular sheet using straightforward and rigorous two-dimensional balance of forces and their corresponding, mutually perpendicular, principal orientations (Supplementary Fig. S2E). We then computed the local tension within the cellular sheet defined as \(σ_{\text{max}} + σ_{\text{min}}/2\) and the maximum shear stress defined as \(σ_{\text{max}} - σ_{\text{min}}/2\).

Ensemble average. Maps of velocity, force and stress fields were obtained at three time points, at \(t = 0\) h (2 h before an expanding edge of a monolayer encountered the island), \(t = 12\) h and \(t = 24\) h, respectively, from six MDCK monolayers. For each monolayer, a circular island on a phase image was fitted to a circle to extrapolate a coordinate of the centre. Using that coordinate, all of the maps measured above from each monolayer were displaced with respect to the one from a chosen reference monolayer. The maps were then averaged over six monolayers at each time point. In Figs 1j,1k and 2a,b,d,e,f,h each map was folded top to bottom in half and averaged. Note that all of the local fields were forced to be zero at locations where all six maps do not overlap. For MCF10A cell lines, each map was averaged over 6 frames during 4 h of measurements for each sample, and was averaged across an ensemble of 4 monolayers for each cell type.

Immunofluorescence microscopy and cell morphology measurements. Immunofluorescence experiments and cell morphology measurements were performed using a protocol described previously\(^{31}\). Briefly, cells were fixed with 3% paraformaldehyde (Sigma-Aldrich) in phosphate buffered saline (PBS), permeabiled with 0.5% Triton X-100 (Sigma-Aldrich) in PBS, and blocked with 10% fetal bovine serum (FBS; Sigma-Aldrich) in PBS. Primary antibody rabbit anti-ZO-1 (Zymed, Invitrogen) diluted at 1:100 in 10% FBS in PBS was incubated for 1 h at room temperature and detected using secondary antibody donkey anti-rabbit (Invitrogen). Actin was visualized using Alexa Fluor 564-conjugated phallolidin (Invitrogen) at 1:1,000 in PBS. Primary antibodies rabbit anti-total-ERK 1/2 and mouse anti-phospho-ERK 1/2 (Cell Signaling Technology) diluted at 1:500 in 1% BSA in PBS were incubated for 1 h at room temperature, and detected using secondary antibodies donkey anti-rabbit and goat anti-mouse (Invitrogen). Cell segmentation based on the ZO-1 immunostainings was implemented in MatLab using a watershed algorithm.

Calculation of tractions normal to the frustrated edge. To compute tractions normal to the frustrated edge, \(T_{\text{f}}\), we defined vectors normal to that edge for each pixel within the monolayer using an approach described previously\(^{35}\). Briefly, we computed for each pixel its shortest distance to the edge. The spatial gradients of the shortest-distance map then defined the normal vectors everywhere in the monolayer. We also used this map to define cell strips with a defined range of distance to the edge; the strip width was chosen to be 20 µm, typically enclosing 1–2 cells. In the leading strip closest to the edge, we quantified the average \(T_{\text{f}}\) and normalized it with the root-mean-square traction magnitude \(T_{\text{rms}}\); this allowed us to obtain the averaged \(T_{\text{f}}/T_{\text{rms}}\) for each sample. To quantify the efficiency of keratixons, we measured at least 4 monolayers for MDCK and MCF10A cell lines. We then evaluated the statistical significance in the difference of \(T_{\text{f}}/T_{\text{rms}}\) between two cell types using the Kruskal–Wallis test. The difference is regarded statistically significant if the P value is less than 0.05.

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Author contributions

Additional information
Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.J.F.

Competing financial interests
The authors declare no competing financial interests.