Supplemental information

Epithelial cells adapt to curvature induction via transient active osmotic swelling

Caterina Tomba, Valeriy Luchnikov, Luca Barberi, Carles Blanch-Mercader, and Aurélien Roux
Supplementary Figures

Figure S1. Tube characterization. Related to Figure 1. (A) Example of formation of multi-rolls: top (top) and side (bottom) views of a 3 concentric PDMS tubes with cells, cell membrane (deep-red CellMask™, orange), and nuclei (Hoechst, cyan). Scale bar, 100 µm. (B) Method applied to measure the PDMS layer thickness of flat regions and of tubes. Top: PDMS layer identified by fluorescence exclusion (dextran solution, green). Scale bar, 50 µm. Bottom: example of plot profile got with a line thickness of 50 pixels (see the orange lines on the top image on the flat region and the tube). The PDMS width is measured between the 2 green circles, which represent the middle height between the min and the max peaks (blue lines). (C) Distribution of PDMS thickness on flat regions and on tubes without cells. Mean values and SD: 13.96 ± 1.06 µm (Flat, n = 12 images), 13.87 ± 1.20 µm (Tubes, n = 44 images). N = 3. (D) Distribution of inner radii of tubes with cells as a function of time (minutes). Mean values and SD: 97.1 ± 18.4 µm (1 min, n = 42 images), 102.3 ± 17.8 µm (5 min, n = 53 images), 100.0 ± 21.8 µm (10 min, n = 45 images), 107.8 ± 26.8 µm (20 min, n = 47 images), 99.2 ± 15.2 µm (30 min, n = 53 images), 105.5 ± 21.5 µm (60 min, n = 44 images), N = 3. N is the number of independent replicates, the horizontal red lines stand for the mean values.
Figure S2. Effects of fixing, rolling, in-plane deforming and cutting a cell monolayer. Related to Figure 2. (A) Representative orthogonal (top) and top (bottom) views of cell monolayers on a flat glass slide before (“Live”) and after (“Fixed”) chemical fixation. Scale bars, 20 µm. (B) Distribution of cell volume on a flat glass slide before (“Live”) and after (“Fixed”) chemical fixation. n = 250 cells; N = 2. (C) Distribution of cell aspect ratio (height/width) on flat regions (black squares) and on tubes over time (minutes) after cutting (red squares). n ≥ 81 cells/timepoint; N ≥ 3. (D) From left to right: representative maximum-intensity z-projections of cell monolayers on a flat region and on tubes from 1 to 60 min after cutting. Scale bars, 50 µm. (E) Distribution of cell volume on an elastomer film before (black symbols) and after compression (left, magenta circles) or stretching (right, green circles) over time, n ≥ 3406 cells/timepoint, N ≥ 3. (F) Schematic of the regions observed over time from a cut of a cell monolayer on a flat PDMS substrate, where 0 µm indicates the position of the cutting. (G) Distribution of cell volume on a flat PDMS substrate (without inner strain gradient) before (black symbols) and after cutting (from 0 to 300 µm from the cutting, magenta circles; from 300 to 600 µm from the cutting, orange circles; from 600 to 900 µm from the cutting, yellow circles) over time. n ≥ 210 cells/timepoint; N = 1. Insets: representative orthogonal views (top) and maximum-intensity z-projections (bottom) of cell monolayers on a flat region before cut and at 0 to 300 µm, 300 to 600 µm and 600 to 900 µm from the cut at 1 min after cutting. Scale bars, 50 µm. In all images: green (cell membrane, Myr-Palm-GFP), magenta (nuclei, H2B-mCherry). N is the number of independent replicates, the horizontal lines stand for the mean values.
Figure S3. Cell volume quantification at different tube radii and initial cell densities. Related to Figure 3. (A,B) Distribution of cell volume on tubes 1 min (A) and 5 min (B) after cutting, as a function of the inner radius of the tube. \( n \geq 93 \) cells/radius (A), \( n \geq 58 \) cells/radius (B). (C) Distribution of cell volume on flat regions (black squares) and tubes (red circles) over time after cutting when the initial cell seeding density is of 0.3·10^6 cells/cm^2, \( n \geq 347 \) cells/timepoint, \( N = 2 \). (D) Representative orthogonal views of cells on tubes at different timepoints after cutting, when the initial cell seeding density is of 0.3·10^6 cells/cm^2. Scale bar, 20 \( \mu \)m. (E) Representative maximum-intensity z-projections of cell monolayers on tubes 24 hours after cell seeding, with an initial cell seeding density of 0.3·10^6 cells/cm^2 (left) and 0.6·10^6 cells/cm^2 (right). Scale bars, 30 \( \mu \)m. In all images: green (cell membrane, Myr-Palm-GFP), magenta (nuclei, H2B-mCherry). N is the number of independent replicates, the horizontal lines stand for the mean values.
Figure S4. Influence of drugs affecting ion channel activity and of hypo- and hyper-osmotic shocks. Related to Figure 4. (A-B) Distribution of cell volume after (A) DCPIB treatment on tubes (left, dark pink circles) over time after cutting and on flat regions (right, light pink circles), n ≥ 1322 cells/timepoint, N = 3; and after (B) EIPA treatment on tubes (left, light orange circles) over time after cutting and on flat regions (right, dark yellow circles). n≥1317 cells/timepoint, N = 3. (C-H) Weighted means and SEWM (with variance weights) of cell volume, representative orthogonal views on flat regions or tubes at 5 and 30 min after cutting, and distribution of cell volume of (C-E) ethanol-treated cells: (C) on flat regions (black squares) and on tubes (black circles) over time after cutting, n ≥ 2360 cells/timepoint, N = 3; (E) on tubes (left, red circles) over time after cutting and on flat regions (right, black circles), n ≥ 699 cells/timepoint, N = 3; and of (F-H) DMSO-treated cells: (F) on flat