Nanoparticles that mimic platelets by rushing towards blood-vessel obstructions could deliver clot-busting drugs for use in stroke treatment.

Blocked blood vessels experience higher shear forces than do healthy vessels, causing platelets to stick to vessel walls near the blockage. Donald Ingber at Harvard University in Boston, Massachusetts, and his team created nanoparticle aggregates (pictured) that break up into their component parts under high shear forces, and swarm the blood clot area.

The nanoparticles are coated with tissue plasminogen activator (tPA), which dissolves blood clots. More than 80% of mice with clots blocking the lungs’ main arteries survived after being treated with the drug-coated nanoparticles — whereas all untreated animals died within an hour.

The nanoparticles reduce by 100-fold the amount of tPA needed to dissolve a clot compared with injection of the protein into the blood. Delivery using the particles also reduces the chances of tPA causing bleeding elsewhere in the body.

Reef fish change sex but not personality

Many traits such as body colour are expressed differently between the sexes to maximize the benefits for each sex. However, this does not seem to be true for animal personality — at least not in the case of a hermaphroditic reef fish.

Dennis Sprenger of the University of Tübingen, Germany, and his team induced the dominant females in 25 social groups of the reef fish, Parapercis cylindrica (pictured), to change sex by removing the only male from each group. The team found that the more active and aggressive females became the more active and aggressive males.

Although aggressive behaviour is beneficial for territorial males, it is probably detrimental to the future reproductive success of females, the authors say. They conclude that such constraints make it difficult for selection to produce a behaviour that is optimal for both sexes — which could explain why variation in behaviour between the sexes has been maintained.

A powerful explosion on a distant star seems to have triggered partial evaporation of the atmosphere of a closely orbiting planet.

Alain Lecavelier des Etangs of the Paris Institute of Astrophysics and his colleagues used data from the Hubble Space Telescope to examine the atmosphere of the hot, Jupiter-like exoplanet HD 189733b on two occasions. The telescope failed to detect the planet’s atmosphere in April 2010, but in September 2011 it revealed an abundance of hydrogen gas rushing away from the planet. Only hours before the 2011 observations, NASA’s Swift satellite had detected a large X-ray flare on the parent star. Energy from this flare could have prompted evaporation of the hydrogen atoms, the authors suggest.

CELL MECHANICS

Wave of migration

Cells migrate en masse to generate and renew tissue — but inadequate resolution and incompatible timescales obscure the mechanism behind this migration. A unique approach reveals that stress mediates collective motion by propagating in a wave from the leading edge to the population centre.

Manuel Théry

We need not delve far into our early development to appreciate the critical role of collective cell migration. At two weeks, the cell monolayer that makes up our entire embryo folds back on itself during the process of gastrulation — separating layers of cells fated for different functions in adulthood, and mapping out the future growth of our form. A few of our cells move out of the monolayer, causing a local deformation that triggers a collective migration along the outer side of the embryo (Fig. 1a). But the exact mechanism supporting such multicellular migration has so far remained obscure.

The production and spatial distribution of mechanical forces during individual cell migration has been comparatively well characterized. Now, writing in Nature Physics, Xavier Serra-Picamal and colleagues report an innovative approach for probing the mechanical rules governing collective migration, which were previously hidden by the complex fluctuations of cellular forces. Their technique has enabled them to precisely quantify the development of intercellular forces in a cohort of migrating cells, revealing the observation that stress propagates in a wave, from the moving front to the centre of the group.

Cell motion is slow — on the order of micrometres per hour — and dominated by friction. At the cell scale, inertia is negligible compared with viscous drag, and forces in moving cells sum to zero. By contrast, the dynamics regulating the spatial organization of the intracellular cytoskeleton is faster — on the order of micrometres per second — and is known to be responsible for the oriented cell deformation and propulsion. It is therefore difficult to relate fast internal reorganization to slow global deformation to formulate an exact description of the physical mechanism regulating cell migration. Furthermore, it seems that cells can migrate by exerting either pulling forces and/or pushing forces on their microenvironment. The scenario gets even more complicated when several cells interact with each other.

Multicellular migration can be studied by assembling colonies of cells, bound together by cell–cell adhesions, which expand by outward migration of the cells on the periphery. However, a complete description of the mechanical forces is compromised by the fact that, although forces exerted on the underlying substrate may be measured, the forces between adjacent cells are difficult to gauge.

Members of the same research team previously developed a method to infer these forces in large multicellular aggregates from measurement of the traction forces on the substrate. In the study, these traction forces were associated with every cell in the colony rather than being restricted to those at the front. This showed that the collective movement was not supported by pulling forces exerted specifically by the leader cells at the front. Moreover, cells seemed to exert strong traction and intercellular forces throughout the colony. The transmission of mechanical constraints from cell to cell generated local correlations in the orientation of forces. However, these forces fluctuated in space and time in response to many parameters, such as local cell density, misaligned cell movement and stochastic

---

**Figure 1** Multicellular migration mechanics. a. The collective movements of cells during gastrulation give rise to the first two distinct layers dictating our body plan. Green arrows show cell migration in the original layer towards the invagination streak and red arrows show the cell migration in the newly formed layer away from it. b. Pulling forces exerted by the front cell stimulate traction forces on the substrate, and each cell counterbalances these forces by applying force to the cell behind it — effectively transmitting the mechanical signal generated by the release of the leading edge throughout the cell population. c. Central cells (purple) initially lengthen in response to pulling forces, then suddenly recover their rest shape via fluidization (blue star), only to become elongated again. These shape changes mediate the observed contraction waves.
internal activities. The distribution of forces within the cell group was dominated by dynamic heterogeneities spanning only a few cells2. How these mechanical dynamic heterogeneities were able to support global cell cooperation during collective movement remained mysterious.

