Embyronic tissues as active foams

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The physical state of embryonic tissues emerges from non-equilibrium, collective interactions among constituent cells. Cellular jamming, rigidity transitions and characteristics of glassy dynamics have all been observed in multicellular systems, but it is unclear how cells control these emergent tissue states and transitions, including tissue fluidization. Combining computational and experimental methods, here we show that tissue fluidization in posterior zebrafish tissues is controlled by the stochastic dynamics of tensions at cell–cell contacts. We develop a computational framework that connects cell behaviour to embryonic tissue dynamics, accounting for the presence of extracellular spaces, complex cell shapes and cortical tension dynamics. We predict that tissues are maximally rigid at the structural transition between confluent and non-confluent states, with actively generated tension fluctuations controlling stress relaxation and tissue fluidization. By directly measuring strain and stress relaxation, as well as the dynamics of cell rearrangements, in elongating posterior zebrafish tissues, we show that tension fluctuations drive active cell rearrangements that fluidize the tissue. These results highlight a key role of non-equilibrium tension dynamics in developmental processes.

M any essential processes in multicellular organisms, from organ formation to tissue homeostasis, require a tight control of the tissue physical state. While tissue mechanics and structure at supracellular scales emerge from the collective physical interactions among the constituent cells, their control occurs at cell and subcellular levels. Bridging these scales is essential to understand the physical nature of active multicellular systems and to identify the processes that cells use to control the physical state of embryonic tissues.

In vitro experiments of cell monolayers on synthetic substrates have revealed characteristics of glassy dynamics and rigidity transitions, which are thought to be linked to biological function and multiple pathologies. In contrast, suspended epithelial monolayers are largely solid-like in vitro and show evidence of fracture in vivo. Experiments with embryonic tissues have shown characteristics of glassy dynamics in cell movements, viscous behaviour at long timescales and also structural signatures reminiscent of jamming transitions, with cell divisions, cell shape and/or changes in cell adhesion suspected to play a role in the control of these emergent behaviours. Recent in vivo experiments in developing zebrafish embryos showed the existence of a rigidity transition underlying the formation of the vertebrate body axis, revealing a functional role of rigidity transitions in embryonic development (Fig. 1a). Both the presence of adhesion-dependent spaces between cells (Fig. 1b–d) and the dynamics of cell–cell contacts (Fig. 1e,f) were shown to influence the physical state of the tissue. However, the relative roles of cell adhesion and cell–cell contact dynamics in the control of posterior tissue fluidization are still unclear. In general, little is known about how different cell behaviours control rigidity transitions and tissue fluidization in embryonic tissues, and whether all these observed emergent phenomena share a common physical origin.

The physical behaviour of multicellular systems has been studied theoretically using various approaches. Vertex models and cellular Potts models account for cell geometry and use equilibrium formulations to describe the physical state of the system. These descriptions predict a density-independent rigidity transition in confluent systems that depends on the balance between actomyosin-generated cortical tension $T_c$ and cell adhesion $W$ (treated as passive, effective tensions), with the resulting cell shape being the control parameter for solid/fluid states. Since cell shape plays a central role in models of confluent systems, the ability to accurately describe complex cell shapes, beyond polygonal shapes, may be important to understand the physical state of the system, as recently suggested. Non-vertex models of deformable particles have instead focused on configurations in non-confluent equilibrium systems, both in the presence and absence of cell adhesion. In contrast, self-propelled particle and Voronoi models that focus on cell movements on synthetic substrates account for the dynamics of the system and predict rigidity transitions that depend on cell density and self-propulsion. All these descriptions capture some important aspects of the problem, but each neglects a subset of key cell behaviours and/or restricts the allowed configurations. No current theoretical description accounts for all observed relevant cell behaviours in a common framework, hindering our understanding of how cells control the emergent physical state of the tissue.

Dynamic vertex model with extracellular spaces

To study the dynamics of embryonic tissues and their physical state, we generalize two-dimensional (2D) vertex models by accounting for (1) extracellular spaces (Fig. 1b,c,g and Methods), (2) the stochastic dynamics of active cortical tensions (Fig. 1e,f) and (3) complex cell shapes (Fig. 1a,g and Methods). Unlike previous vertex models, we do not assume the existence of a preferred cell perimeter, as experimental evidence for this constraint is lacking. Instead, since cells actively control adhesion, cortical tension and osmotic pressure, we derive a physical description reminiscent of foams but with active tension dynamics. Tissue dynamics and structure, as well as cell movements and their shapes, are all determined by the dynamics of vertices (Fig. 1g), which follow from force balance, namely

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Fig. 1 | Characteristics of multicellular systems and simulation framework. a, Confocal section through embryonic tissues showing the intensity (I) of fluorescent reporter protein secreted extracellularly as well as cell membranes (green), indicating the presence of extracellular spaces (red range). b, Schematics defining the cell size $L_c$, cell number $N$ and simulation box area $A$, which specify cell density. c, Top: kymograph of membrane signal intensity ($I$) along a tissue region (pink, left) containing a cell–cell contact, showing contact length fluctuations (bottom). d, Schematics showing contact length fluctuations. e, f, Top: simulated tension fluctuations causing cell–cell contact length fluctuations. Bottom: increasing (decreasing) tension shortens (lengthens) cell–cell junctions. g, Schematics of the dynamic vertex model formulation. Triple vertices (physical vertices, red) and non-physical intermediate vertices (blue) are shown. Scale bars, 10 μm.

\[ \eta_\alpha \frac{dR_\alpha}{dt} = \sum_{j \in F(\alpha)} \left( T_{ij} \Theta(T_{ij}) + N_{ij} \right), \]  

where $t$ is time, $R_\alpha$ is the position of the vertex $\alpha$, $\eta_\alpha$ is a friction coefficient characterizing the dissipation associated with moving a vertex, $T_{ij}$ is the effective tension at the contact between cell $i$ and $j$ (with $F(\alpha)$ representing the set of all cells sharing vertex $\alpha$) and $N_{ij}$ is the normal forces acting on vertex $\alpha$. $\Theta(\cdot)$ represents the Heaviside step function and prevents unrealistic negative tensions. Normal forces arise from osmotic pressure differences in adjacent cells and are given by $N_{ij} = (\Delta P_i - \Delta P_j) L_c / 2$ (Methods), where $L_c$ and $\Delta P_i$ and $\Delta P_j$ are, respectively, the contour length of the contact between cells $i$ and $j$ and the osmotic pressure difference across cell $i$ ($j$).