regions (black squares) and on tubes (black circles) over time after cutting, n ≥ 17 images/timepoint, N ≥ 3; (H) on tubes (left, red circles) over time after cutting and on flat regions (right, black circles), n ≥ 2630 cells/timepoint, N ≥ 3. (I) Distribution of cell volume after Bumetanide treatment on tubes (left, blue circles) over time after cutting and on flat regions (right, dark blue circles), n ≥ 1311 cells/timepoint, N = 3. (J-L) Weighted means and SEWM (with variance weights) of cell volume, representative orthogonal views on flat regions or tubes at 5 and 30 min after cutting, and distribution of cell volume of Furosemide-treated cells: (J) on flat regions (blue squares) and on tubes (light blue circles) over time after cutting, n ≥ 4 images/timepoint, N = 3; (L) on tubes (left, light blue circles) over time after cutting and on flat regions (right, blue circles), n ≥ 33 cells/timepoint, N = 1. Scale bars, 20 µm. In all images: green (cell membrane, Myr-Palm-GFP). N is the number of independent replicates, the horizontal lines stand for the mean values.
Figure S5. Influence of drugs affecting actin depolymerization and myosin contractility, of outward rolling and of 15% and 30% in-plane deformations. Related to Figure 5. (A) Distribution of cell volume after Jasplakinolide treatment on tubes (left, light violet circles) over time after cutting and on flat regions (right, dark violet circles) over time, n ≥ 1911 cells/timepoint, N = 3. (B) Distribution of cell volume after Blebbistatin treatment on tubes (left, green circles) over time after cutting and on flat regions (right, dark green circles) over time, n ≥ 2171 cells/timepoint, N = 3. (C) Mean values and SD of apical to basolateral actin intensity ratio on flat regions (0 min) and on tubes with inverted sign of curvature over time after cutting, n≥227 images/timepoint; N = 1. (D) Mean values and SD of apical to basolateral actin intensity ratio on flat regions (0 min, white circle) and after compression (magenta squares) or stretching (green rhombuses) of 15% over time, n≥6 images/timepoint, N = 1. (E) Mean values and SD of fluorescence lifetime (difference values of each timepoint $-T_i$ – with the mean initial timepoint $-T_0$) on flat regions (0 min, white circle) and after compression (magenta squares) or stretching (green rhombuses) of 15% over time, n ≥ 6 images/timepoint, N = 1. (F) Weighted means and SEWM (with variance weights) of cell volume on flat regions (black circle) and after compression (light magenta squares) or stretching (light green rhombuses) of 30% over time, n ≥ 4 images/timepoint, N = 1. (G) Mean values and SD of apical to basolateral actin intensity ratio on flat regions (0 min, white circle) and after compression (light magenta squares) or stretching (light green rhombuses) of 30% over time, n ≥ 12 images/timepoint, N = 1. (H) Mean values and SD of fluorescence lifetime (difference values of each timepoint $-T_i$ – with the mean initial timepoint $-T_0$) on flat regions (0 min, white circle) and after compression (light magenta squares) or stretching (light green rhombuses) of 30% over time, n ≥ 5 images/timepoint, N = 1. N is the number of independent replicates.
Figure S6. Influence of drugs affecting actin polymerization, mTORC1&2 activity and cell membrane tension and E-cadherin organization within rolled epithelia. Related to Figure 6. (A) Weighted means and SEWM (with variance weights) of cell volume on flat substrates before (-1 min) and over time (minutes) after Latrunculin A treatment, n ≥ 6 images/timepoint, N = 1. (B) Mean values and SD of fluorescence lifetime (difference values of each timepoint – Ti – with the initial timepoint – T0) on flat regions before (-1 min) and over time (minutes) after Latrunculin A treatment, n ≥ 7 images/timepoint, N = 1. (C) Weighted means and SEWM (with variance weights) of cell volume after Latrunculin A treatment on flat regions (violet squares) over time and on tubes (dark violet circles) over time after cutting, n ≥ 3 images/timepoint, N = 1. (D) E-cadherin-GFP MDCK cells on tubes at different time points after rolling. Schematics show the black rectangular position on the tube corresponding to the observation point of view reported on their right: top, orthogonal side and longitudinal side. Arrows point to the apical side of the cell monolayer. Scale bars, 20 µm. (E) Distribution of cell volume after Torin1 treatment on tubes (left, dark grey circles) over time after cutting and on flat regions (right, grey circles) over time, n ≥ 1861 cells/timepoint, N=3. (F) Distribution of cell volume after Rapamycin treatment on tubes (left, dark blue circles) over time after cutting and on flat regions (right, blue circles) over time, n ≥ 2199 cells/timepoint, N = 3. (G) Mean values and SD of fluorescence lifetime (difference values of each timepoint – Ti – with the initial timepoint – T0) on flat regions before (-1 min) and over time (minutes) after PalmC treatment, n ≥ 4 images/timepoint, N = 2. (H) Volume distribution of live cells on flat regions observed over time, where “0 min” represents the condition just before treating cells with PalmC. n ≥ 288 cells/timepoint. (I) Distribution of cell volume after PalmC treatment on tubes (left, violet circles) over time after cutting and on flat regions (right, light violet circles) over time, n ≥ 1251 cells/timepoint, N = 3. N is the number of independent replicates, the horizontal lines stand for the mean values.
Section 1: Estimation of the compressive force within the cell monolayer due to rolling of the PDMS substrate.

