Active wetting of epithelial tissues

Carlos Pérez-González1,2,10, Ricard Alert3,4,10, Carles Blanch-Mercader5,6, Manuel Gómez-González1, Tomasz Kolodziej7, Elsa Bazellieres1, Jaume Casademunt3,4,* and Xavier Trepat1,2,8,9,*

Development, regeneration and cancer involve drastic transitions in tissue morphology. In analogy with the behaviour of inert fluids, some of these transitions have been interpreted as wetting transitions. The validity and scope of this analogy are unclear, however, because the active cellular forces that drive tissue wetting have been neither measured nor theoretically accounted for. Here we show that the transition between two-dimensional epithelial monolayers and three-dimensional spheroidal aggregates can be understood as an active wetting transition whose physics differs fundamentally from that of passive wetting phenomena. By combining an active polar fluid model with measurements of physical forces as a function of tissue size, contractility, cell–cell and cell–substrate adhesion, and substrate stiffness, we show that the wetting transition results from the competition between traction forces and contractile intercellular stresses. This competition defines a new intrinsic length scale that gives rise to a critical size for the wetting transition in tissues, a striking feature that has no counterpart in classical wetting. Finally, we show that active shape fluctuations are dynamically amplified during tissue dewetting. Overall, we conclude that tissue spreading constitutes a prominent example of active wetting—a novel physical scenario that may explain morphological transitions during tissue morphogenesis and tumour progression.

Living tissues are active materials with the ability to undergo drastic transitions in shape and dimensionality. When properly controlled, such morphological transitions enable development and regeneration. When regulation fails, however, aberrant morphological transitions underlie developmental defects and tumour formation. Transitions in tissue shape are regulated by a myriad of molecular processes that act upon a limited number of physical properties to ultimately determine tissue dynamics. To understand the nature of these physical properties and their impact on tissue shape, extensive research has focused on how a three-dimensional (3D) cell aggregate spreads on a substrate. Besides mimicking biological processes such as epiboly in zebrafish, the spreading of a cell aggregate is amenable to theoretical and experimental access, and has become a widespread model process.

Given the fluid behaviour of cell aggregates at long times, their spreading on a substrate has been studied as a wetting problem. In analogy with the case of a fluid drop, the extent to which the aggregate spreads on the substrate has been proposed to rely on a competition between cell–cell (Wcc) and cell–substrate (Wcs) adhesion energies, encoded in the so-called spreading parameter S = Wcc - Wcs. This parameter changes sign at the wetting transition that separates tissue spreading (S > 0) from retraction into a droplet-like aggregate (S < 0). This analogy with the classical theory of wetting has successfully explained aspects of tissue wetting such as changes in contact angle as a function of cell–cell and cell–extracellular matrix (ECM) adhesion. However, this conceptual framework overlooks the active nature of living tissues and, hence, it does not explicitly account for the ability of cells to polarize, generate traction forces, and couple such forces with adhesion dynamics. To a great extent, this limitation stems from the lack of direct measurements of cell–cell and cell–matrix forces during tissue wetting and dewetting.

To overcome these experimental and theoretical limitations, we performed a systematic quantitative study of the mechanics of tissue wetting as a function of cell–cell and cell–matrix adhesion, ECM ligand density, ECM stiffness, tissue size, and contractility. Our results cannot be explained solely in terms of the physics of passive fluids. Instead, we show that the tissue wetting transition and dewetting dynamics are well captured by a new framework for active wetting based on an active polar fluid model of tissue spreading.

E-cadherin expression induces cell monolayer dewetting

We designed an experimental assay to study wetting transitions of epithelial clusters induced by controlled changes in tissue mechanics. The idea behind the experimental approach is to progressively increase cell–cell adhesion in an epithelial monolayer while measuring its effect on cellular forces and tissue spreading. To this end, we use human breast adenocarcinoma cells (MDA-MB-231) stably transfected with a dexamethasone-inducible vector containing the human E-cadherin coding sequence. In the absence of dexamethasone, these metastatic cells do not express significant levels of cell–cell adhesion proteins. On adding dexamethasone, the concentration of E-cadherin increases almost linearly in time for ~24 h and plateaus thereafter (Fig. 1c, Supplementary Fig. 1). To study tissue wetting, MDA-MB-231 cells are seeded on a 12 kPa polyacrylamide gel coated with collagen I (Fig. 1a). Initially, the cells form a monolayer within a circular opening of a polydimethylsiloxane (PDMS) membrane deposited on the gel. Eight hours after E-cadherin induction, the confining membrane is removed and the monolayer spreads. However, after ~20 h, the monolayer often starts retracting, eventually becoming a spheroidal cell aggregate (Fig. 1f; Supplementary Fig. 2; Supplementary Fig. 3).
Fig. 1 E-cadherin expression causes an increase in traction forces and monolayer tension, and induces dewetting. a,b, Scheme of the experimental set-ups. For spreading experiments, cells form a monolayer within the circular opening of a PDMS membrane. After eight hours in the dexamethasone-containing medium, the PDMS membrane is removed and the monolayer spreads on the collagen-coated substrate (a). For confined monolayers, cells are seeded in circular islands of collagen on the substrate and allowed to cover them for three hours. Dexamethasone is then added to induce E-cadherin expression and time-lapse imaging starts (b). c, Quantification of E-cadherin following addition of dexamethasone (inset, up to three days). Tub = tubulin. d,e, Illustration of traction forces (d) and monolayer tension (e). f, Spreading monolayer exhibiting a wetting transition at time \( t = 25 \) h. Scale bar = 100 \( \mu m \). g–i, Phase contrast images (g), and maps of traction forces (h) and average normal monolayer tension (i) for a representative confined cell island of radius 100 \( \mu m \). Monolayer dewetting starts at ~25 h. Scale bar = 40 \( \mu m \). j–l, Evolution of monolayer area (j), mean traction magnitude (k) and mean average normal monolayer tension (l). Data are presented as mean ± s.e.m. \( n = 18 \) cell islands.
Videos 1, 2). Thus, the monolayer undergoes a transition from wetting to dewetting, which we refer to hereafter generically as wetting transition.