Examples of reproducible organizational processes are few and far between in cultured cells, even though the same cells order readily in vivo. This artefact is thought to stem from a lack of geometrical boundary conditions, because the deterministic rules guiding intracellular organization or force distribution in multicellular groups8 are only reproducible under defined geometrical boundary conditions. One way to overcome this limitation in migrating cells involves controlling the geometry such that the initial conditions, when relaxed, trigger oriented migration10.

In their new study, Serra-Picamal et al. have combined this spatio-temporal control of cell group migration with a quantification of mechanical forces2. Force fluctuations persisted in the system, but by controlling the geometry, the authors were able to project two-dimensional force distributions into one dimension, and calculate the local average force field at any given moment. Repeating over several time points enabled them to quantify and visualize the temporal variations of the averaged force field.

The investigation revealed that the original intercellular stress generated close to the free group edge propagates backward as the edge moves forward (Fig. 1b). For a given cell within the group, the study shows that the pulling force exerted by the front cell towards the free edge is not the sole propelling force. Instead, it stimulates traction forces on the cell substrate along the direction of intercellular forces. These traction forces are internally counterbalanced by each cell applying force to the cell immediately behind it, and thereby, the mechanical signal triggering forward cell migration is transmitted throughout the group.

Using their accurate force-quantification method, involving thousands of cells, migrating in geometrically controlled conditions, Serra-Picamal et al. also revealed important individual cell-mechanical properties critical to eventual dynamic large-scale force patterning2. They found that the cells along the midline, subject to increasing isotropic stress as the group extends, initially manage to sustain the stress and simply deform. But above a critical stress, these cells suddenly relax and recover their rest shape (Fig. 1c). Cytoskeletal reinforcement and the idea of a positive feedback loop generating force production in response to external stress are well-known cell behaviours11. But force-induced relaxation has been underappreciated by other researchers using precise tools to probe single cells.

Previous work by the same team shows that cell cytoskeleton can fluidize without reinforcing in the specific case of short symmetric external stretch12. Now, the authors have proposed that such a fluidization could be responsible for the observed relaxation at the group centre2. This would support the reiteration of wave propagation throughout the group, and the consequential formation of alternate stripes of stress accumulation.

This new piece of work demonstrates that local movement could have reproducible mechanical consequences hundreds of micrometres away from the moving edge, through the propagation of stress waves. It is tempting to speculate that such waves occur during embryonic gastrulation. As cell contraction has been shown to direct stem cell fate13, alternating stripes of contraction could be a robust way to structure the newly formed cell layers and specify complex geometrical differentiation patterns.

References

Published online: 8 July 2012
Mechanical waves during tissue expansion

Xavier Serra-Picamal, Vito Conte, Romaric Vincent, Ester Anon, Dhananjay T. Tambe, Elsa Bazellieres, James P. Butler, Jeffrey J. Fredberg and Xavier Trepat

The processes by which an organism develops its shape and heals wounds involve expansion of a monolayer sheet of cells. The mechanism underpinning this epithelial expansion remains obscure, despite the fact that its failure is known to contribute to several diseases, including carcinomas, which account for about 90% of all human cancers. Here, using the micropatterned epithelial monolayer as a model system, we report the discovery of a mechanical wave that propagates slowly to span the monolayer, traverses intercellular junctions in a cooperative manner and builds up differentials of mechanical stress. Essential features of this wave generation and propagation are captured by a minimal model based on sequential fronts of cytoskeletal reinforcement and fluidization. These findings establish a mechanism of long-range cell guidance, symmetry breaking and pattern formation during monolayer expansion.

Epithelial monolayer expansion is increasingly regarded as a mechanical phenomenon in which physical forces not only drive cell motions but also trigger and feedback to signalling pathways. Each cell in the sheet is now known to generate forces on its underlying substrate, to transmit forces through intercellular junctions so as to create long-ranged gradients of tension, and to migrate preferentially along the direction of maximum principal stress. Despite such recent discoveries, the ultraslow dynamics of epithelial expansion remain poorly understood.

To study such dynamics, we developed an experimental approach that combines soft lithography, traction force microscopy and monolayer stress microscopy. A polydimethylsiloxane (PDMS) membrane was fabricated with a rectangular opening and deposited on a polyacrylamide gel that had been coated with collagen I. We then seeded Madin–Darby Canine Kidney (MDCK) epithelial cells and allowed them to adhere and proliferate. On reaching confluence and a relatively high cell density, the monolayer sheet exhibited features typical of a stable epithelium including apico-basal polarity and accumulation of cortical actin at cell–cell junctions. Moreover, whereas a number of genes traditionally associated with the epithelial to mesenchymal transition such as those encoding paxillin, vinculin and β-catenin exhibited changes in expression, others such as those encoding E-cadherin, vimentin and ZO-1 did not. A similar evolution of cellular velocities was recently reported after monolayer wounding, thus supporting the notion that progressive cell mobilization away from the leading edge is a general response of cell collectives to the release of physical boundaries. Traction forces exerted by cells on the underlying substrate exhibited similar spatial organization, with boundary layers of large tractions at both edges and virtually no traction at the centre. Fluctuations of both velocities and tractions increased with time and progressively expanded towards the monolayer midline.