To capture the observed fluctuating nature and finite persistence of tension dynamics, $T_\alpha$, we describe them as an Ornstein–Uhlenbeck process, with a tension that fluctuates around a fixed point $T^0_{ij}$ and has a persistence time $\tau_i$ (refs. 32,33), specifically

\[ \tau_i \frac{dT_{ij}}{dt} = -(T_{ij} - T^0_{ij}) + \Delta T \xi, \]  

where $\Delta T$ is the amplitude of tension fluctuations and $\xi$ is Gaussian white noise (Fig. 1f and Methods). The fixed point effective tensions depend on both the average cortical tension, $T_\alpha$, and average strength of cell–cell adhesion, $W$, which, like $\Delta T$, are different at cell–cell contacts and free cell boundaries (Fig. 1b and Methods).

Scaling all quantities, we obtain the relevant dimensionless parameters (Table 1 and Methods). Since $P_c L_c / T_i$ and $\tau_i / T_i$ (Table 1) can be estimated from existing experimental data (Methods), we focus on the parameter space spanned by $\Delta T / T_0$, $W / T_\alpha$ and the normalized cell density $\rho$ (Table 1).

**Structural transitions and mechanics of equilibrium systems**

We first explore how the states and mechanics of the system change if spaces between cells are allowed. In the absence of tension
fluctuations ($\Delta T/T_0 = 0$), the amount of extracellular spaces in equilibrium configurations is determined by force balance and varies with both the cell density $\rho$ and the relative cell adhesion strength $W/T_0$ (Fig. 2a). Increasing cell density results in larger cellular volume fraction $\phi$ (Fig. 2b) and cell contact number $z$ (Fig. 2c), with the system eventually becoming confluent at an adhesion-dependent critical density, $\phi_c(W/T_0)$. For any fixed cell density, the system undergoes a non-confluent to confluent transition as $W/T_0$ is increased, since higher adhesion promotes stronger cell–cell contacts (Fig. 2a,b). These results extend previous studies for purely repulsive deformable particles\textsuperscript{4} to arbitrary adhesion levels.

Changes in relative cell adhesion affect not only the volume fraction but also the structural characteristics of extracellular spaces. Equilibrium configurations sharply transition from a large number of small extracellular spaces to a few large extracellular spaces at $W/T_0 \approx 0.23$ (Fig. 2a,d), as small triangular extracellular spaces can only be stabilized below this value (Supplementary Section 1). Concomitant to the presence of large extracellular holes, which are reminiscent of epithelial fracture in vivo\textsuperscript{9}, the spontaneous clustering of cells strongly resembles flocculation in sticky emulsions\textsuperscript{14,15} (Fig. 2a). Moreover, the system displays bistability between two possible equilibrium configurations for $W/T_0 \geq 0.23$, namely a confluent state with stretched cells and a non-confluent state with sparse and large extracellular holes (Fig. 2c), with strong hysteresis in

### Table 1: Definition of the relevant dimensionless parameters in the problem

| Dimensionless parameters | Description |
|--------------------------|-------------|
| $\Delta T/T_0$           | Magnitude of tension fluctuations (Fig. 1a). |
| $W/T_0$                  | Relative strength of cell–cell adhesion $W$ and average cortical tension $T_0$ (Fig. 1b). |
| $\rho \equiv N L_0^2/A_t$ | Normalized system density (with $A_t$ and $N$ being the total area of the system and the number of cells, respectively; Fig. 1d). |
| $P_{\tau \gamma}/T_0$     | Relative magnitude of normal to tensional forces. |
| $\tau_c/\tau_s$          | Ratio of persistence time of tension fluctuations and characteristic timescale $\tau_s \equiv T_0/\tau_\delta$ of dissipation at vertices. |