To reproducibly study this transition, we seed cells on adherent (collagen I-coated) circular islands of controlled size (100μm in radius) surrounded by an uncoated surface that cells cannot invade (Fig. 1b). We use traction force microscopy to measure traction forces on the substrate (Fig. 1d)\(^4\), and monolayer stress microscopy to measure tension within and between cells (Fig. 1e)\(^18,19\). After hours after E-cadherin induction, monolayers become cohesive (Fig. 1g). Cells at the edge polarize by extending lamellipodia towards the exterior of the island, generating radially oriented inward-pointing tractions (Fig. 1h; Supplementary Fig. 3)\(^20,21\). Monolayer tension increases from the edge of the monolayer and reaches a maximum at the centre (Fig. 1i). Note that monolayer tension is a bulk property and should not be confused with the interfaceal surface tension that plays a central role in classical wetting phenomena. During the first ~25 h of the experiment, tractions (Fig. 1k) and tension (Fig. 1l) rise in parallel with the increase in E-cadherin. As for unconfined spreading monolayers, the monolayer eventually retracts, decreasing its area (Fig. 1j) and dewetting the substrate to form a spheroidal aggregate, thus completing a transition from a two-dimensional (2D) to a 3D tissue geometry (Supplementary Fig. 4; Supplementary Videos 3, 4).

### Formation of E-cadherin junctions activates myosin

To study the mechanisms underlying the increase of tension we measure myosin levels and activity. During the first 24 h of E-cadherin expression, myosin levels remain constant but di-phosphorylated myosin light chain (ppMLC) exhibits an approximately threefold increase (Fig. 2a,b). Untransfected cells (CT) or transfected cells lacking dexamethasone in their medium (labelled E-cad) show constant ppMLC levels (Fig. 2a), indicating that the observed response is not attributable to a secondary effect of dexamethasone addition or to transfection artefacts. Unlike in cohesive monolayers, expression of E-cadherin does not lead to an increase of tension in single cells (Fig. 2c). Consistently, monolayers show higher levels of ppMLC than single cells several hours after induction (Supplementary Fig. 5). Moreover, abrogating cell–cell adhesions with the calcium chelator egtazic acid (EGTA) (2 mM) prevents the buildup of traction and tension, as well as the wetting transition (Supplementary Fig. 6, Supplementary Video 5). Thus, we conclude that E-cadherin regulates myosin-generated contractility through a mechanism dependent on cell–cell junction formation. Notably, E-cadherin not only affects intercellular forces but also tractions, ultimately determining the global mechanics of the monolayer\(^22,23\).

### Tissue tension induces the wetting transition

Next, we study the reorganization of adhesive and cytoskeletal structures in the monolayer. Upon induction of its expression, E-cadherin progressively accumulates at cell–cell contacts (Fig. 2h) and colocalizes with β-catenin (Fig. 2i,j), confirming the formation of adherens junctions. In parallel, the focal adhesion protein paxillin redistributes to the periphery of the monolayer (Fig. 2k), and supracellular stress fibres rich in active myosin massively form (Supplementary Fig. 7, Fig. 2l,m). These results suggest that dewetting is not directly caused by an increase in cell–cell adhesion, but rather by an increase in tension, which eventually causes the failure of cell–substrate adhesions. To test this hypothesis, we incubate the cells with blebbistatin (25 μM) to hinder the increase in contractility without impairing the over-expression of E-cadherin (Fig. 2d,e). This treatment reduces cellular forces (Supplementary Fig. 8) and delays dewetting (Fig. 2g; Supplementary Video 6). Conversely, the addition of the ROCK inhibitor Y27632 (25 μM) during dewetting causes the monolayer to rewet the substrate (Fig. 2f,g; Supplementary Video 6), thus demonstrating the active origin and reversibility of the transition. Together, these results show that the wetting transition results from a competition between active cellular forces, rather than simply between cell–cell and cell–substrate adhesion energies.

### An active polar fluid model of tissue wetting

To understand how the wetting transition emerges from active cellular forces, we build upon a continuum mechanical model of epithelial spreading\(^40\). Given the long timescales of the wetting/dewetting processes, we neglect the elastic response of the tissue\(^11,25–29\), assuming that it has a purely viscous behaviour\(^26,30–32\). Thus, taking a coarse-grained approach, the model describes the cell monolayer as a 2D active polar fluid\(^26–39\), namely in terms of a polarity field \(p(\mathbf{r},t)\) and a velocity field \(\mathbf{v}(\mathbf{r},t)\) (Supplementary Information). Our 2D model does not aim at describing the out-of-plane flows and shape of the tissue, nor the dynamics of the contact angle. However, it allows us to predict the onset and initial dynamics of the wetting transition, which is the focus of our study.

The cell monolayer is unpolarized in the bulk and polarized at the edge (see Fig. 1g,h). Hence, we take a free energy for the polarity field that favours the unpolarized state \(p = 0\) with a restoring coefficient \(a > 0\), and that introduces a cost for polarity gradients, with \(K\) the Frank constant of nematic elasticity in the one-constant approximation\(^40\):

\[
F = \int \left[ \frac{a}{2} \rho_0 p_0^2 + \frac{K}{2} \left( \partial_\alpha p_\alpha \right) \left( \partial_\alpha p_\beta \right) \right] d^2 \mathbf{r} \tag{1}
\]

We assume that the polarity field is set by flow-independent mechanisms, so that it follows a purely relaxation dynamics, and that it equilibrates fast compared to the spreading dynamics. Hence, \(\delta F/\delta p_\alpha = 0\), which yields

\[
L_\alpha \nabla^2 p_\alpha = p_\alpha \tag{2}
\]

where \(L_\alpha = \sqrt{K/a}\) is the characteristic length with which the polarity decays from \(p_\alpha(R) = 1\) at the edge of the monolayer of radius \(R\) to \(p_\alpha(0) = 0\) at the centre (red shade in Fig. 3a).