If each cell in the monolayer has the capability to exert tractions on its substrate across focal or fibrillar adhesions, it also has the capability to exert stresses on its immediate neighbours across cell–cell junctions. Using monolayer stress microscopy, we measured corresponding inter- and intra-cellular stresses, which we refer to together simply as monolayer stresses. Shortly after the stencil was lifted, the average normal stress was largely tensile (positive) but was restricted to thin boundary layers at the leading edges, whereas the centre of the monolayer remained relaxed.

1Institute for Bioengineering of Catalonia, Barcelona 08028, Spain, 2Facultat de Medicina, Universitat de Barcelona, and Ciber Enfermedades Respiratorias, Barcelona 08036, Spain, 3Laboratoire Matière et Systèmes Complexes (MSC), Université Paris Diderot, and Unité Mixte de Recherche 7057 CNRS, F-75205 Paris Cedex 13, Paris, France, 4School of Public Health, Harvard University, Boston, Massachusetts 02115, USA, 5Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA, 6Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona 08010, Spain. *These authors contributed equally to this work. †e-mail: xtrepat@ibecbarcelona.eu.

© 2012 Macmillan Publishers Limited. All rights reserved.
cells; cell–cell tension transmission exhibited a growing scale of length (Fig. 2k), and the maximum intercellular shear stress ($\mu$) followed a similar pattern (Fig. 2m,n). Taken together, these findings demonstrate that force transmission from cell-to-cell, and cellular migration across the epithelial sheet, are initiated at the leading edge and progressively penetrate towards the centre (Supplementary Movie S3).

Moreover, these stress fields were anisotropic. At each position in the monolayer plane, the maximum ($\sigma_{\text{max}}$) and minimum ($\sigma_{\text{min}}$) principal stresses were represented as an ellipse aligned with corresponding principal orientations (Fig. 2p). Throughout epithelial expansion, stress ellipses tended to be spindle-shaped and thus revealed pronounced stress anisotropy. The maximum principal stress orientation tended to be perpendicular to the leading edge and thus roughly parallel to local cell motion (Fig. 2q). As described previously, this mode of local cell guidance defines plithotaxis.$^{19}$

Superposed on systematic monolayer spreading were large-scale spatio-temporal fluctuations of tractions, monolayer stresses and cellular velocities (Fig. 2f,i,l,o). To better characterize the systematic evolution of mechanical patterns, we averaged these variables over the observable monolayer length (corresponding to the $y$ coordinate), thereby reducing the dimensionality of the system to only one spatial dimension and one temporal dimension.

All data could then be represented as kymographs in the $x$-$t$ plane (Methods). Kymographs of cellular velocity ($v_y$) revealed motility patterns that were not restricted to the initial phase of inward mobilization (Fig. 3a). To the contrary, after reaching the monolayer midline at $\sim$150 min, the two fronts of cell motility coalesced and then continued towards the leading edge. When cells are cohesive and mass is conserved, cellular velocities must be linked to the rate of cell deformation (strain rate, $\varepsilon_{xx}$; ref. 11) through the expression $\varepsilon_{xx} = \partial v_y / \partial x$. Remarkably, kymographs of $\varepsilon_{xx}$ revealed clear evidence of wave-like crests of strain rate that were launched at each leading edge, propagated away from and back to the leading edge at roughly twice the speed of the advancing front edge, and spanned the entire monolayer (Fig. 3b). To distinguish these mechanical waves from other known types of mechanical wave, and because they inscribe an X-shape on the kymograph, we call them X-waves.

To study the physical origin of the X-wave, we next focused on traction generation and stress transmission in the monolayer. Whereas traction kymographs demonstrated extrema at the leading edge, monolayer stresses were highest at the monolayer midline, indicating that local force generation was globally integrated and transmitted through cell–cell junctions to give rise to a stress build-up (Fig. 3c,d). Importantly, monolayer stress at the midline oscillated in time (Fig. 3g,h and Supplementary Movie S4); these oscillations were in phase with fluctuations of cell area (Fig. 3f,h) and demonstrated phase quadrature with strain rate (Fig. 3e). Contrary to long-held assumptions (reviewed in ref. 12), these observations establish that on the ultraslow timescales of cellular migration the dominant cellular stresses in the monolayer are elastic, not viscous.

In the absence of appreciable inertia, there can exist no exchange between kinetic and potential energy storage as is usually associated with propagation of passive mechanical waves, thus suggesting that the mechanism underlying the observed propagation might be active. To investigate this possibility, we inhibited myosin using blebbistatin. Blebbistatin prevented the formation of stress fibres (Supplementary Fig. S2) and had little effect on the velocity of the leading edge, thus confirming previous reports in wound scratch assays.$^{8}$ Blebbistatin caused traction forces and intercellular stresses to be abrogated, however (Supplementary Fig. S2 and Movie S5). A well-defined front of strain rate could be clearly identified nonetheless, but this front was stationary, did not propagate

---

**Figure 1** | **Experimental model.** **a,** A PDMS membrane is deposited on a collagen-coated polyacrylamide (PA) gel. Cells are seeded and allowed to attach only over the gap defined by the PDMS membrane. When confluent cells reach a relatively high confluence, the PDMS membrane is peeled off and cells start invading the surrounding space. **b,** Transversal view of LifeAct MDCK cells at the specified time points after the PDMS membrane was removed; scale bar, 20 µm. **c,** Basal actin (LifeAct–GFP), E-cadherin, ZO-1 and paxillin immunofluorescence micrographs before, 1 h after and 4 h after removing the membranes. Scale bars, 15 µm. **d,** Cell sheet area (filled circles) and cell sheet area due to proliferation (white circles) at different time points. The area due to proliferation was calculated by counting the number of new cells in the monolayer at distinct time points and then multiplying the number of new cells by the average cell area. Data are mean ± s.d. ($n = 5$). Inset: relative contribution of cell proliferation to cell sheet area.