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**Fig. 2 | Equilibrium configurations and structural transitions.** a–c, Representative equilibrium configurations (a), volume fraction $\phi$ (b) and mean number of neighbours $z$ (c) for varying values of relative adhesion $W/T_0$, and cell density $\rho$. Green and purple boxes in a correspond to parameter values specified as circle dots of the same colour in b and c. d, Number $N_c$ (top) and average area $A_c$ (bottom) of extracellular spaces for varying relative cell adhesion and different cell densities, showing a sharp structural transition at $W/T_0 \approx 0.23$ (grey line) leading to the opening of large extracellular spaces. e, Lowering (dashed line) and increasing (dash-dotted line) relative adhesion quasi-statically shows bistable states and strong hysteresis in equilibrium configurations. Equilibrium quenched states are also shown (solid line). f, Average neighbour number (cell contacts) $z$ as the system volume fraction changes at vanishing cell adhesion, showing the existence of a jamming transition at $\phi_c \approx 0.83$ and $z_c = 4$ (configuration shown inset). Power-law fits $z - z_c = z_0 (\phi - \phi_c)^{\gamma 2} + z_1 (\phi - \phi_c)$ with coefficients $z_0 \approx 1.45$ and $z_1 \approx 10.45$ (red line) and with $z_0 \approx 4.85$ and $z_1 \approx 0$ (grey line) are shown. g, Average shape factor ($\Delta$) for varying relative adhesion showing a sharp increase at $W/T_0 = 2$ (vanishing tensions), leading to anisotropic cell shapes (inset), recapitulating density-independent transitions. h, Schematics of a simple shear deformation imposing a large strain step ($\varepsilon_{\text{strain}} = 1.5$), with associated temporal evolution of both strain and shear stress. i, Temporal relaxation of shear stress $\sigma_{\text{y}}$ (normalized to $\sigma_0 \equiv \sqrt{T_0/\lambda_0}$) after the imposed strain step for varying adhesion levels. j,k, Dependence of the yield stress $\sigma_{\text{y}}$ on the relative adhesion strength (j) and on both cell density and relative adhesion (k), showing a maximum at the structural transition between confluent and non-confluent states (green line). Error bands, s.d.; $N = 20$ (b–f,g) and $N = 10$ (e–k) independent simulations for each parameter set.
Fig. 3 | Tissue dynamics with finite tension fluctuations. a. MSD for varying magnitudes of tension fluctuations ΔT/T_s showing subdiffusive (0 < α < 1) and diffusive (α = 1) behaviours as tension fluctuations increase (inset). b. Snapshots of dynamic configurations with examples of cell trajectories over t/τ_1 = 10^2. c. MSD at long timescales (t = 10^3 τ_1 ≫ τ_2 > τ_3), showing non-monotonous behaviour for varying relative adhesion strength and minimal values at the structural transition. d. Cellular NE rate for varying relative adhesion. Distinct types of NE event: gain/loss of cell contacts (left) and T1 transitions (right). Cells and junctions undergoing NE events are highlighted as yellow/blue and red colour, respectively. Error bands, s.d.; N = 10 independent simulations for each set of parameters.

equilibrium configurations if adhesion or cortical tension are varied in a quasi-static manner (Fig. 2e, Supplementary Video 1 and Methods). Some of the predicted system configurations (Fig. 2a) are analogous to those previously obtained for equilibrium systems with open boundaries27, but the bistability and hysteresis of configurations reported here can only be observed in closed systems that more closely resemble epithelial tissues.

In the limit of vanishing cell adhesion, the system should behave as foams/emulsions, which display a jamming transition at a critical value φ_c. The isostatic condition (z_i = 4 in 2D) sets the critical volume fraction φ_c ≈ 0.83 of the system18 (Fig. 2f). Both this φ_c value and the power-law dependence of z (Fig. 2f) are in agreement with recent equilibrium simulations of deformable particles36, indicating that our description accurately describes the foam limit. In the confluent limit, previous works reported density-independent rigidity transitions, which are controlled by the cell shape factor s (s = P/√A), with P and A being the cell perimeter and the area, respectively. The system switches from a solid to a fluid state at approximately s_c ≈ 3.81, with cells transitioning from isotropic to anisotropic shapes17,25-27. The fluid state in these descriptions is characterized by vanishing effective tensions (T_0 = 0), allowing neighbour exchanges (NEs) at no energetic cost17. Setting W/T_s = 2 in our framework leads to vanishing effective tensions and a sharp increase in average shape factor due to the emergence of anisotropic cell shape (Fig. 2g), recapitulating density-independent transitions.

To directly assess the physical state of the system, we monitor shear-stress relaxation after imposing an affine deformation (Fig. 2h and Methods). The initial stress jump is largest with no adhesion and vanishes when effective tensions vanish (Fig. 2i and Supplementary Fig. 5). Subsequently, shear stress relaxes with a characteristic timescale τ_2 towards a constant value at long timescales, namely the yield stress σ_Y (ref. 39), which depends non-monotonically on cell adhesion (Fig. 2j and Supplementary Video 2). For low relative adhesion W/T_s, the system is non-confluent and the yield stress increases with increasing adhesion as extracellular spaces close down. In contrast, for adhesion values leading to confluence, increasing W/T_s leads to a decreasing yield stress due to lower effective tensions. Vanishing yield stress indicates a fluid tissue state, which occurs only for W/T_s = 0 and W/T_s = 2 and corresponds to the jamming transition (Fig. 2f) and the density-independent rigidity transition (Fig. 2g), respectively, with the tissue being solid for all other values in the 0 < W/T_s < 2 range. These results show that equilibrium systems are maximally rigid at the structural transition between confluent and non-confluent states (Fig. 2j,k), with increasing adhesion rendering the system more rigid in non-confluent systems and doing the opposite in confluent states.

Dynamics of active multicellular systems

Unlike equilibrium systems, tension dynamics at cell–cell contacts can drive cell movements, NEs and cell shape changes (Supplementary Videos 3 and 4). At timescales longer than all characteristic timescales (t ≫ τ_1 > τ_3), cell movements are caged for small ΔT/T_s, as indicated by the saturation of the mean squared displacement (MSD) and bounded cell trajectories (Fig. 3a,b and Methods). For increasing magnitudes of tension fluctuations, cell uncaging starts to occur and the asymptotic behaviour of the MSD
for $t \gg \tau_1$ becomes a power law ($\text{MSD} \propto t^\alpha$), with an exponent $\alpha$ that increases with activity (Fig. 3a, inset). This evidences subdiffusive ($\alpha < 1$) cell movements for intermediate activities, and diffusive ($\alpha = 1$) behaviour for large enough tension fluctuations (Fig. 3a, b).