Then, force balance imposes

\[
\partial_\alpha \sigma_{\alpha\beta} = T_\alpha \tag{3}
\]

where \(\sigma_{\alpha\beta}\) and \(T_\alpha\) are the components of the monolayer tension and traction stress fields, respectively. We relate these forces to the polarity and velocity fields via the following constitutive equations for a compressible active polar fluid\(^41\):

\[
\sigma_{\alpha\beta} = \frac{\sigma_{\alpha\beta}}{h} = \eta \left( \partial_\alpha v_\beta + \partial_\beta v_\alpha \right) - \zeta p_\alpha p_\beta \tag{4}
\]

\[
f_\alpha = \frac{T_\alpha}{h} = - \zeta v_\alpha + \zeta p_\alpha \tag{5}
\]

Here, \(h\) is the monolayer height, \(\eta\) is the monolayer viscosity, \(\zeta\) is the active stress coefficient, \(\xi\) is the cell–substrate viscous friction coefficient, and \(\zeta\) is the contact active force coefficient. These parameters are assumed to be time-dependent to account for the evolving mechanical properties of the monolayer. Note that \(\zeta < 0\) for contractile behaviour, and hence we call \(-\zeta\) ‘contractility’. In
addition, we define the maximal traction stress exerted by polarized cells, \( T_0 = \zeta h \).

Assuming radial symmetry, neglecting cell–substrate viscous friction, and imposing stress-free boundary conditions, we analytically solve the model (Supplementary Information). Thus, we obtain the spreading velocity \( V = \nu(R) = dR/dt \) and, hence, the spreading parameter \( S = \eta V \). In the experimentally relevant limit \( L_\beta \ll R \), it reads

\[
S \approx \frac{T_0 L_\beta}{h} R + \left( \frac{3 T_0 L_\beta}{2 h} \right) \frac{L_\beta}{h} \tag{6}
\]
Strikingly, the spreading parameter depends on the monolayer radius \( R \), which entails the existence of a critical radius

\[
R^* \approx \frac{1}{2} \left( 3L_\gamma \zeta \frac{h}{T_\mu} \right)^{\frac{1}{2}} ~ \frac{1}{2} \frac{\zeta}{\zeta_i} \quad (7)
\]

above which the tissue spreads \((S>0)\) driven by traction forces \( T_\mu \) and below which it retracts \((S<0)\) driven by tissue contractility \( \zeta < 0 \) (Fig. 3b). The competition between bulk and contact active forces defines a novel intrinsic length scale \( L_\gamma \equiv -\zeta/\zeta_i \) of active polar fluids that naturally gives rise to the critical radius for the wetting transition, a striking property that has no counterpart in the classical wetting scenario.

Unlike for ordinary fluids, the wetting properties of tissues are not determined by local forces at the contact line but by the balance of forces across the entire monolayer, which results in the size-dependent wetting. Specifically, the internal, unpolarized region of the monolayer is subject to almost no external forces, and hence it is under a uniform tension set by traction forces at the polarized boundary layer. Because of the viscous rheology of the tissue, this uniform tension generates an outwardly directed flow with a linearly increasing velocity profile (Supplementary Figs. 9, 10; Supplementary Video 7). Thus, larger monolayers exhibit higher velocity right behind the boundary layer, which requires a higher contractility to induce monolayer dewetting (Fig. 3c; Supplementary Information). Finally, we suggest that the predicted non-monotonous flow profiles might induce the formation of 3D cell rims observed at the edge of epithelial monolayers\(^{2,41}\).

**Tissue wetting depends on tissue size and substrate properties**

The model predicts that the wetting transition depends on monolayer size and tissue forces (Fig. 3b,c). To assess the role of these variables in the experiments, we generate circular islands of different radii \((50, 100, 150 \text{ and } 200 \mu m)\) on substrates of different ECM ligand densities \((100, 10 \text{ or } 1 \mu g \text{ ml}^{-1})\) of collagen in the coating solution (Fig. 4a,b). We also study cell monolayers on substrates of different rigidities \((3, 12, \text{ and } 30 \text{ kPa})\) (Supplementary Fig. 11). With the sole exception of 30 kPa gels, on which dewetting does not occur in the timescale of the experiment, monolayers in all conditions feature a tension buildup phase and a dewetting phase (Supplementary Figs. 11b–d,12a–k; Supplementary Videos 8, 9). However, the duration of each phase presents large quantitative differences (Fig. 4a,b; Supplementary Fig. 11a). To assess these differences, we implement a robust user-blind method to measure the time \( \tau \) at which dewetting starts (Supplementary Fig. 13; see Materials and Methods). This analysis establishes that smaller monolayers dewet earlier than larger ones, and so do monolayers on softer and/or less densely coated substrates.
Fig. 4 | The wetting transition depends on substrate ligand density and monolayer radius. a, Time evolution of epithelial monolayers of different initial radius. Larger monolayers dewet later. b, Time evolution of monolayers on substrates with different ligand density. Monolayers on substrates with higher ligand density dewet later. Islands are 100 μm in radius. The red dashed line and shade in a and b indicate dewetting. Scale bars = 40 μm. c, Wetting transition time as a function of monolayer radius and substrate ligand density. d, Critical traction as a function of monolayer radius and substrate ligand density. Horizontal lines show the average critical tractions at different collagen concentrations, with shadows indicating error margins. e, Average critical traction as a function of the relative amount of collagen on the substrate. f, Critical contractility as a function of monolayer radius and substrate ligand density. Lines show the critical contractility corresponding to the average critical traction for each collagen concentration, with shadows indicating error margins. g, Phase diagram of tissue wetting as a function of monolayer radius, contractility and substrate ligand density. The plotted surface corresponds to the observed wetting–dewetting transition. Data are presented as mean ± s.e.m. For islands on 100 μg ml⁻¹ collagen: n=17 (200 μm radius), n=15 (150 μm radius), n=18 (100 μm radius), and n=11 (50 μm radius). For islands on 10 μg ml⁻¹ collagen: n=17 (200 μm radius), n=15 (150 μm radius), n=17 (100 μm radius), and n=10 (50 μm radius). For islands on 1 μg ml⁻¹ collagen: n=11 (200 μm radius), n=10 (150 μm radius), n=8 (100 μm radius), and n=8 (50 μm radius).
should thus be interpreted as the maximum force that cells can of the long-wavelength modes increase with (Fig. 5e; D
stark contrast with the known isotropic dewetting of passive flu-
ting perturbations, especially of mode 
we obtain experimental growth rates from the structure factors (Fig. 4c). In fact, these two quantities linearly correlate (Fig. 5g), which suggests that monolayer viscosity increases with time in our experiment, probably due to a combination of cell–cell junction formation, increasing contractility, and increasing cell density.

