SUPPLEMENTARY INFORMATION

APPENDICES

Appendix 1. A 0-D model for simulating the mechanical response of an epithelial tissue to compressive strain

Here, we derive a zero-dimensional model for the evolution of contour length l of an epithelial monolayer, which reproduces the experimentally observed dynamics.

A – Description of the model

Based on experimental observations, we assume that the behaviour of the monolayer can be described by a simple rheological model consisting of three branches in parallel (Fig. 4a). The first branch consists of an active stress contribution $\sigma_a > 0$ which models cellular contractility observed in experiments (Fig. 3b) and brings the material to a tensile state at zero strain. The second branch behaves as a solid with an elastic modulus E, denoting the observed behaviour at long time-scales (Fig. 3d). The third branch behaves as a viscous liquid with an elastic modulus Y and relaxation time τ. The product $Y\tau$ corresponds to a bulk viscosity η. Finally, in line with experimental measurements, we hypothesise that the tissue is unable to sustain any compressive force and buckles when the stress σ reaches zero. This leads to different dynamic behaviours depending on tensile or compressive stress (cases B1 and B2 below).

For simplicity, we chose to simplify the dynamics of the tissue by modelling its relaxation with a single characteristic time τ, as our aim is to capture the effects of the buckling non-linearity and tissue pre-tension on the epithelium dynamics.

B – Constitutive behaviour

We have shown that epithelial tissues can buckle and adopt a contour length that is larger than the device plate-to-plate distance. Thus, we define two different strains in our experiments relating to the epithelial tissue and the applied strain related to the device:

Tissue strain: $\varepsilon=\frac{l-l_0}{l_0}$ where l is the contour length of the tissue.

Device strain: $\varepsilon_d=\frac{d-l_0}{l_0}$ where d is the plate-to-plate distance.

These two strains are defined with respect to a contour length l_0 which is associated to the initial monolayer length before the application of any compression and is equal to the initial plate-to-plate distance. Such a choice implies that, as verified experimentally (Fig. 3b), the initial stress on the coverslips is mainly of active origin.

The response of the material to imposed stress or strain is governed by the following equations:

B1 – Under tensile stress:

- *Stress* $\sigma>0$: $\frac{\sigma}{E}=\varepsilon-\varepsilon_b+r\,\varepsilon_1$ with $r=\frac{Y}{E}$, $\varepsilon_b=\frac{-\sigma_a}{E}<0$ and ε_1 the internal strain in the spring E which satisfies the equation:

  $\dot{\varepsilon}_1+\frac{\varepsilon_1}{\tau}=\dot{\varepsilon}$

- *Strain*: $\varepsilon=\varepsilon_d$

B2 – Under compressive stress:

- *Stress*: $\sigma = 0$, this is an assumption of the model based on experimental measurements.
- *Strain*: $\varepsilon = \varepsilon_b - r\varepsilon_1$, where ε_1 satisfies the same equation as in B1. Note that in this case $l > d$ i.e. $\varepsilon > \varepsilon_d$.

C – Steady state behaviour

To begin with, we compute the stress versus device strain, and the tissue strain versus device strain relation $\sigma(\varepsilon_d)$ and $\varepsilon(\varepsilon_d)$ in steady state. This behaviour corresponds to the compressions performed at 0.5 %.s^{-1} and below (see Fig. 1b (iii), Fig. 1e, Fig. 3d, Fig. 4b and Fig. 5b). When the driving parameter ε_d changes at a low rate, the viscous branch does not contribute, and we obtain the following relation: $\sigma / E = \varepsilon_d - \varepsilon_b$, if $\sigma > 0$.

This relation is valid until the tissue reaches its buckling threshold for $\varepsilon_d = \varepsilon_b$. At this point, the stress on the tissue vanishes. For $\varepsilon_d < \varepsilon_b$, the stress remains at zero (Fig. 5a). Similarly, for the tissue strain, $\varepsilon = \varepsilon_d$ as long as $\varepsilon_d > \varepsilon_b$ and plateaus at ε_b for $\varepsilon_d \leq \varepsilon_b$.