Comparing MSD values at long timescales ($t = 10^2 \tau_1 \gg \tau_1 > \tau_2$) shows that increasing tension fluctuations always leads to higher cell movements, regardless of cell adhesion strength, as recently reported for confluent systems. However, for a fixed level of activity, MSD values vary non-monotonically as adhesion increases, displaying very reduced cell movements at the transition between non-confluent and confluent states (Fig. 3c). Similar to the MSD, the NE rate displays analogous non-monotonic behaviour, with minimal NE events at the structural transition. Close to confluence for high adhesion levels, the NE rate is dominated by T1 transitions and related to the MSD by $\text{MSD} \times \text{cell area} \propto k_N^2$ (Extended Data Fig. 1), with the timescale for T1 transitions, $1/k_N$, diverging as the activity vanishes, a signature of glassy dynamics. In contrast, at low adhesion levels leading to non-confluent states, loss and formation of new cell contacts dominate NE events (Fig. 3d) and the MSD and NE behaviours differ close to jamming. The non-monotonic behaviour of both MSD and NE events, with minimal values at the transition between non-confluent and confluent states, suggests that the non-equilibrium system is also maximally solid-like at the structural transition.

**Physical state of active multicellular systems**

To study the rigidity of active systems, we apply a shear-strain step and monitor stress relaxation, as described above (Fig. 2h). The presence of finite tension fluctuations qualitatively changes stress relaxation at long timescales compared with equilibrium systems, displaying a slow stress decay rather than plateauing to a yield stress (Fig. 4a and Supplementary Video 5). This long timescale stress relaxation is driven by actively induced T1 transitions (Fig. 4b), and can be accurately described by a stretched-exponential function (Fig. 4a, inset), as previously done to explain the dynamics of systems with a large number of intrinsic timescales. The stress relaxation timescale, $\tau_{SR}$, at which shear stress reaches the level of active shear stress in the unperturbed system (Fig. 4a, inset, and Supplementary Fig. 2), varies by over five orders of magnitude as the magnitude of tension fluctuations or relative adhesion change slightly (Fig. 4d).

While larger activity values reduce $\tau_{SR}$ monotonically, increasing the relative adhesion strength leads to non-monotonic changes in $\tau_{SR}$, which rapidly increases in non-confluent states and displays the opposite behaviour in confluent states. The largest stress relaxation timescale occurs at the structural transition between non-confluent and confluent states, indicating that tissues are minimally fluid at long timescales close to this structural transition (Fig. 4d), consistent with the predicted low cell movements and NE rates close to the transition (Fig. 3c, d).

In contrast to previous equilibrium vertex models, our results show that a given tissue can behave as a fluid or a solid depending on the time necessary to form embryonic structures. If the characteristic timescale $\tau_d$ of developmental processes is larger (smaller) than the stress relaxation timescale $\tau_{SR}$, namely $\tau_d/\tau_{SR} > 1$ ($\tau_d/\tau_{SR} \ll 1$), the tissue behaves as a fluid (solid). Using typical developmental timescales ($\tau_d \approx 1-2$), we obtain the tissue phase diagram (Fig. 4e). Over a critical value of tension fluctuations, the tissue is always fluid regardless of cell adhesion levels. Below that critical activity value, the tissue is fluid at both low and high adhesion levels, but solid in between, in the region of the phase
diagram surrounding the structural transition between non-confluent and confluent states. This non-monotonic behaviour mirrors the behaviour of NE and cell movements, but care must be taken in inferring the physical state of the system solely from cellular movements (Extended Data Fig. 2). While cell density and cell shape are the control parameters associated with jamming transitions and density-independent transitions, respectively, our results show that tension fluctuations control a distinct rigidity transition in both confluent and non-confluent states, as suggested recently for confluent states.

To relate the mechanics of the system to its structure in the presence of tension dynamics, we study the configurations of the system. Tension fluctuations generally promote transitions from confluent to non-confluent states by opening up extracellular spaces at weak regions (Fig. 4i), implying that larger adhesion values are required to reach confluent states for increasing \( \Delta T/T_a \). This effect becomes negligible for large cell densities (\( \rho > 1 \)) and volume fraction is then solely determined by cell density \( \rho \) and relative cell adhesion \( W/T_a \) (Supplementary Fig. 3). While cell shapes are only moderately affected by tension fluctuations in non-confluent regimes, with low shape factors associated with rounder cells, increasing tension fluctuations in confluent regimes lead to substantially larger shape factors (Fig. 4g,h). In contrast to density-independent transitions, where large shape factors (above approximately 3.81) result from vanishing junctional tensions in fluid states, the large shape factors reported here are due to spatiotemporal tension fluctuations (Fig. 4h), with cell–cell contacts maintaining finite tensions (Supplementary Fig. 4 and Supplementary Video 6), as observed experimentally\(^{1,42} \). In particular, we find the presence of both fluid and solid tissue states for adhesion values in the range \( 0 < W/T_a < 2 \) that would correspond solely to solid states in previous equilibrium descriptions. These results indicate that the tissue fluid or solid state cannot be inferred from static measurements of cell shape factor only, as the tissue physical state and its structure depend strongly on the magnitude of tension fluctuations (Fig. 4g,h).

**Fluidization of embryonic tissues**

Our theoretical results indicate that in the presence of active tension fluctuations, active NE events control stress relaxation at long timescales both in confluent and non-confluent tissues. To experimentally address the role of tension fluctuations in stress relaxation and tissue fluidization, we employed magnetically responsive oil droplets to directly measure strain and stress relaxation in posterior tissues of developing zebrafish embryos (Fig. 5a), as these tissues have been previously shown to be in a fluid-like state\(^{44} \). After injecting a single droplet in the mesodermal progenitor zone (MPZ), we induced large droplet deformations of multiple cell diameters by applying a controlled magnetic field for a 15 min period. 