Active forces govern tissue morphology during dewetting
Our analysis thus far shows that an active polar fluid model captures the onset of dewetting as a function of the material properties and geometry of the tissue. Next, we focus on the early dynamics of tissue dewetting. Immediately after the onset of dewetting, the monolayer loses its circular symmetry, acquiring an elliptic-like shape before collapsing into a spheroidal cell aggregate (Fig. 5a; Supplementary Video 11). This striking symmetry breaking is in stark contrast with the known isotropic dewetting of passive fluids. Although pinning of the contact line may contribute to breaking the circular symmetry, the fact that monolayer retraction systematically tends to start at diametrically opposed points of the tissue boundary (Supplementary Video 11) suggests the presence of a morphological instability of active origin. Indeed, from our active polar fluid model, we analytically predict a long-wavelength instability of monolayer shape during dewetting (Supplementary Information).

To test the predictions, we characterize the evolution of tissue morphology by tracking the contour of the monolayer (Fig. 5a). The local radius perturbation \( \delta R(0,t) = R(0,t) - R_e \) quantifies the loss of circular symmetry (Fig. 5c), and its Fourier transform dissects the contribution of each perturbation mode to the overall shape of the monolayer (Fig. 5b). Consistent with the predicted instability, the amplitudes \( |\tilde{\delta R}_n| \) of the long-wavelength modes increase with time following the onset of dewetting (Fig. 5d). Their predicted growth rates \( \omega_n \) depend on a single yet-unmeasured parameter, the monolayer viscosity at the wetting transition, \( \eta \). Its value can be inferred from the retraction rate of the monolayer, \( \omega_a \), which we experimentally measure by fitting the exponential growth of the zeroth perturbation mode: \( \delta \tilde{R}_0(t) = \delta \tilde{R}_0(t_\text{start}) e^{\omega_a(t-t_\text{start})} \) (Fig. 5e; Supplementary Fig. 15). Comparing with the theoretical prediction (Supplementary Information)

\[
\omega_b \approx \frac{T_{\text{ex}}}{2\eta h}
\]

we obtain viscosities that increase with monolayer radius and substrate ligand density, spanning from 3 to 30 MPa s (Fig. 5f). The tendency exhibited by the viscosity is similar to that of transition times (Fig. 4c). In fact, these two quantities linearly correlate (Fig. 5g), suggesting that monolayer viscosity increases with time in our experiment, probably due to a combination of cell–cell junction formation, increasing contractility, and increasing cell density.

Once the theoretical growth rates \( \omega_n \) are known, we can predict the amplitudes of the different shape modes. Assuming that monolayer shape fluctuations are fast compared to the dewetting dynamics, we compute the structure factor of the monolayer boundary

\[
S_n(t) = \langle |\tilde{\delta R}_n(t)|^2 \rangle = \frac{D}{\omega_n} [2\omega_n(t-t_\text{start}) - 1]
\]

where \( D \) is the noise intensity of mode amplitudes (Supplementary Information). By fitting this prediction to the experimental data (Fig. 5j; Supplementary Fig. 16b; Supplementary Information), we infer the value of \( D \), which increases with tissue size but decreases with substrate ligand density (Fig. 5b). This behaviour is consistent with active shape fluctuations driven by the total traction force in the tissue, which scales linearly with monolayer radius (see equation (6)), and damped by cell–substrate friction, which increases with substrate ligand density. Finally, we obtain experimental growth rates from the structure factors (Fig. 5j; Supplementary Fig. 16b; Supplementary Information). Despite their expected variability, our experimental results agree with the predictions, confirming that the growth of shape-changing perturbations, especially of mode \( n = 2 \), is responsible for the elliptic-like shape of the monolayer (Fig. 5i, j). Overall, these results show that active forces and shape fluctuations determine the morphological evolution during monolayer dewetting.

Discussion and outlook
Our results illustrate how E-cadherin adhesion regulates tissue mechanical properties and forces, and, in turn, how these forces determine tissue shape, dynamics, fluctuations and dimensional-ity as a function of tissue size, contractility, substrate stiffness, and cell–cell and cell–substrate adhesion. In vivo, transitions in tissue morphology are characterized by changes in cell contractility, cell adhesion and ECM composition. It is appealing to think that these changes translate into different wetting states. For example, this could explain increased tumour invasiveness when contractility decreases or critical traction increases due to an enhanced cell–substrate adhesion, ECM deposition or ECM stiffening. This scenario is supported by previous experiments associating E-cadherin-dependent epithelial retraction and
suppression of tumour invasion in vivo. Furthermore, tumour growth per se implies an increase in tissue radius, possibly leading to a dewetting–wetting transition even if contractility and critical traction remain unaltered. In this line, the nucleation of a spreading monolayer from a growing cell aggregate has been previously reported.