D – Response of the epithelium to a step of compressive strain

Next, starting from a tissue at its original length, we abruptly shorten it and determine the transitory regime towards establishment of the steady-state stress computed above. The step shortening occurs at t = 0 and changes the device strain from $\varepsilon_d = 0$ to $\varepsilon_d = \varepsilon_d^f$. The

initial stress state is σ_a. We then distinguish three cases depending on the magnitude of compressive strain. Fig. S4c shows the evolution in a range of magnitudes of device strain.

D1 – Case 1 (low device strain): $\varepsilon_d^f > \frac{\varepsilon_b}{1+r}$

In this case, stress in the tissue is always positive and the tissue never buckles. The stress relaxes exponentially. We have:

- *Stress:* $\frac{\sigma(t)}{E} = \varepsilon_d^f - \varepsilon_b + r\,\varepsilon_d^f e^{-t/\tau}$
- *Strain:* $\varepsilon(t) = \varepsilon_d^f$

D2 – Case 2 (intermediate device strain): $\varepsilon_b < \varepsilon_d^f < \frac{\varepsilon_b}{1+r}$

This case corresponds to the experiments shown in Fig. 1b(i) and Fig. 1c. Here, the tissue reaches zero stress and buckles immediately after the step of device strain. After the flattening of the tissue (Phase 1), the tissue returns to a tensional stress state (Phase 2). We thus split the dynamics into two phases:

- *Phase 1 - Stress:* $\sigma = 0$

  - Strain: $\varepsilon(t) = \varepsilon_b\left(1 - \frac{r}{1+r} e^{\frac{-t}{\tau(1+r)}}\right)$

- *Transition time:* Phase 1 comes to an end when ε reaches ε_d^f at time:

$$T = (1+r)\,\tau log\left(\frac{\varepsilon_b r}{(\varepsilon_b - \varepsilon_d^f)(1+r)}\right)$$

- *Phase 2 - Stress:* $\frac{\sigma(t)}{E} = (\varepsilon_d^f - \varepsilon_b)\left(1 - e^{-(t-T)/\tau}\right)$

  - Strain: $\varepsilon(t) = \varepsilon_d^f$

D3 – Case 3 (large device strain): $\varepsilon_d^f < \varepsilon_b$

This case corresponds to the experiments shown in Fig. 1b(ii) and Fig. 1d. In this case, the tissue buckles immediately after strain application but cannot flatten sufficiently to restore positive stress. Thus we have:

- *Stress:* $\sigma(t)=0$
- *Strain:* $\varepsilon(t)=\varepsilon_b\left(1-\frac{r}{1+r}e^{\frac{-t}{\tau(1+r)}}\right)$

  Note that ε converges to the value ε_b for large times indicating that the tissue remains longer than the coverslip-to-coverslip distance, forming a stable fold.

E – Response to cycles of compressive strain

To investigate the duration over which an epithelial tissue can 'remember' its previous mechanical state, we apply a sequence of cycles of compressive strain. From the initial state, the device strain is initially shortened to ε_d^f. This is followed, after a time $T_1 > T$, by a lengthening of $-\varepsilon_d^f$ back to the initial length which is maintained for a duration Δ_1. This is then followed by second cycle of shortening back to ε_d^f. The magnitude of the step of shortening ε_d^f is chosen to be in D-Case 2 so that the contour length shows a relaxation dynamic which is not instantaneous while still reaching a final shape of the tissue that is flat.

In general, because the visco-elastic branch could not fully relax during the period of lengthening, immediately after the second shortening (occurring at $t = T_1 + \Delta_1$), the value of the tissue strain is:

$\varepsilon = \frac{\varepsilon_b + r\alpha\varepsilon_d^f}{1+r}$ where $\alpha = e^{-\Delta_1/\tau}$

This leads to a second recovery with different dynamics that is of the form:

$$\varepsilon(t) = \varepsilon_b + \frac{r\left(\alpha\varepsilon_d^f - \varepsilon_b\right)}{1+r} e^{\frac{-t-(T_1+\Delta_1)}{\tau(1+r)}}$$

until ε reaches ε_d^f after a duration of:

$$T_\alpha = (1+r)\tau log\left(\frac{r\left(\varepsilon_b - \alpha\varepsilon_d^f\right)}{\left(\varepsilon_b - \varepsilon_d^f\right)(1+r)}\right)$$

F – Evolution of the transient buckling point with device strain and strain rate

We now consider the effect of the device strain rate on the buckling properties of the epithelium. This is in order to establish the phase diagram of Fig. S4a, defining the planar or buckled state of the tissue with respect to the device strain and strain rate imposed on tissue boundaries.