With time, these boundary layers became markedly heterogeneous but systematically grew to encompass increasing numbers of
and dispersed or attenuated after about 450 min. Together, these findings indicate that the propagating mechanical wave involves a contractile component.

To study the role of intercellular adhesion in monolayer stress transmission and wave propagation, we disrupted cell–cell junctions by chelation of extracellular calcium\(^5\) after 280 min of monolayer expansion (Supplementary Fig. S3 and Movie S6). Within 20 min of calcium chelation, the monolayer lost its structural integrity and isolated cells were seen to escape from the leading edge. Monolayer stress exhibited a sharp drop and wave crests vanished (Supplementary Fig. S3). Restoration of calcium levels 45 min later rescued monolayer stresses and wave propagation but, remarkably, wave propagation restarted at the very edge the monolayer. These data highlight a central role for cell–cell junctions in the generation and propagation of X-waves. They show, further, that these waves are not restricted to the case of sudden release of a physical constraint.

Across experiments, monolayer expansion exhibited various levels of symmetry breaking (Supplementary Fig. S4). As in the symmetric case (Fig. 3), experiments in which symmetry was spontaneously broken showed propagation of velocity fronts back from each leading edge but, on collision, one of the two fronts penetrated past the midline into the opposite side of the monolayer before propagating back towards the leading edge (Supplementary Fig. S4a,b). As such, the characteristic time for the velocity front to complete one cycle of inward and outward propagation was longer than in the symmetric case. Symmetry breaking in multicellular systems is a widespread process that remains poorly understood\(^13-15\). Our findings suggest that symmetry breaking during monolayer expansion originates at the very edge of the monolayer boundaries at the very onset of migration (Supplementary Fig. S4 and Movie S7), with the highest stresses restricted to the first few rows of the fastest leading edge.

Waves in chemical systems, as in the Belousov–Zhabotinsky reaction, rely on temporal competition between reaction times and diffusion times, and a generalization of this idea to non-equilibrium mechano-chemical systems was recently proposed\(^16\).
Figure 3 | Dynamic behaviour of a cell sheet expanding nearly symmetrically. a–d. Kymographs of velocity $v_x$ (a), strain rate $\dot{\epsilon}_{xx}$ (b), tractions $T_x$ (c) and monolayer stress component $\sigma_{xx}$ (d). Boundary artefacts attributable to particle image velocimetry have been suppressed from b (Supplementary Fig. S5). e–g. Average value of $\dot{\epsilon}_{xx}$ (e), cell area (f) and $\sigma_{xx}$ (g) at the monolayer midline (average over a strip of width 125 μm centred at the midline). h. Overlay of monolayer stress component $\sigma_{xx}$ on phase-contrast images. The right square panels are a magnification of the highlighted region in the left panels. Scale bar, 50 μm.

Alternatively, wave propagation can also originate from threshold phenomena that are rapid, coupled with refractory phenomena that are slow, as in propagation of the action potential\cite{17}. Although it remains unclear how comparable mechanisms might account for the mechanical waves reported here, a minimal one-dimensional mechanical model captures the observed phenomenology without invoking chemical factors or their associated reaction, diffusion and advection. The model treats the monolayer as a collection of springs (cells) of elastic constant $k$ connected in series (Fig. 4a). Each cell is allowed to generate a self-propelling force $F_i$. This propelling force can be transmitted through elastic forces to neighbouring cells and by frictional forces to the underlying gel substrate. The position $x_i$ of a generic node (cell–cell junction) is affected by the force $F_i$, by the elastic responses $f_e^i$ and $f_{e,1}^i$ of the two cells joined at that node, and by the viscous friction $f_v^i$ between those two cells and the gel substrate (Fig. 4b), represented as a dashpot of constant viscosity $\eta$. 


wave with many of the same features as the X-waves demonstrated experimentally (Fig. 4d–f and Supplementary Movie S8). A role for reaction–diffusion–advection mechanisms or for gene oscillators cannot be ruled out, but to explain these mechanical waves, such mechanisms need not be invoked.

In view of this relationship, pattern formation during development is widely attributed to cellular sensing of local chemical differentials that become reiterated over the span of a great many cells. Although there is little doubt that such reiterated chemical differentials are necessary to explain patterning, it remains unclear whether they are sufficient. For example, large multicellular systems are typically heterogeneous, dynamic and noisy. In such systems, can the reaction–diffusion–advection mechanisms act with sufficient precision to transmit requisite information over distances spanning a great many cells? This question and others have led to the hypothesis that pattern formation requires another feedback mechanism, and that such a mechanism is provided by physical forces. It is now well established that local physical forces can be transduced into local intracellular signals to activate local regulatory protein networks, but patterns of stress and strain reiterated in time and over space across a multicellular tissue have never before been observed. Our finding of a slow mechanical wave constitutes the first direct evidence of such reiterated mechanical patterns and thus provides a natural candidate to trigger mechanotransduction.
pathways during wound healing, morphogenesis and collective cellular invasion in cancer.