Our analysis unveils fundamental features of tissue wetting that differ qualitatively from the classical wetting paradigm. We account
for these differences by developing a theoretical framework for active wetting, which explicitly relates the wetting properties of tissues to active cellular forces. This framework, based on active gel theory, captures the mechanics of the wetting transition as well as the dynamics of monolayer morphology during the early stages of tissue dewetting. Furthermore, it allows the quantification of active stresses, viscosity, and active fluctuations in the tissue. In light of these results, we propose that tissue spreading can be understood as the wetting process of an active polar fluid, constituting the defining example of the general phenomenon of active wetting.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41567-018-0279-5.

Received: 13 December 2017; Accepted: 17 August 2018; Published online: 24 September 2018

References
1. Gonzalez-Rodriguez, D., Guevorkian, K., Douezan, S. & Brochard-Wyart, F. Soft matter models of developing tissues and tumors. Science 338, 910–917 (2012).
2. Friedl, P. & Gilmour, D. Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Cell Biol. 10, 445–457 (2009).
3. Jülicher, F. & Eaton, S. Emergence of tissue shape changes from collective cell behaviours. Semin. Cell. Dev. Biol. 67, 103–112 (2017).
4. Ryan, P. L., Foty, R. A., Kohn, J. & Steinberg, M. S. Tissue spreading on implantable substrates is a competitive outcome of cell–cell vs. cell–substratum adhesivity. Proc. Natl Acad. Sci. USA 98, 4323–4327 (2001).
5. Douezan, S. et al. Spreading dynamics and wetting transition of cellular aggregates. Proc. Natl Acad. Sci. USA 108, 7315–7320 (2011).
6. Douezan, S., Dumond, J. & Brochard-Wyart, F. Wetting transitions of cellular aggregates induced by substrate rigidity. Soft Matter 8, 4578–4583 (2012).
7. Beaune, G. et al. How cells flow in the spreading of cellular aggregates. Proc. Natl Acad. Sci. USA 111, 8055–8060 (2014).
8. Beaune, G. et al. Reentrant wetting transition in the spreading of cellular aggregates. Soft Matter 13, 8474–8482 (2017).
9. Behrndt, M. et al. Forces driving epithelial spreading in zebrafish gastrulation. Science 338, 257–260 (2012).
10. Campinio, P. et al. Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly. Nat. Cell Biol. 15, 1405–1417 (2013).
11. Morita, H. et al. The physical basis of coordinated tissue spreading in zebrafish gastrulation. Dev. Cell 40, 354–366.e4 (2017).
12. Wallmeyer, B., Trinschek, S., Yigit, S., Thiele, U. & Betz, T. Collective cell migration in embryogenesis follows the laws of wetting. Biophys. J. 114, 213–222 (2018).
13. Douezan, S. & Brochard-Wyart, F. Dewetting of cellular monolayers. Eur. Phys. J. E 35, 34 (2012).
14. Smeets, B. et al. Emergent structures and dynamics of cell colonies by contact inhibition of locomotion. Proc. Natl Acad. Sci. USA 113, 14621–14626 (2016).
15. Ravanis, A. et al. Regulation of epithelial cell organization by tuning cell–substrate adhesion. Integr. Biol. 7, 1228–1241 (2015).
16. Sarrió, D. et al. Functional characterization of E- and P-cadherin in invasive breast cancer cells. BMC. Cancer 9, 74 (2009).
17. Trepat, X. et al. Physical forces during collective cell migration. Nat. Phys. 5, 430–436 (2009).
18. Serra-Picamal, X. et al. Mechanical waves during tissue expansion. Nat. Phys. 8, 628–634 (2012).
19. Tambe, D. T. et al. Collective cell guidance by cooperative intercellular forces. Nat. Mat. 10, 469–475 (2011).
20. Mertz, A. F. et al. Cadherin-based intercellular adhesions organize epithelial cell–matrix traction forces. Proc. Natl Acad. Sci. USA 110, 842–847 (2013).
21. Mertz, A. F. et al. Scaling of traction forces with the size of cohesive cell colonies. Phys. Rev. Lett. 108, 198101 (2012).
22. Harris, A. R., Daened, A. & Charras, G. T. Formation of adherens junctions leads to the emergence of a tissue-level tension in epithelial monolayers. J. Cell. Sci. 127, 2507–2517 (2014).
23. Bazellères, E. et al. Control of cell–cell forces and collective cell dynamics by the intercellular adhesives. Nat. Cell Biol. 17, 409–420 (2015).
24. Lecuit, T. & Yap, A. S. E-cadherin junctions as active mechanical integrators in tissue dynamics. Nat. Cell Biol. 17, 533–539 (2015).
25. Muhamed, I. et al. E-cadherin-mediated force transduction signals regulate global cell mechanics. J. Cell. Sci. 129, 1843–1854 (2016).
26. Blanch-Mercader, C. et al. Effective viscosity and dynamics of spreading epithelia: a solvable model. Soft Matter 13, 1235–1243 (2017).
27. Köpf, M. H. & Pismen, L. M. A continuum model of epithelial spreading. Soft Matter 9, 3727–3734 (2013).
28. Banerjee, S., Utujo, K. J. C. & Marchetti, M. C. Propagating stress waves during epithelial expansion. Phys. Rev. Lett. 114, 228101 (2015).
29. Nothnohn, J. et al. Cellular contraction and polarization drive collective cellular motion. Biophys. J. 110, 2729–2736 (2016).
30. Lee, P. & Wolgemuth, C. W. Crawling cells can close wounds without purse strings or signaling. PLoS Comput. Biol. 7, e1002007 (2011).

Acknowledgements
We thank D. Sarrió and G. Moreno-Bueno for providing the E-cadherin inducible cells; N. Castro for technical assistance; A. Eloségui, V. González, E. Latorre, L. Valon and R. Vincent for stimulating discussions. R.A. thanks G.Torrents for assistance.