In this section, we use a continuum elastic description of the PDMS substrate and cell monolayer to estimate the pressure experienced by the cell monolayer due to the spontaneous curvature of the PDMS substrate, as well as the extent of the PDMS substrate unrolling due to the elastic resistance of the cell monolayer to bending.

We focus on the most curved region of the substrate + monolayer system in Fig. 1 of the main text, in which we assume its shape to be cylindrical. We also assume translational invariance along the axial direction of the cylinder, which allows us to focus only on the circular cross-section of the cylinder in the following. The substrate has a preferred radius of curvature $R_0 \approx 100 \mu m$ that is much larger than the thicknesses of the PDMS substrate and of the cell monolayer (both in the order of $h \approx 10 \mu m$), therefore we describe substrate and cell monolayer as thin shells.

To estimate the pressure experienced by the cell monolayer due to the curvature of the substrate, we focus on a short time window (< 10 min) that follows the substrate’s self-rolling, in which we assume that the cell monolayer behaves as a simple elastic material. When the cell monolayer sticks to the rolled substrate, it is both bent and laterally compressed, and we postulate its surface energy density as

$$ e_m = \frac{k_b}{2} \left( \frac{1}{R} \right)^2 + \frac{\lambda}{2} \varepsilon^2 $$  \hspace{1cm} (1)

where $k_b = 0.5 \mu N \cdot \mu m$ (A. Trushko et al, Dev. Cell, 2020) is the monolayer bending rigidity, $R$ is its radius of curvature, $\lambda = 0.15 \mu N/\mu m$ (A. Trushko et al, Dev. Cell, 2020) is its rigidity to lateral compression and $\varepsilon$ is its lateral strain. We estimate the strain as the relative variation of the length of the cell monolayer mid-line before ($l_{h/2}^o$) and after ($l_{h/2}$) bending, $\varepsilon = \left( l_{h/2}^o - l_{h/2}^o \right) / l_{h/2}^o$. We assume that the length of the cell-substrate contact line is the same before and after bending, and that $R$ is the radius of curvature there, in which case one finds that $\varepsilon = -h/(2R)$. Note that at $R = R_0$ we have $\varepsilon = 0.05$. We can now rewrite the surface energy density of the cell monolayer as