Methods

Cell culture. MDCK strain II cells were cultured in minimum essential media with Earle’s Salts and L-glutamine (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin.

Microfabrication of the PDMS membranes. PDMS membranes were fabricated according to procedures described previously. Briefly, SU-8 50-masters containing rectangles of 300 x 2,500 µm were raised using conventional photolithography. Uncured PDMS was spin-coated on the masters to a thickness lower than the height of the SU-8 feature (35 µm) and cured for 2 h at 60 °C. A thicker border of PDMS was applied at the edges of the membranes for handling purposes. PDMS was then peeled off from the master and kept in ethanol at 4 °C until use.

Preparation of polyacrylamide gels. Polyacrylamide gel preparation was adapted from protocols described in refs 34, 35. Glass-bottom dishes were activated by using a 1:1:14 solution of acetic acid/bind-silane/ethanol. The dishes were washed twice with ethanol and air-dried for 10 min. For 3 kPa gels, a stock solution containing a concentration of 5.5% acrylamide, 0.09% bisacrylamide, 0.5% ammonium persulphate, 0.05% tetramethylthylendiamine, 0.4% of 200-nm-diameter red fluorescent carboxylate-modified beads (Fluospheres, Invitrogen) and 2 mg ml⁻¹ NH-acrylate was prepared. A drop of 10 µl was added to the centre of the glass-bottom dishes, and the solution was covered with 12-mm-diameter glass coverslips. After polymerization, gels were washed with PBS and incubated with 100 µl of a collagen I solution (0.1 mg ml⁻¹, Millipore) overnight at 4 °C. Gels were washed afterwards with PBS and incubated with cell culture media with 10% FBS for 6 h.

Cell patterning on soft substrates. One hour before seeding the cells, the PDMS membranes were air dried and incubated in a solution of 2% Pluronic F-127 (Sigma-Aldrich) in PBS to avoid damage of the gel coating due to the PDMS membrane. The membranes were then washed twice with PBS and air dried for 20 min, and they were deposited on the surface of the polyacrylamide gel. A small volume (8 µl) containing 15,000 cells was placed on the exposed region of the polyacrylamide gel defined by the PDMS membrane. Once the cells were attached to the polyacrylamide gel (20 min), the unattached cells were washed away and 200 µl of medium was added. Twelve hours after seeding the cells, 2 ml of medium was added and the PDMS membranes were carefully removed with tweezers before the beginning of the experiment.

Time-lapse microscopy. Multidimensional acquisition routines were performed on an automated inverted microscope (Nikon Eclipse Ti) equipped with thermal, CO₂ and humidity control, using MetaMorph (Universal Imaging) software. Time-lapse recording started approximately 30 min after removing the PDMS membrane. The interval between image acquisition was 1 min and a typical experiment lasted for 20 h. To capture the full width of the expanding cell sheet, two images were acquired at 10 every time point, approximately overlapping laterally by 10%. The two images were accurately stitched with subpixel resolution using custom-made Matlab software.

Fluorescence microscopy. Immunofluorescence microscopy experiments were carried out by fixing the cells with 3% paraformaldehyde (Sigma-Aldrich) in PBS, permeabilizing with 0.5% Triton X-100 (Sigma-Aldrich) in PBS, and blocking with 10% FBS (Sigma-Aldrich) in PBS.

Primary antibodies mouse anti-E-cadherin (BD Transduction Laboratories), rabbit anti-ZO-1 (Zymed, Invitrogen) and mouse anti-paxillin (BD Transduction Laboratories) diluted at 1:1,000, 1:500 and 1:100, respectively, in 10% FBS in PBS were incubated for 1 h at room temperature, and were detected using secondary antibodies goat anti-mouse and donkey anti-rabbit (Invitrogen). A spectral confocal microscope (Nikon Eclipse C1si) was used for high-resolution image acquisition.

Cell area measurements. The contour of each cell was determined using a segmentation algorithm (Greyscale Watershed for Image, D. Sage, Biomedical Image Group, EPFL). Phase-contrast images were pre-processed by contrast enhancement followed by a Gaussian blur. To limit the over-sampling inherent to water-sheding algorithms, we set up the appropriate limits for the cell area and eccentricity.

Velocity measurements. Velocity fields were computed using custom-made particle image velocimetry software on the phase-contrast images. The interrogation window was either 64 x 64 pixels or 96 x 96 pixels, and the time interval between consecutive analysed images was 1 min. Monolayer boundaries were computed using a home-made algorithm based on the standard deviation of each interrogation window in the phase-contrast images.

Traction microscopy. Traction forces were computed using Fourier transform traction microscopy with a finite gel thickness. Gel displacements between any experimental time point and a reference image obtained after monolayer trypsinization were computed using home-made particle image velocimetry software. To reduce systematic biases in subpixel resolution and peak-locking effects, we implemented an iterative process (n = 4 iterations) based on a continuous window shift technique.

Monolayer stress microscopy. Monolayer stresses were computed using monolayer stress microscopy. Monolayer stress microscopy uses traction forces and straightforward force balance demanded by Newton’s laws to map the two-dimensional stress tensor σ in the monolayer:

\[ \sigma = \frac{\sigma_{xx}}{2} + \frac{\sigma_{yy}}{2} + \sqrt{\left(\frac{\sigma_{xx} - \sigma_{yy}}{2}\right)^2 + \sigma_{xy}^2} \]

By rotating these stress components at each point in the cell sheet, we computed the magnitude of the two principal stress components σ_max and σ_min and their corresponding, mutually perpendicular, principal orientations. For each point in the monolayer, we computed the average normal stress within and between cells defined as \( \hat{\sigma} = \frac{\sigma_{xx} + \sigma_{yy}}{2} \) and the maximum intercellular shear stress defined as \( \hat{\mu} = \frac{(\sigma_{max} - \sigma_{min})}{2} \).