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### Fig. 5 | Stress relaxation and tissue fluidization in posterior tissues during body axis elongation.

**a.** Lateral view of a zebrafish embryo (scale bar, 200 \( \mu m \)) and sketches showing lateral and dorsal views of posterior tissues, indicating the fluid-like MPZ and solid-like PSM (ventral, V; dorsal, D; anterior, A; posterior, P; medial, M; lateral, L). A confocal section showing a portion of the MPZ (cell membranes, yellow) with a magnetic droplet (magenta) is highlighted. **b.** Schematic sketches and confocal snapshots of magnetic droplet actuation in the MPZ tissue. **c.** Time evolution of the strain, \( (b-b_0)/R \), before, during and after magnetic actuation. **d.** Relaxation of anisotropic stress \( \sigma_a(t) \) after magnetic actuation in both linear and log-linear scales, with \( \sigma_a^0 = \sigma_a(t = 0) \). The fit (black line) corresponds to a stretched-exponential function. **e.** Example of NE events: cells in contact (blue line) before the T1 transition (cyan; top) and not in contact after the transition (bottom). **f.** Normalized frequency of amplitude of junctional length fluctuations for the MPZ, both in the absence and presence of blebbistatin (bleb; average amplitudes in inset). **g.** Cumulative NE events in the MPZ away from the droplet (cyan; top) and not in contact after the transition (bottom). **h.** Normalized frequency of amplitude of junctional length fluctuations for the MPZ, both in the absence and presence of blebbistatin (bleb; average amplitudes in inset). **i.** Cumulative NE events in the MPZ (away from the droplet, cyan; dashed black line shows linear fit) and in the close neighbourhood of the droplet (around droplet, red). Inset: visual definition of droplet neighborhood (red). **j.** Temporal evolution of NE rate in the MPZ (cyan) and in the region around droplet (red). Initial \( (t < 15 \text{ min}) \) and final \( (t > 40 \text{ min}) \) stages of droplet relaxation are highlighted as green and pink shaded regions, respectively. **k.** Average NE rates in the region around droplet (AD, both during initial droplet relaxation (green) and at its final stages (pink)) and in the MPZ, both in the absence (cyan) and presence (orange) of blebbistatin. **l.** Experimentally measured and simulated dynamics of cell shapes in both the MPZ and the PSM, showing faster dynamics in the MPZ and largely static cell boundaries in the PSM. Experimental data are average intensity projection of a confocal section timelapse. **m.** MSRD shows uncaging behaviour for the MPZ but caged for the PSM of both wild-type and cdh2\(^{-/-} \) embryos. Error bands, s.d. (d.g) and s.e.m (i.k); \( N = 10 \) (d), \( N = 298 \) from 4 embryos (g-i, AD), \( N = 396 \) from 3 embryos (g-i, MPZ). Data in f, MPZ bleb in i and MSRD for MPZ and PSM in wild-type embryos in k were reanalysed from ref.\(^{11} \). Scale bars, 10 \( \mu m \), unless otherwise stated.
(Fig. 5bc and Methods). We subsequently monitored the droplet relaxation for 1 h after removing the magnetic field and measured the decay of stress in the tissue (Fig. 5d and Methods). We observed an initial fast decay (~30 s) followed by a very slow relaxation at long timescales that displays the stretched-exponential behaviour predicted theoretically for a fluctuation-induced stress relaxation (Fig. 4a). This slow relaxation was previously interpreted as a yield stress due to limitations in the measurement timescales\(^1\), a well-known limitation in mechanical measurements of complex materials\(^8\). The present long timescale measurements reveal a very slow stress relaxation and indicate that posterior tissues completely fluidize on timescales of approximately 1 h, enabling nearly complete remodelling of posterior tissues during axis elongation, as this occurs at rates of approximately 45 \(\mu\)m h\(^{-1}\) (refs. \(^{44,45}\)).

To address the role of active NE (T1 transitions) in tissue fluidization (Fig. 4c), we quantified the characteristics of NE events (Fig. 5e) during droplet relaxation in the MPZ, as cells in this region display considerable cell–cell contact fluctuations (Fig. 5f and Methods). Measurement of cumulative NE events in the MPZ (away from the droplet) shows a linear increase over time (Fig. 5g and Methods), indicating an approximately constant NE rate in the MPZ with characteristic timescales of tens of minutes (Fig. 5h,i). Inhibiting myosin II activity with blebbistatin causes a reduction in the magnitude of cell–cell contact fluctuations (Fig. 5f and Methods) and a decrease of nearly 50% in the NE rate (Fig. 5i), indicating that the measured NE events in the MPZ are actively induced by actomyosin-generated tension fluctuations. While NE events in the tissue directly adjacent to the droplet display the same NE rate as the rest of the MPZ in the final stages of droplet relaxation (40–55 min; Fig. 5g–i), the NE rate close to the droplet is higher than the rest of the MPZ during the beginning of droplet relaxation (0–15 min; Fig. 5g–i). This observed initial excess of NE events close to the droplet indicates the presence of passive NE events caused by the capillary stresses imposed by the droplet on the tissue during its relaxation, as predicted theoretically (Fig. 4c). The measured low and constant rate of active NE events in the MPZ is consistent with the measured stretched-exponential behaviour of the stress relaxation at long timescales (tens of minutes), and indicates that tissue fluidization is largely caused by active NE events arising from tension fluctuations at cell–cell contacts.