$$ e_m = \frac{K_m}{2R^2} $$  \hspace{1cm} (2)

where $K_m = k_b + \lambda(h/2)^2$ is an effective bending rigidity of the cell monolayer. Note that $\lambda(h/2)^2 \approx 3.8 \mu N \cdot \mu m \gg k_b$, implying that most of the effective resistance of the cell monolayer to the rolling of the PDMS substrate comes from its resistance to lateral compression.

We now calculate the pressure required to stabilize the cell monolayer at a radius of curvature $R = R_0$, which is:

$$ P_{R=R_0} = \left( \frac{d e_m}{dR} \right)_{R=R_0} = -\frac{K_m}{R_0^3} \approx -4 Pa $$  \hspace{1cm} (3)

In conclusion, we expect that shortly after self-rolling the substrate exerts a pressure in the order of 4 \( Pa \) onto the cell monolayer in order to stabilize it.

Next, we estimate the extent of the PDMS substrate unrolling due to the elastic response of the cell monolayer. We start by evaluating the bending rigidity $K_s$ of the PDMS substrate, which can be written in terms of its thickness, Young modulus $E$ and Poisson ratio $\nu$, by resorting to the classical theory of thin shells (Landau and Lifshitz, 1975, Theory of elasticity):

$$ K_s = \frac{E h^3}{12(1-\nu^2)} $$  \hspace{1cm} (4)

Using $E = 1 \text{ MPa}$ (Egunov et al, Soft Matter, 2016) and $\nu = 0.5$ for PDMS, we get $K_s \approx 100 \mu N \cdot \mu m$. Then, we postulate the surface energy density of the substrate
\[ e_s = \frac{K_s}{2} \left( \frac{1}{R} - \frac{1}{R_0} \right)^2. \]

In the absence of a cell monolayer, the equilibrium radius of the substrate is \( R_0 \). In the presence of a cell monolayer, the equilibrium radius is the one that minimizes the total energy density \( e = e_m + e_s \), which is

\[ R^* = R_0 \frac{K_s + K_m}{K_s} \approx 104 \, \mu m. \]  

To conclude, we expect that the unrolling of the substrate due to the resistance of the cell monolayer to bending deformations is \( (R^* - R_0)/R_0 = 4\% \).

Methods S2. Related to Figures 1 and 2.

Section 2: Estimation of the maximal thickening of the PDMS and cell layer upon rolling.

To calculate the cells monolayer thickening due to geometrical reasons, and non-biological reasons, we split PDMS film relaxation on two steps: (i) in-plane shrinking of the film upon the release from the PDMS-glass substrate (Fig. 1a of the main text), and (ii) curling of the film due to internal bending moment in the PDMS film. We assume that the cell monolayer is much softer than the PDMS film, therefore we neglect its elastic response and assume it adapts its shape to the spontaneous curvature of the film. We model the substrate and cells monolayer as three-dimensional materials, with initial thicknesses \( H_0 \) and \( h \), respectively, and long sides with typical initial lengths \( L_0 \) (cf. schematic on the left). We determined experimentally that the PDMS layer experiences isotropic shrinking along its long sides when released from the substrate, when the rolling is hindered by placing a slippery glass slide atop of the film, such that the typical length of its long sides gets resized to \( L = L_0(1 - \delta L) \), with \( \delta L \approx 0.02 \). Let \( h \) and \( H \) be the thicknesses of the cell monolayer and PDMS, respectively, after lateral isotropic shrinking. Assuming that both cells and PDMS are incompressible, \( h \) and \( H \) can be calculated by enforcing the conservation of their volumes, yielding:

\[ H = \lambda H_0, \]
\[ h = \lambda h_0, \]

where

\[ \lambda = \frac{1}{(1-\delta L)^2}. \]

Let \( H' (h') \) be the thickness of the PDMS film (layer of the cells) after bending (Fig. S5). In order to find the upper limit on the thickening of the layer of cells due to the film bending, we make the following assumptions: (1) the neutral surface upon curling corresponds to the outer surface of the tube; (2) the tube does not elongate along its axial direction upon bending due to the Poisson effect. Under these assumptions, we calculate the thickening of the PDMS substrate and cell monolayer upon bending by enforcing the conservation of the areas of their cross-sections.