Kymographs. For each pixel in the monolayer, we computed the distance to the closest leading edge. Next we computed the median values of velocities, tractions, monolayer stresses and strain rates of all pixels located at a given distance from the leading edge. These median values were then represented on a unidimensional segment whose width was the mean width of the monolayer. This operation was repeated for each experimental time point.

Quantification of gene expression. MDCK monolayers were collected at 0, 3, and 7 h of monolayer expansion. The total RNA was extracted using the PARIS kit (Applied Biosystems) according to the manufacturer’s instructions. RNA was quantified by the absorbance at 260 nm, and reverse transcribed into complementary DNA using the high-capacity RNA-to-cDNA master mix (Applied Biosystems). Quantitative PCR was performed with the 7500 fast real-time PCR system and software (Applied Biosystems). TaqMan gene expression assay C02668852_m1, C02651495_m1, C02628470_m1, C02626248_m1, C02667774_m1, C02645536_m1, and C03023880_g1 were used to detect vimentin, paxillin, ZO-1, E-cadherin, β-catenin, vinculin and β-actin, respectively. Quantitative real-time PCR values were normalized to an internal control s18 (TaqMan probe C02824915_g1, 18S), averaged and expressed relative to gene expression before cell migration (0 h).

Received 31 January 2012; accepted 30 May 2012; published online 8 July 2012

References


Acknowledgements

We thank M. Bintanel for technical assistance, S. Garcia and A. Carreras for help with polyacrylamide gels and micropatterning, the Nanotechnology Platform from Barcelona Science Park, J. J. Munoz for help with the numerical implementation of the model, and P. Roca-Cusachs, D.G. Miguez, D. Navajas, R. Farre and J. Alcaraz for discussions. This research was supported by the Spanish Ministry for Science and Innovation (BFU2009-07595 and FPU fellowship XS), the European Research Council (Grant Agreement 242993) and the National Institutes of Health (R01HL102373, R01HL107561).

Author contributions

X.S.-P. and X.T. designed experiments; X.S.-P. and X.T. performed gene expression experiments; X.S.-P., R.V., V.C. and X.T. analysed data; D.T.T. contributed software; V.C. built the computer model and performed simulations; X.S.-P. and X.T. performed all experiments. E.B. and X.S.-P. helped with polyacrylamide gels and micropatterning, the Nanotechnology Platform from Barcelona Science Park, and commented on the manuscript; X.T. supervised the project.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturephysics. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to X.T.
Mechanical waves during tissue expansion

Supplementary Figures

**Fig. S1. Relative changes in mRNA levels during monolayer expansion.** Results are plotted as mean ± SD of two independent experiments, each performed in triplicate. For each independent experiment, the cells from 5 independent cell sheets were collected.
Fig. S2. Blebbistatin increases velocity of monolayer expansion but abrogates traction generation, monolayer stress transmission and wave propagation. Kymographs of velocity \( v_x \) (a), strain rate \( \dot{\varepsilon}_{xx} \) (b), tractions \( T_x \) (c), and monolayer stress component \( \sigma_{xx} \) (d). Blebbistatin prevented stress fiber formation. (e) Control. (f) Blebbistatin treatment. Scale bar = 20 \( \mu \text{m} \).
**Fig. S3.** Effect of a sudden disruption of cell-cell junctions with EGTA during monolayer expansion. Kymographs of velocity $v_x$ (a), strain rate $\dot{\varepsilon}_{xx}$ (b), tractions $T_x$ (c), and monolayer stress component $\sigma_{xx}$ (d). EGTA was added at 280 min and removed at 325 min (dashed lines).
**Fig. S4. Dynamic behavior of an asymmetrically expanding cell sheet.** Kymographs of velocity $v_x$ (a), strain rate $\dot{e}_{xx}$ (b), tractions $T_x$ (c), and monolayer stress component $\sigma_{xx}$ (d).

**Fig. S5. Boundary effects of PIV.** When PIV is applied to the monolayer boundaries, cells only occupy a fraction of the interrogation window. The velocity vector corresponding to that interrogation window has one contribution from the motile monolayer and another contribution from the immobile substrate. As a consequence, the resulting velocity is smaller than the real monolayer velocity. This effect leads to a systematic gradient of velocity at the very edge of the monolayer and, as such, to an artifactual negative strain rate. We have suppressed this artifactual data from Fig. 3b and Supplementary Fig. S4b. (a) and (b) show the unedited data corresponding to Fig. 3b and Supplementary Fig. S4b, respectively.
Supplement 1: Monolayer Stress Microscopy and material properties of the cell monolayer.