NATURE PHYSICS | VOL 15 | JANUARY 2019 | 79-88 | www.nature.com/naturephysics

87
with mathematical details. C.P.-G. and R.A. were funded by Fundació 'La Caixa'. R.A. thanks J. Prost and acknowledges EMBO (Short Term Fellowship ASTF 365-2015), The Company of Biologists (Development Travelling Fellowship DEVTF-151206), and Fundació Universitària Agustí Pedro i Pons for supporting visits to Institut Curie. This work was supported by the Spanish Ministry of Economy and Competitiveness/FEDER (BFU2015-65074-P to X.T., FIS2016-78507-C2-2-P to J.C.), the Generalitat de Catalunya (2014-SGR-927 and CERCA Program to X.T., 2014-SGR-878 to J.C.), the European Research Council (CoG-616480 to X.T.), European Commission (H2020-FETPROACT-01-2016-731957 to X.T.) and Obra Social 'La Caixa'. IBEC is recipient of a Severo Ochoa Award of Excellence from the MINECO.

**Author contributions**

C.P.-G., R.A., J.C. and X.T. conceived the study and designed experiments. C.P.-G. performed the experiments with the help of T.K. and E.B. C.P.-G. and M.G.-G. developed computational analysis tools. C.P.-G. processed and analysed the experimental data. R.A. developed the active wetting theory with the help of C.B.-M. and fitted the model predictions to the experimental data. J.C. and X.T. supervised the study. C.P.-G., R.A., J.C. and X.T. wrote the manuscript. All authors contributed to the interpretation of the results and commented on the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41567-018-0279-5.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.C. or X.T.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
E-cadherin induction. Immediately before starting an experiment ($t_0$), normal cell media was replaced by media containing 10% of dexamethasone to induce the expression of E-cadherin.

Polyacrylamide gel substrate. Polyacrylamide (PAA) gels of 3, 12 and 30 kPa (Young modulus) were produced as described previously18. Briefly, a solution containing 5.5% acrylamide, 0.09% bis-acrylamide (3kPa); 7.5% acrylamide, 0.16% bis-acrylamide (12 kPa); or 12% acrylamide, 0.15% bis-acrylamide (30 kPa); plus 0.5% ammonium persulfate, 0.03% tetramethylethylenediamine and 0.64% of 200-nm-diameter red fluorescent carboxylate-modified beads was prepared and allowed to polymerize. The PAA gel surface was then incubated with a solution of 2 mg ml$^{-1}$ Sulfo-SANPAH under ultraviolet light for 5 min (wavelength of 365 nm at a distance of 5 cm). After that, three washes of 3 min each were performed to remove the excess of Sulfo-SANPAH. At this point, the gel was ready to add the ECM protein.

PDMS stencils. Polydimethylsiloxane (PDMS) membranes were fabricated as explained previously19. Briefly, SU-8 master containing arrays of circles of different sizes (200, 150, 100 and 50 μm radius) were raised using conventional photolithography. Importantly, all the different sizes were included in the same array to allow having different conditions in the same gel, thus decreasing experimental variability. Uncured PDMS was spin coated on top of the masters to allow having different conditions in the same gel, therefore decreasing experimental variability. Uncured PDMS was spin coated on top of the masters to allow having different conditions in the same gel, therefore decreasing experimental variability.

Cell patterning on PAA gels. The PDMS stencils were incubated with a solution of pluronic acid F127 (2%) for one hour. After that, they were washed twice in phosphate-buffered saline (PBS) and allowed to dry for 20 min. For confined areas, the PAA gel surface was then incubated with a solution of 2 mg ml$^{-1}$ Sulfo-SANPAH under ultraviolet light for 5 min (wavelength of 365 nm at a distance of 5 cm). After that, three washes of 3 min each were performed to remove the excess of Sulfo-SANPAH. At this point, the gel was ready to add the ECM protein.

Time-lapse microscopy. Multidimensional acquisitions were performed on an automatic inverted microscope (Nikon Eclipse Ti) using a 20× objective (NA 0.75, air) for traction force microscopy experiments. MetaMorph (Universal Imaging) was used to image every hour during the duration of the experiment. Around 50 cell islands were imaged in parallel using a motorized stage. In the case of the 3D reconstruction, Supplementary Fig. 4, Supplementary Video 4) and nuclei position analysis, Supplementary Fig. 10, Supplementary Video 8), multidimensional acquisitions were performed on a Nikon microscope with a spinning disk confocal objective (NA 1.40, oil).

Traction force microscopy. Traction forces were computed using Fourier-transform traction microscopy with a finite gel thickness from a gel displacements field1. Gel displacements were obtained using a custom-made particle image velocimetry set-up. In brief, the fluorescent beads in any experimental timepoint were compared to a reference image obtained after cell trypsinization at the end of the experiment.

Monolayer stress microscopy. Monolayer tension was obtained using monolayer stress microscopy, as described previously18. Force balance with tractions yields the traction field in the monolayer, as a second-rank symmetric tensor. We computed the average normal stress as the mean of the xx and yy components. In this 2D approximation, tension has units of surface tension, namely N m$^{-1}$.

Western blot. Approximately 500,000 cells were seeded on 12 kPa (Young Modulus) PAA gels (for MLIC and ppMLC) or plastic (for E-cadherin). After three hours, E-cadherin expression was induced and cells were sequentially lysed with 20% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. After that, proteins were transferred to a nitrocellulose membrane and disaggregated using a syringe and centrifuged at 20,000 rpm for 15 min. Samples were heated at 95°C for 5 min and loaded on polyacrylamide gels (Any kdu, Bio-rad) for electrophoresis. After that, proteins were transferred to a nitrocellulose membrane (Whatman, GE Healthcare Life Sciences) overnight. Membranes were blocked with 3% dry milk-Tris buffer saline–0.2% Tween, incubated with primary antibodies overnight at 4°C and, later, incubated with horseradish-peroxidase-coupled secondary antibodies for one hour at room temperature. Bands were revealed using an ECL system (Roche) and imaged using ImageQuant LAS 4000 and quantified using ImageJ software. Tubulin was used as an endogenous control for normalization.