To characterize the relative roles of tension fluctuations and cell–cell adhesion in rigidity transitions, we used N-cadherin (\(cdh2\))–/– mutants lacking N-cadherin mediated cell–cell adhesion\(^ {36}\) (Methods). While the MPZ tissue in wild-type embryos is fluidized by active NE events (Fig. 5d–i) and displays cellular movements and mean squared relative displacements (MSRD) compatible with cell uncaging (Fig. 5j,k), the presomitic mesoderm (PSM) has been shown to be in a solid-like state\(^ {15}\), with cells caged by their neighbours (MSRD \(\ll\) at 1 min; Fig. 5j,k). Our measurements indicate that cell movements in the PSM of N-cadherin (\(cdh2\))–/– mutants, a tissue that has been shown to be characterized by reduced cell volume fraction and lower yield stress but similar cell–cell contact fluctuations as wild-type embryos\(^ {36,37}\), display the same MSRD behaviour as wild-type embryos (Fig. 5k). These results indicate that lowering cell–cell adhesion while maintaining low cell–cell contact fluctuations does not allow cells to uncage themselves to remodel the tissue.

Altogether, both our computational and experimental results indicate that actomyosin-generated tension fluctuations actively drive structural rearrangements that cause stress relaxation in the tissue, thereby controlling tissue fluidization and enabling tissue remodelling.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41567-021-01215-1.

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Methods

Pressure and volume relation. The (osmotic) pressure \( P \) of a cell has been experimentally shown to vary with its volume according to \( P = k/V \) (ref. 4), meaning that changes in the osmotic pressure difference between the inside (\( P_i \)) and outside (\( P_o \)) of the cell lead to changes in the cell volume \( V \), with \( k \) characterizing the cell compressibility (we assumed for simplicity that cells are never compressed close to their dry mass limit). In our 2D description, the cell area \( A \) plays the role of the volume, so that \( P = k/A \), with the characteristic cell size \( L_c \) (or preferred area \( A_{c} \equiv L_{c}^{2} \)) being set by this relation, namely \( L_{c} \equiv \sqrt{k/P} \). However, while we use the experimentally measured relation between osmotic pressure and cell volume (area in 2D), the specific \( P = V \) (or \( P = A \)) functional form does not change our results qualitatively as long as the cell volume decreases with increasing applied osmotic pressure, namely \( \Delta P = P_i - P_o (A - A_r) \) (with \( I \) being a coefficient characterizing the cells' compressibility), and the deviations from the preferred cell area in 2D (\( A_r \)) are mild.

Fixed point tension and tension dynamics. The fixed point tension, \( T_{fp} \), corresponds to an average effective tension at cell–cell contacts and has contributions from the average actomyosin-generated cortical tension \( T_{c} \) in each cell and the average cell–cell adhesion strength \( W \), so that \( T_{fp} = 2T_{c} - W \) (Fig. 1b). At free cell boundaries (cell boundaries contacting the extracellular space), the average effective tension is just the average cortical tension of the cell, namely \( T_{fp} = T_{c} \). Here we assume the average tension \( T_{c} \) not to change with the cell perimeter, as no current experimental observations suggest that dependence. In situations where the cell shape becomes very anisotropic or contorted enough to have very large perimeters, a dependence of the average effective tension on the perimeter could potentially arise from limitations in plasma membrane availability.

The stochastic dynamics of tensions depends on the magnitude of tension fluctuations, which is \( \Delta T \) at cell–cell contacts and \( \Delta T/2 \) at free cell boundaries (cell boundaries contacting the extracellular space).

Parameter estimation. Since the relaxation timescale \( \tau_{r} \) has been measured to be much smaller than the persistence timescale of tension variations (\( \tau_{c} \lesssim 20 \) s (refs. 42,43); \( \tau_{c} \gtrsim 90 \) s (ref. 44)), we set the ratio to \( \tau_{r}/\tau_{c} = 10 \). While the values of osmotic pressure are unknown in vivo, they are expected to be larger than cortical stresses\(^\text{42,43}\), so that \( P_{c}/T_{c} > 1 \). Consequently, we fix \( P_{c}/T_{c} = 10 \) for all simulations. This ensures relatively mild cell size variations, as observed experimentally\(^\text{41}\). Fixing these parameters reduces the parameter space to the normalized amplitude of tension fluctuations \( \Delta T/T_{c} \), the ratio \( W/T_{c} \) of average adhesion strength and average cortical tension, and the ratio \( \Delta T_{c}/A_{c} \) of the cells’ total preferred area and the total available area.

Numerical integration. The dimensionless version of the governing equations (equations (1) and (2)) was integrated numerically using the Euler–Maruyama method with a time step, \( \Delta t \). We used \( \Delta t = 0.005 \mu \text{s} \) for all simulations to have a time resolution much smaller than the smallest characteristic timescale in the system, \( \tau_{c} \).

Initial configuration generation protocol. A polygonal tiling of confluent states is first generated by random Poisson Voronoi tessellation in a square periodic box of total area \( A_{t} \). Before the introduction of extracellular spaces, the initial confluent configuration is first annealed to a local equilibrium state to prevent sudden adjustment of cell shape with extracellular spaces. Extracellular spaces are introduced by replacing each vertex in the confluent configurations with a small triangular extracellular space ‘cell’ centred around the original vertex position, and with the three new vertices located on the each of the original edges and of 1% their original length. Small tension fluctuations (\( \Delta T/T_{c} = 0.5 \)) are applied for a duration of \( \tau_{r} \). The annealing configuration is used as an initial configuration for simulations of both equilibrium configurations and dynamics.