Consider a segment of the layer of the length \( L \), which curls in a segment with the opening angle \( \alpha \), inner radius \( R \), and outer radius \( R_N \), corresponding to the neutral surface, \( R_N = R + h' + H' \) (cf. schematic above). Conservation of the areas of the cross-sections of the PDMS and cells layers implies:
\[ \frac{\alpha}{2} [(R + h' + H')^2 - (R + h')^2] = LH \]

\[ \alpha \frac{1}{2} [(R + h')^2 - R^2] = Lh \]  

(9)

Substituting \( \alpha = \frac{L}{R_N} = \frac{L}{R+h'+H'} \) in (9), we get

\[ \left\{ \begin{array}{l}
(R + h' + H')^2 - (R + h')^2 = 2H(R + h' + H') \\
(R + h')^2 - R^2 = 2h(R + h' + H')
\end{array} \right. \]

(10)

which can be recast as

\[ \left\{ \begin{array}{l}
2R(H' - H) + 2h'(H' - h) + h'(H' - 2h) = 0 \\
2R(h' - h) + h'(h' - 2h) - 2hH' = 0
\end{array} \right. \]

(11)

Note that the system (11) is solved for \( H' = H \) and \( h' = h \) to leading order when \( R \to \infty \), which corresponds to the case of flat layers. Given that in the experiments \( R \) is much larger than all other length scales, we exploit the large \( R \) limit to solve (11) perturbatively, close to the flat solution. We define the perturbed thicknesses \( H' = H(1 + \delta H/R) \) and \( h' = h(1 + \delta h/R) \), where \( \delta H \) and \( \delta h \) are of the same order of \( H \) and \( h \). We substitute these expressions in (11) and keep only the leading order terms in the limit \( R \to \infty \), which yields:

\[ \delta H = \frac{H}{2} \]  

(12)

\[ \delta h = \frac{1}{2}(h + 2H) \]  

(13)

Using the experimental values (Fig. 1d, S1c, 2b) \( R \approx 100 \mu m, H_0 \approx 14 \mu m, h_0 \approx 10 \mu m, \) and \( \lambda \approx 1.04 \), we finally get \( H' \approx 15.6 \mu m \) and \( h' \approx 12.5 \mu m \). Therefore, the thickness increase due to shrinking and curling of the PDMS substrate does not exceed 12% for PDMS and 25% for the cell monolayer. Note that if a cell thickness increases of 25%, conservation of area implies that its width decreases of 20%, in this model.

However, the estimated PDMS layer thickening is comparable with the standard deviation of the experimental measurements \( (14 \pm 1 \mu m) \) and the PDMS thickness that we observed (Fig. S1c) is not significantly different before and after rolling. This confirms the prediction for a maximal PDMS thickness change upon self-rolling, which results in the same range than the experimental fluctuations.

The estimated cell thickening is less than the experimentally observed, suggesting that cell monolayers do not behave as elastic incompressible materials.
Methods S3. Related to Figure 2.

Section 3: Estimation of the maximal compression of the PDMS layer upon rolling.

To calculate the maximum PDMS layer compression due to the shrinking and the curling of the film, we assume that the neutral surface upon curling corresponds to the outer surface of the tube.

We consider a segment of the layer with initial length $L$ and thickness $H$, which curls into a segment with opening angle $\alpha$, thickness $H'$, inner radius $R_i$, and outer radius $R_N = R_i + H'$ (cf. schematic on the left). We call $L'$ the length of the inner face of the tube, such that, by construction:

$$\alpha (R_i + H') = L$$
$$\alpha R_i = L'$$

(14)

We call $\Delta L = L - L'$, and obtain the relative variation of the PDMS layer length from system (14):

$$\frac{\Delta L}{L} = \frac{H'}{R_i + H'}$$

(15)

Substituting the estimated and the experimental values $H' \approx 15\mu m$ and $R_i \approx 100\mu m$ in (7) gives $\frac{\Delta L}{L} \approx 0.13$. Therefore, the compression of the PDMS layer due to shrinking ($\approx 2\%$) and curling of the PDMS substrate does not exceed 15%.