Immunostaining. MDA-MB-231 cells were washed with PBS, fixed with 4% paraformaldehyde (PIFA) for 10 min and permeabilized in 0.1% Triton X-100 for 5 min. After washing, cells were blocked in 10% PBS for one hour and incubated with primary antibodies for three hours. Cells were then washed and incubated with the appropriate secondary antibody for one hour. After washing, cells were mounted in Mowiol reagent. Images were acquired using a Nikon microscope with a spinning disk confocal unit (CSU-W1, Yokogawa) using a 60× objective (NA 1.40, oil).

Antibodies. The primary antibodies used were: anti-E-cadherin monoclonal antibody (clone 36, BD Transduction Laboratories, no. 610181), anti-$\alpha$-tubulin (clone B-5-1-2, Sigma-Aldrich, no. T5168), anti-$\beta$-catenin (clone 14, BD Transduction Laboratories, no. 610154), anti-paxillin (clone 349, BD Transduction Laboratories, no. 610051), anti-rat collagen type I (EMD Millipore, AB755P), anti-ppMLC (Cell Signaling Technology, #3674), and anti-MLC (Cell Signaling Technology, #3672). The secondary antibodies were: peroxidase-conjugated anti-mouse IgG (Jackson Immuno Research, no. 715-035-151) and peroxidase-conjugated anti-rabbit IgG (Jackson Immuno Research, no. 111-035-003). Western blot and Alexa Fluor 488 anti-rabbit (Invitrogen, Molecular Probes, no. A-21206), Alexa Fluor 488 anti-mouse (Invitrogen, Molecular Probes, no. A-11029), Alexa Fluor 555 anti-mouse (Invitrogen, Molecular Probes, no. A-28180), Alexa Fluor 640 anti-rabbit (Invitrogen, Molecular Probes, no. A-21245), Alexa Fluor 405 anti-mouse (Invitrogen, Molecular Probes, no. A-31553) for immunostaining. For western blot, anti-$\alpha$-E-cadherin was diluted 1:2,000, anti-$\alpha$-Tubulin was diluted 1:5,000; anti-ppMLC was diluted 1:500; anti-MLC was diluted 1:200; secondary antibody was diluted 1:5,000. For immunofluorescence, primary antibodies were diluted 1:200 and secondary antibodies were diluted 1:400. F-actin was labelled with Phalloidin–TRITC (Sigma-Aldrich, no P1951) diluted 1:2,000.

Cell island segmentation. At every timepoint, cell islands were semi-automatically segmented using custom-made Matlab software. First, a preliminary mask of the island contour was performed automatically based on changes in contrast of phase contrast images. The errors in the automatic segmentation were manually corrected.

Immunostaining fluorescence intensity quantification. Both for ppMLC and collagen intensity quantifications, the region of interest (ROI) was segmented as explained above. The mean or median intensity in the ROI was calculated and the background intensity was subtracted from every individual measurement.

Kymography. We obtained the radial coordinates of each pixel of the cell island masks by calculating its shortest distance to the edge. The radial direction of the edge was calculated and expanded to the inner pixels of the mask to decompose traction vectors in radial and tangential components. Finally, tractions and tensions were averaged according to their distance to the edge at every timepoint to build spatiotemporal kymographs.

Wetting transition definition. We defined an objective criterion to detect the wetting transition in different experimental conditions. First, cell islands are automatically segmented on the basis of changes in contrast of the phase contrast images, followed by a manual correction of the errors in segmentation. Every cell island mask is divided into a specific number of circular sectors on the basis of its initial radius (24 for 200 μm radius, 18 for 150 μm, 12 for 100 μm, and 6 for 50 μm). Using this strategy, each sector has an approximately equal arc length at time 0 (~52μm). The average radius of every sector is computed over time, obtaining a curve with a roughly constant value at the first time points that suddenly drops at the time of dewetting (Supplementary Fig. 6c). This curve is fitted with a negative sigmoidal function $R(t)=\frac{a}{1+b \exp((d+ln(2\times\sqrt{3}))/c+x)}$ using the nonlinear least squares method. The transition time for every circular sector is defined as the time point at which the fitted function reaches 95% of its initial value (open circles in Supplementary Fig. 13c). For the whole island, we define the onset of dewetting as the moment at which one sixth of the circular sectors are dewetting according to the criterion above.

Collagen amount quantification. Rat tail type I collagen immunostainings were performed on patterns made on polyacrylamide gels coated with three different collagen concentrations (100, 10 and 1 μg ml$^{-1}$). The patterns were automatically segmented. Their median intensity was calculated and corrected by subtracting the mean background intensity.
Model parameters fit. We fit the predicted radial traction force profile
\[ T_r(r) = -T_{rr}(r) = -\frac{\partial}{\partial r} \left( \frac{L_1}{L_2} \right) \]
where \( L_1 \) is the modified Bessel function of the first kind and first order (Supplementary Information), to the experimentally measured profiles at different times, as represented in kymographs in Supplementary Fig. 3a. At each time point, the fitting algorithm searches for the radial position of the maximum of the experimental traction force profile, which sets the monolayer radius \( R(t) \). Then, the theoretical prediction is fitted up to this point, discarding the outer region due to the long-range propagation of deformations in the elastic substrate used in this outer region may arise from poorly attached protrusions or be an artefact of the theoretical prediction being fitted up to this point, discarding the outer region (Supplementary Information), to the experimentally measured profiles at different times.