As for any model of multicellular systems, some of the observed configurations depend on the initialization protocol. Regular hexagonal packings can be obtained by choosing regularly arranged initial cellular configurations. The fixed point tension, \( T_{fp} \), is obtained by quenching the system from an initial state with tension fluctuations (\( \Delta T/T_{c} = 0.5 \)) to an equilibrium state (\( \Delta T/T_{c} = 0 \)) for each parameter set. While we observe small changes in the resulting configurations if the magnitude of the initial tension fluctuations is varied in the annealing protocol, our results, including the existence of the structural transition, remain qualitatively unchanged. Quasi-static changes of the relative adhesion at equilibrium where performed by first quenching the system to equilibrium at a given parameter value, and then performing a small change in the parameter (\( W/T_{c} \)) and letting the system relax. Specifically, the system was initialized at a large value of the relative adhesion strength that ensured a confluent state (\( W/T_{c} = 1 \)) and then quenched to a local equilibrium state. The relative adhesion strength was then progressively reduced to \( W/T_{c} = 0 \) by small changes (\( \Delta W/T_{c} = -0.02 \)). Similarly, the other branch was found by initializing the system at zero adhesion strength (\( W/T_{c} = 0 \)) and progressively increasing it to \( W/T_{c} = 1 \) by small increments (\( \Delta W/T_{c} = 0.02 \)). After each adhesion adjustment, the system is relaxed to local equilibrium states.

Introducing spaces between cells. Extracellular spaces are first introduced to initial configurations of confluent states as cells with different properties. Each vertex is replaced by a small triangular extracellular space centred around the original vertex position, and with the three new vertices located on the each of the original edges and of 1% their original length. These extracellular spaces then behave like ‘cells’ with different properties (see above) and their size and geometrical features are determined by force balance at the vertices, as is the case for cells too. When two extracellular space ‘cells’ become neighbours, they are merged. This implementation allows non-confluent states in vertex models and is different from previous descriptions of non-confluent states, which used centre particles\(^\text{29,30}\) or deformable particles models\(^\text{31,32}\).

Intermediate non-physical vertices. Intermediate vertices are introduced for both cell–cell contacts and free cell boundaries to allow for more realistic cell shapes. With intermediate vertices, individual edges consist of linear segments joined together to form a piecewise linear edge. The desired segment length is introduced as a parameter and the number of intermediate vertices for each edge is equal to the closest lower integer given by the ratio of instantaneous edge length to the segment length criterion. When the edge length increases (decreases), an intermediate vertex can be added (deleted) following the criterion just described. As the number of intermediate vertices increases, intermediate vertex positions are reassigned uniformly along the edge. If the longest segment is longer than twice the shortest segment in a given edge, intermediate vertex positions are also reassigned uniformly along the piecewise linear line. This implementation of intermediate vertices is similar to the one used for confluent equilibrium systems\(^\text{8,25}\).

While we considered all vertices to have the same drag coefficient, physical and non-physical vertices may, in general, be characterized by different drag coefficients. This approximation is not due to technical reasons, as the simulation framework presented here can simulate different drag coefficients at different vertices different drag coefficients. Previous studies have shown that the measured stress relaxation timescales for movement of vertices is much smaller (~1–20 s (refs. 42,43)) than the persistence timescale of active tension fluctuations, which is approximately 100 s (ref. 44). Since we are interested in the behaviour of the system at long timescales (longer than the persistence timescale of active tension fluctuations (\( \tau_{r} \)) and the timescales associated with viscous relaxation at vertices are much smaller than the persistence timescale of active stresses (\( \tau_{r} \)), we neglected the differences in drag coefficient at the different vertices, as they are irrelevant in this limit.

Treatment of topological transitions. T1 transitions occur in our description when a given edge length is shorter than a critical length \( L_c \approx 0.01 \times 2\sqrt{A_c} \).

When a T1 transition leads to the formation of a new vertex between three cells, we introduce a small triangular extracellular space ‘cell’ and let it evolve in time, as described above. Free cell boundaries (a boundary of a cell and the extracellular space) occasionally intersect each other due to the system dynamics. This event corresponds to the formation of a new contact between two cells. Therefore, when an intersection between any two free boundary edges is detected, a new cell–cell contact is introduced, splitting the extracellular space in two. While not simulated here, cell divisions and cell death, which would also introduce topological transitions, are straightforward to simulate in this framework. Since these events would also induce cell rearrangements, they are also likely to enable tissue fluidization, as previously suggested\(^\text{10,11,14}\).

Cell trajectories and MSD. To obtain the MSD, we first computed the cell centres (centroid of polygon), \( r_{c,i} \), for each time point, \( t \), based on vertex positions. Then, the cell trajectories were obtained by monitoring the changes of cell centres. Using the cell trajectories \( r_{c,i}(t) \), we obtained the MSD according to

\[
\text{MSD}(t) = \frac{1}{N} \sum_{i} (r_{c,i}(0) - r_{c,i}(t))^{2}.
\]

MSD values were averaged over all cells and also all initial timepoints \( t_i \) for any given set of model parameters.

Application of a step strain. Since tissue fluidization in biological tissues is associated with their nonlinear mechanical response\(^\text{36}\), we impose large strains \( \epsilon_{y} \) of 150%. The large strain was applied by deforming the simulation box from a square to a parallelogram of the target shear strain, namely 150%. An affine deformation is applied to all vertex positions when imposing the step strain. Lees–Edwards periodic boundary conditions were imposed throughout the simulation to avoid mismatch of cell geometry across system boundaries.
Stress calculation. The non-dimensional stress tensor can be computed from the transient tissue geometry with knowledge of junctional tensions and cell pressures\(^{1,15}\) (lowercase indicates normalized quantities), namely

\[
\sigma_{ij\alpha\beta} = \rho \left( \sum_{i=0}^{N} \Delta\rho_{i,j} \delta_{\alpha\beta} + \sum_{j=0}^{N} \delta_{\alpha\beta} \frac{\partial l_{\alpha\beta}}{\partial \phi_{j}} \right),
\]