To obtain the distribution of stresses throughout the monolayer, MSM uses the map of traction forces together with the two-dimensional balance of forces that is demanded by Newton’s laws. Because this force balance must be obeyed at every instant, the computation of monolayer stresses from traction forces does not depend on whether the monolayer is elastic, viscous, or viscoelastic. Without loss of generality, we thus chose to treat the monolayer as a 2D elastic homogeneous material with Young’s modulus $E$ and Poisson’s ratio $\nu$. In our previous report we used precisely the same approach with $\nu$ assigned to be 0.5, but in that report we asserted - incorrectly - that if the traction forces are specified then recovered stress distribution within the monolayer is independent of constitutive properties of the monolayer. While that statement is rigorously true in one-dimensional systems, it is not true in two-dimensional systems, wherein there are two degrees of freedom in the external tractions ($T_x$ and $T_y$) but three components to the stress tensor ($\sigma_{xx}$, $\sigma_{yy}$, $\sigma_{xy}$). In that case, the coupling between tractions and stresses does not depend on the Young’s modulus but does depend on the Poisson’s ratio $\nu$.

That being the case, we assessed the sensitivity of stress maps to the assumed value of $\nu$ by computing $\sigma_{xx}$, $\sigma_{yy}$, and $\sigma_{xy}$ from a set of experimental traction fields $T_x$ and $T_y$ with $\nu$ ranging from 0 to 0.5 (Fig. S6). These results demonstrate that variations in $\nu$ have negligible effects on $\sigma_{xx}$ and $\sigma_{xy}$ but substantial effects on $\sigma_{yy}$ as $\nu$ approached zero. This coupling between $\sigma_{yy}$ and $\nu$ has its origin in the boundary conditions of zero normal displacement at the top and bottom edges of the field of view, but has little influence when $\nu > 0.4$. Moreover, our findings of wave propagation and pattern formation during monolayer growth are restricted to the direction of expansion ($x$) and are thus largely independent of the choice of $\nu$. Therefore, throughout this report we assume incompressibility ($\nu=0.5$).
Fig. S6. Dependence of computed monolayer stresses on assumed Poisson’s ratio. Phase contrast image (a), traction maps (b, c), and monolayer stress maps (d–o) for different values of Poisson’s ratio.
Supplement 2: Monolayer Stress Microscopy and cell height

Monolayer Stress Microscopy performs a formal two-dimensional balance of line tension that is converted to familiar units of stress by using the average height ($h$) of the monolayer. To account for changes in $h$ during monolayer expansion we used confocal microscopy. We performed z-stack confocal imaging on MDCK cells stably expressing LifeAct-GFP and computed the average fluorescence intensity $<I_{(z,t)}>\text{ for each time point and for each } z\text{ plane, where brackets denote average over } x \text{ and } y \text{ within the monolayer. We calculated } h \text{ as the characteristic height obtained from fitting an exponential decay to the tail of } <I_{(z,t)}>\text{. The average cell height was roughly homogeneous across the direction of expansion (Fig. S7b) and exhibited a sharp decrease with time that stabilized at ~8 } \mu m \text{ (Fig. S7a).}

![Figure S7](image)

**Fig. S7. Monolayer height.** (a) Average cell height during monolayer expansion. Error bars represent the SD. (b) Average cell height as a function of the distance from the monolayer midline at different time points. Dashed lines are mean±SD.
Supplement 3: one-dimensional model of monolayer expansion

Using the model described in Fig. 4, we consider here four distinct loading scenarios and analyze the extent to which each of these scenarios captures the central features of our observations including 1) constant velocity of the monolayer leading edge, 2) propagation of a strain rate front, 3) monolayer stress buildup, and 4) temporal reiteration of both strain-rate propagation and stress buildup.

In every case, a numerical solution was obtained by using Newton-Raphson reiterations. In each node, residual force contributions from loads \( \mathbf{F}_l \) along with forces of discrete elements that join at that node (springs and dashpot) were required to satisfy Eq. 1 (main text).

We first considered the simple case where only the two front cells (one on each side of the monolayer) generate self-propelling forces (Fig. S8, Supplementary movie 9). We simulated this scenario by applying two forces of constant magnitude and opposite sign to the front nodes of the monolayer. In response to these forces, leading cells begin stretching immediately, but viscous friction with the substrate prevents instantaneous transmission of the leading force to the follower cells (Fig. S8d). Consequently, the further a cell is from the leading edge, the longer that cell takes to begin stretching. This delay translates into a pulse of strain rate that penetrates the monolayer and attenuates progressively (Fig. S8c). The system tends asymptotically to a state in which stress and strain of each cell are equal across the monolayer. Accordingly, the system never displays a stress buildup nor constant velocity of the leading edges of the monolayer, thus showing that an active leader/passive follower scenario does not capture the experimental features we observed.

We next considered the case in which all cells have the capacity of generating self-propelling forces simultaneously. (Fig. S9, Supplementary movie 10). We simulated this scenario by applying outward pointing forces of constant magnitude at each node of the monolayer at the same instant of time. Under these circumstances, each half of the monolayer begins moving outwards uniformly and cohesively (Fig. S9b). The elastic stress is highest at the center of the monolayer and decreases towards its edges (Fig. S9d), thus causing a progressive decrease of cell velocities away from the monolayer midline (Fig. S9b). When the model was run in such a configuration we could simulate constant velocity at the leading edges (Fig. S9b), stress buildup in its
central regions (Fig. S9d), and outward propagation of a strain rate pulse (Fig. S9c), all features we observed experimentally. Nevertheless, the model is not able in such circumstances to reproduce an initial phase where stress and strain rate propagate inward from the leading edges towards monolayer midline.

![Diagram]

**Fig. S8**: (a) Cartoon illustrating epithelial dynamics induced by migrating cells located at the leading edges only. At instant $t = t_0$ the two front cells are subject to self-propelling forces of same modulus and opposite direction. (b) Kymograph of epithelial velocity field vs. time; (c) kymograph of epithelial strain rate field vs. time; (d) kymograph of epithelial elastic stress field vs. time.