For this, instead of assuming instantaneous shortening, we impose a ramp of deformation at constant strain rate:

$$\varepsilon_d(t) = \begin{cases} -\dot{\varepsilon}_d t \; if \; t \le -\varepsilon_d^f/\dot{\varepsilon}_d \\ \varepsilon_d^f if \; t \ge -\varepsilon_d^f/\dot{\varepsilon}_d \end{cases}$$

where $\acute{\varepsilon}_d$ is the (positive) device strain rate. We then ask what are the conditions such that buckling occurs during the shortening phase. Using the expression for $\varepsilon_d(t)$, starting from a tensile state, we compute the time dependent stress:

$$\frac{\sigma(t)}{E} = -\dot{\varepsilon}_d t - \varepsilon_b + \tau r\,\dot{\varepsilon}_d\left(1 - e^{-t/\tau}\right)$$

This stress monotonically decreases in time and we then ask if there is a value of time $t^a \in \left[0, -\varepsilon_d^f/\dot{\varepsilon}_d\right]$ in the interval of shortening such that σ reaches zero.

An analytical result can be derived in two limiting cases. When $\tau \dot{\varepsilon}_d \ll 1$ (i.e. device strain rate is very slow), we expect $t^a$to be much larger than τ. Thus

$0 = -\acute{\varepsilon} t^a - \varepsilon_b$

and $t^a \leq -\varepsilon_d^f / \dot{\varepsilon}_d$ leads to the buckling condition $\varepsilon_d^f \leq \varepsilon_b$.

This is indeed the condition we expect during quasi-static shortening.

When, on the contrary, if $\tau \dot{\varepsilon}_d \gg 1$,

$0 = -\acute{\varepsilon} t^a - \varepsilon_b - r \acute{\varepsilon} t^a$

and the condition $t^a \leq -\varepsilon_d^f / \acute{\varepsilon}$ leads to the buckling condition $\varepsilon_d^f < \frac{\varepsilon_b}{1+r}$ which is the one derived in the previous section when considering a sudden shortening. The general case is shown in Fig. S4a.

Appendix 2. Determination of model parameters from experiments

The model contains 4 parameters, which we extract as follows:

- The pre-stress in the tissue σ_a corresponds to the stress as measured before any mechanical perturbation is applied to the tissue, i.e at zero strain (Fig. 3b).

- The elasticity E of the tissue was extracted from the slope of the linear phase in slow compression experiments (Fig. 3d).

The two other parameters were extracted from stress relaxation experiments at low device strains ($\varepsilon_d \leq 10\%$, n = 8, Fig. S4b). At these strains, the model predicts that the tissue does not buckle and that the stress relaxation follows a single exponential (see Case 1, Part C, Appendix 1).

- The elastic constant Y in the viscous branch is extracted from the peak value of the stress σ_i immediately after the fast step of device strain:

  $$Y = \frac{\sigma_i - \sigma_a}{\varepsilon_d} - E$$

  Here, the elasticity E of each sample is extracted from the steady-state value of the stress σ_f in the plateau region (from 100 to 300s, (Fig. S4b)) and through the relation:

  $$E = \frac{\sigma_f - \sigma_a}{\varepsilon_d}$$

  (We verified that the value of E obtained through this method is the same on average as the one we could extract from the slope of the first phase in the slow strain rate experiments.)

- The time-scale $\tau = Y\eta$ was extracted from the characteristic time-scale of stress recovery. An exponential fit function could not perfectly capture the fast stress relaxation occurring at very short time-scales. Therefore, we defined τ as the half-life of stress recovery (see Fig. S4b).

The average values of these parameters were introduced in the equations derived in Appendix 1 to perform the *in silico* experiments shown in Fig. 4 and S4.