(4)

where \(\Delta\rho_{i,j} \equiv \rho_{i,j} - \rho_{i,j} \), and \(m\) and \(n\) are indices indicating the spatial direction (\(m = x,y\) and \(n = x,y\)), \(\phi_i\) is the dimensionless cell area, \(I_{\alpha\beta}\) is the vector form of the edge length between cells \(i\) and \(j\), and \(\delta_{\alpha\beta}\) is the Kronecker delta. The shear stress term can be written as

\[
\sigma_{\alpha\beta} = \rho \left( \sum_{j=0}^{N} \delta_{\alpha\beta} \frac{\partial l_{\alpha\beta}}{\partial \phi_{j}} \right).
\]

(5)

Active shear stress. Due to tension fluctuations, the macroscopic shear stress shows fluctuations around zero. To quantify the magnitude of these active shear-stress fluctuations, the shear-stress values are monitored over a time interval of \(10^2\) and their standard deviation is computed for each parameter set (no macroscopic imposed strain). The level of active shear stress corresponds to the computed standard deviation of shear stress, and increases approximately linearly with the magnitude of tension fluctuations for a given relative cell adhesion strength (Supplementary Section 2).

Zebrafish husbandry, lines and experimental manipulations. Zebrafish (Danio rerio) were maintained under standard conditions\(^{1,15}\). The cdh2\(^{1,51}\) mutant line\(^{1}\) was used to disrupt adhesions between cells, otherwise phenotypically wild-type lines were used. All husbandry and experiments were done according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California Santa Barbara. Transgenic lines Tg(hsp70:secP-mCherry)p1 (refs. 51) and Tg(bact2:mem-neonGreen-neonGreen)hm40 and embryo were taken every 5 s. Ferrofluid droplets were fluorescently written as Supplementary Section 2).

Ferrofluid droplets were monitored over time by measuring the change in the droplet long semi-axis \(a\) and \(b\), respectively, as previously described\(^{1,15}\). The droplet strain, \(\left( b - b_0 / a \right)\), is defined as the ratio of its elongation along the direction of applied magnetic field, namely \(b - b_0\) (with \(b_0\) being the value of \(b\) just before magnetic actuation), and the droplet radius \(R\), and can be measured over time by monitoring the change in the droplet long semi-axis \(b(t)\). The stresses at supracellular (tissue) scales associated with a particular droplet deformation were obtained from the elliptical droplet deformation, as previously described\(^{1,15}\). The droplet interfacial tension \(\sigma_m\) and \(H_b\) are the mean curvatures of the droplet ellipsoidal shape at the intersection of the principal axis with the fitted ellipsoid. Since the droplet shape is that of a prolate spheroid, \(H_b\) and \(H_a\) read \(H_b = b/a'\) and \(H_a = 1/2a + a'/(2b')\). By monitoring the changes in droplet shape over time, we obtained the time evolution of both the mean curvatures, namely \(H_{b(t)}\) and \(H_{a(t)}\). The droplet interfacial tension \(\gamma\) was measured in each experiment in situ and in vivo, as previously described\(^{1,15}\). Using the measured value of the interfacial tension for each droplet and the time evolution of the mean curvatures \(H_{b(t)}\) and \(H_{a(t)}\), we obtained the time evolution of the stresses during relaxation using \(\sigma_m(t) = 2\gamma (H_{b(t)} - H_{a(t)})\).

NE analysis. One-hour-long confocal timelapses of the MPZ region of membrane-labelled embryos containing a previously inserted droplet were acquired at 1 frame every 5 s. A region of the MPZ away from the droplet and a region adjacent to the droplet were cropped and the time at which T1 transitions occurred in each region was recorded. T1 transitions were detected manually by inspecting cell configurations between frames. To improve the accuracy of the analysis, smaller sections of each region containing approximately 10 to 15 cells were further cropped and analysed separately. The cumulative NE were obtained by adding up the NE events occurring over time in each region. The NE rate was obtained by taking the derivative of the cumulative NE function.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Source data are provided with this paper.

Code availability

The code developed for this paper is available in Supplementary Software 1.

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Author contributions
S.K. and O.C. designed research; S.K. implemented and performed the simulations; M.P. and G.A.S.-V. performed experiments; S.K., M.P. and G.A.S.-V. analysed data; S.K. and O.C. wrote the paper; O.C. supervised the project.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | Power law relation between NE rate and MSD at long timescales. Power law relation between long time MSD values and NE rate when the systems are close to confluence for high adhesion levels. NE rate and longtime MSD show a power law relation with an exponent of 0.75.
Extended Data Fig. 2 | Comparison of solid/fluid phase diagrams obtained from stress relaxation and from cell movements. Solid/fluid phase diagrams determined by mechanical measurement of stress relaxation (left) and cell movements, MSD=1/2 (middle) and MSD=1/4 (right). Green region indicates confluent states.
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Software and code

Policy information about: availability of computer code

| Data collection | Commercial Zeiss Zen software was used to perform confocal imaging. Computer simulations were done in Matlab 9.6 (MathWorks). |
|-----------------|--------------------------------------------------------------------------------------------------------------------------|
| Data analysis   | Commercial software used to analyze data: Imaris 9.3 (Bitplane) and Matlab 9.6 (MathWorks). ImageJ was also used to analyze data. |

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Zebrafish (Danio rerio) were used in this study. Since only embryos were studied, sex-specific experiments were not necessary, as zebrafish embryos at the studies stage have not yet undergone sex determination.

None.

None.

Animal husbandry and experiments were done according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California Santa Barbara.

Note that full information on the approval of the study protocol must also be provided in the manuscript.