We next considered a third case in which a cell is able to generate a self-propelling force only if its immediate front neighbor has deformed more than a given strain $\varepsilon_0$ (Fig. S10, Supplementary movie 11). This is equivalent to assume that each cell can only acquire a motile phenotype if it senses a force larger than $k\varepsilon_0$ on its front intercellular junction. This type of mechanism has been recently invoked to explain the collective migration of cell clusters$^{2,3}$ and the underlying physics can be understood in analogy to “unjamming” phenomena$^{4-6}$. Under such an assumption, the monolayer achieves its initial expansion by successive spreading of each cell row, which results in a strain rate pulse propagating away from the leading edge (Fig. S10c). Once the strain rate pulse reaches the monolayer midline, the system tends to its equilibrium configuration in which stress decreases away from the monolayer midline (Fig. S10d). However, the rate at which every cell tends to its equilibrium
length also decreases with the distance from the midline, thus giving rise to outward propagation of a strain rate pulse (Fig. S10c).

**Fig. S9:** (a) Cartoon illustrating epithelial dynamics induced by cells that migrate by generating outward self-propelling forces simultaneously at the initial instant \( t_0 \). (b) Kymograph of epithelial velocity field vs. time; (c) kymograph of epithelial strain rate field vs. time; (d) kymograph of epithelial elastic stress field vs. time.

Unjamming by itself captures central features of monolayer expansion including propagation of a strain rate pulse away from and back to the leading edge, stress buildup, and linear leading edge velocity, but it does not exhibit spatiotemporal reiteration of local differentials of stress and strain rate. Instead, the system tends asymptotically to an equilibrium state in which the length of each cell decreases with the distance from the leading edge. Spatiotemporal reiteration of local differentials in mathematical models of tissue patterning is typically obtained by considering two competing phenomena coupled through a time delay. In traditional reaction-diffusion models, the competing phenomena are activation and inhibition of morphogens and the time delay originates from their distinct diffusion and reaction rates\(^7,8\). This idea was recently generalized to the case of mechanochemical systems, where the competing phenomena are diffusion and contractile advection and the time delay originates in their distinct rates\(^9,10\).
Fig. S10: (a) Cartoon illustrating epithelial dynamics induced by cells that migrate by generating outward self-propelling forces only when their immediate front neighbor has deformed more than a given threshold strain $\varepsilon_s$. (b) Kymograph of epithelial velocity field vs. time; (c) kymograph of epithelial strain rate field vs. time; (d) kymograph of epithelial elastic stress field vs. time.

Here we propose an alternative mechanism in which the two competing phenomena are two recently described non-linear responses of the cytoskeleton to stretch\textsuperscript{11}: reinforcement, which causes increases of stiffness\textsuperscript{12}, and fluidization, which causes decreases of stiffness\textsuperscript{13,14}. While both phenomena remain poorly understood, they have been shown to coexist but play out over different time scales. For example, after a single stretch-unstretch cycle cells fluidize suddenly, but hundreds of seconds later they reinforce slowly\textsuperscript{13,14}. In order to introduce reinforcement and fluidization in our simple 1D model, we build upon the “unjamming” scenario previously analyzed (Fig. S10), now allowing $k$ to vary with time. Specifically, we assume that stiffness $k$ of each spring is constant only for strains $\varepsilon_i$ below an arbitrary threshold $\varepsilon_{th} > \varepsilon_s$. As soon as $\varepsilon_{th}$ is reached, a period of reinforcement begins in which $k$ increases with time for a duration $\Delta t$. This reinforcement phase is followed by a fluidization phase during which $k$ decreases with time for a duration $\Delta t$ (Fig. 4c).

Simulations show that for the first $\sim$400 min no cell reaches $\varepsilon_{th}$ and thus the evolution of the monolayer (Fig. 4, Supplementary movie S8) is identical to the case described previously (Fig. S10). Progressively from the monolayer midline towards the leading
edge, cells begin to undergo strains greater than $\varepsilon_{th}$ and thus elicit an outward wave of reinforcement. As this wave propagates outwards, it causes a progressive stalling paralleled by buildup of stress, as in a tug-of-war, toward the center. The reinforcement wave is followed by a fluidization wave with a delay $\Delta t$. This second wave enables further cell stretching and thus causes a second pulse of positive strain rate to propagate outwards and a second peak of stress at the monolayer midline. These simulations establish that cycles of reinforcement and fluidization triggered by a strain threshold result in reiterated propagation of strain rate fronts through the monolayer and oscillations of intercellular stress at the monolayer midline.

**Simulation parameters**

The values of $F$, $k$, and $\eta$ were chosen to capture the experimental kymographs of velocity, stress, and strain-rate while remaining within the order of magnitude reported in the literature. Specifically, we chose $F=4$ nN, $k=4$ nN, and $\eta=9.2$ nN.min/µm. Variables with units of force were transformed to more familiar units of stress by assuming a cross-sectional area of 100 µm$^2$. Strain thresholds were chosen to be $\varepsilon_s = 0.5$ and $\varepsilon_{th} = 1$. We note that as long as $\varepsilon_s < \varepsilon_{th}$ the model captures the generation and propagation of waves. During a reinforcement/fluidization cycle, the maximum stiffness was set to 3 times its baseline value.

**References**