The local monolayer radius as function of the radial coordinate at which the stress vanishes, \( \sigma_r(R) = 0 \). Second, the contractility can be obtained from fits of the radial tension profile in the monolayer (Supplementary Information):
\[ \sigma_r(R) = \frac{L_1}{L_2} \frac{L_1}{L_2} + \frac{L_2}{L_1} \frac{L_2}{L_1} - \frac{L_1}{L_2} \frac{L_1}{L_2} - \frac{L_2}{L_1} \frac{L_2}{L_1} \]

where \( L_1 \) are modified Bessel functions of the first kind and order \( n \). To check the values of the contractility given by equation (12), we extracted the contractility via two other methods. First, this parameter can be obtained from fits of the radial traction forces. Second, the contractility can be obtained from fits of the radial traction forces. Six times the mean amplitude of all the other modes from the radial coordinate at which the stress vanishes, \( \sigma_r(R) = 0 \). Finally, as derived in the Supplementary Information, the retraction rate calculation.

The growth rate of the perturbation mode \( n = 0 \) is obtained by fitting the exponential function \( \delta R(t) = \delta R_0 e^{\omega_0 t} \) to the evolution of its amplitude, from the last timepoint before the transition to seven hours after the onset of dewetting. By choosing this time span, we ensured there were enough time points to perform reliable fits (Supplementary Fig. 16) while still having most of the perturbation modes in almost all monolayers within the linear regime of the instability. The error of \( \omega_0 \) is defined as the 95% confidence interval.

All three methods yield fully compatible results. Note that, at the lowest order in the small dimensionless parameter \( L_1 / R \), the average tension is completely given by traction forces: \( \sigma = T_{rr} + \sigma_0 (L_1 / R) \). Therefore, the contractility contributes only to the average stress at the first-order level in \( L_1 / R \), which explains the large values of this parameter compared to the stress in the monolayer.

Monolayer boundary Fourier transform. The local monolayer radius as function of the polar angle, \( R(\theta) \), was computed via the same method as for the wetting–dewetting transition definition. However, in this case, the number of segments was systematically multiplied by eight to increase the spatial resolution. We obtained the radius perturbations as \( \delta R(\theta) = R(\theta) - R_0 \), where \( R_0 \) is the average initial radius. This function was Fourier-transformed to obtain the amplitude of every Fourier mode. Two Fourier modes were calculated in different channels. To obtain the evolution of mode \( n = 0 \), we systematically subtracted the mean radius of the island during the last seven time points before wetting–dewetting transition from the current average radius. Respectively, mode \( n = 1 \) is the direct measure of the centrosymmetric motion. To average different replicates, we referred all times to the transition time of each island, namely that we used shifted times \( t - t* \) to the evolution of its amplitude, from the last timepoint before the transition to seven hours after the onset of dewetting. By choosing this time span, we ensured there were enough time points to perform reliable fits (Supplementary Fig. 16) while still having most of the perturbation modes in almost all monolayers within the linear regime of the instability. The error of \( \omega_0 \) is defined as the 95% confidence interval.

Code availability. All computer codes used for this study are available upon request to the corresponding authors.

Data availability
All the data used for this study is available upon request.

References
58. Casares, L. et al. Hydraulic fracture during epithelial stretching. Nat. Mater. 14, 343–351 (2015).
59. Tambe, D. T. et al. Monolayer stress microscopy: Limitations, artifacts, and accuracy of recovered intercellular stresses. PLoS One 8, e55172 (2013).
### Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - A minimum of n=3 independent experiments were carried out.

2. **Data exclusions**
   - Describe any data exclusions.
   - Patterns that did not meet a circularity criterion before the onset of dewetting were excluded from the analysis of shape dynamics (Fig. 5) using a user-blind automated algorithm (detailed in the manuscript). In all the other cases, no data was excluded from the analysis.

3. **Replication**
   - Describe the measures taken to verify the reproducibility of the experimental findings.
   - All attempts to replicate data were successful.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - n/a

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - n/a

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).
   - n/a
   - Confirmed
     - ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
     - ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
     - ☒ A statement indicating how many times each experiment was replicated
     - ☒ The statistical test(s) used and whether they are one- or two-sided
       - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
     - ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
     - ☒ Test values indicating whether an effect is present
       - *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
     - ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
     - ☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.
Software

7. Software

Describe the software used to analyze the data in this study.

All the experimental analysis was performed using custom-made software developed in Matlab R2014b. The fits of the model to experimental measurements was performed using Gnuplot 5.0. Averaging was performed in Excel and GraphPad Prism 6.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

Dexamethasone-inducible MDA-MB-231 cells should be requested Dr. Gema Moreno-Bueno from Universidad Autónoma de Madrid, Spain.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The primary antibodies used were: anti-E-cadherin monoclonal antibody (clone 36, BD Transduction Laboratories, no. 610181), anti-α-tubulin (clone B-5-1-2, Sigma-Aldrich, no. T5168), anti-beta-catenin (clone 14, BD Transduction Laboratories, no. 610154), anti-paxillin (clone 349, BD Transduction Laboratories, no. 610051), anti-rat collagen type I (EMD Millipore, AB755P), anti-ppMLC (Cell Signaling Technology, #3674), and anti-MLC (Cell Signaling Technology, #3672). The secondary antibodies were: peroxidase-conjugated anti-mouse IgG (Jackson Immuno Research, no. 715-035-151) and peroxidase-conjugated anti-rabbit IgG (Jackson Immuno Research, no. 211-032-171) for western blot and Alexa Fluor 488 anti-rabbit (Invitrogen, Molecular Probes, no. A-21206), Alexa Fluor 488 anti-mouse (Invitrogen, Molecular Probes, no. A-11029), Alexa Fluor 555 anti-mouse (Invitrogen, Molecular Probes, no. A-28180), Alexa Fluor 640 anti-rabbit (Invitrogen, Molecular Probes, no. A-21245), Alexa Fluor 405 anti-mouse (Invitrogen, Molecular Probes, no. A-31553) for immunostaining.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Cells were provided by Gema Moreno-Bueno.

b. Describe the method of cell line authentication used.

Cells used were authenticated by providers.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell lines tested negative for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by iCLAC, provide a scientific rationale for their use.

No commonly misidentified cells were used.

Animals and human research participants

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This research did not involve human participants.