Supporting Information

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SI Text

SI Discussion Langevin model for tissue motility. To illustrate our interpretation of the observed interplay between cellular growth, motility, and colony expansion, we formulate and analyze a simple growth model for adherent epithelial tissues. We choose as a point of departure a one-dimensional version of the “vertex model” as shown in Fig. 5A. Physical position of cell i is specified by its vertices $r_i$ and $r_{i+1}$ which it shares with its neighbors. In addition to the interaction between neighboring cells we also include adhesion to the substrate. The attachment of cells to the extracellular matrix is mediated by many focal adhesions on the basal membrane of the cell. In our model we represent the cumulated effect of the focal adhesions by a single attachment point $R_i$ for each cell. We assume the mechanical properties of the tissue to be elastic on short time scales so that for a given set of intrinsic cell lengths and attachment points, the vertices of the cells are determined by minimizing the energy

$$
H(r_1, ..., r_{N+1}) = k \sum_{i=1}^{N} (r_{i+1} - r_i - L_i(t))^2 
+ \sum_{i=1}^{N} |R_i - (r_{i+1} + r_i)/2|^2.
$$

where $L_i(t)$ is the intrinsic preferred length of the cell. The first term describes the mechanical interaction between cells, the second term accounts for the attachment of the cells to the substrate, and the units are chosen so as to make attachment stiffness unity.

In principle, depending on the mechanical stress $\sigma_i \propto k(r_{i+1} - r_i - L_i(t))$ a cell can adapt its intrinsic length scale $L_i$ resulting in an effective plasticity of the tissue that would relax the stress at long times. Here, for simplicity, we will only include the effect of cell growth, which also manifests itself as a change—an increase—of $L_i$. We assume that $L_i$ resists compression $\sigma_i < 0$ and grows with rate $\alpha$ for $\sigma_i > 0$, e.g., $\frac{d}{dt} L_i = \alpha \sigma_i$, where $\alpha$ is the step function. This implements the assumption that cells grow in size only when under tension, with the consequence in the absence of tension, cell divisions will reduce intrinsic cell size $L_i$.

The dynamics of the attachment points is driven by relaxation of elastic stress and by random forces generating cell motility

$$
\frac{d}{dt} R_i = -\frac{\partial H}{\partial R_i} + \eta_i(t),
$$

where $\eta_i$—a Langevin-type random force representing motility—is a Gaussian, white random function of time defined by its second moment $\langle \eta_i(t) \eta_j(0) \rangle = \Gamma(t) \delta_{ij}$. The relevant time scale for the attachment dynamics is given by $\Gamma$—which acts like friction. While we assume that the random force representing cell motility has a zero average in the bulk of the tissue, to represent the observed directed (outward) crawling of cells on the boundary, we allow $\langle \eta_i \rangle = -\langle \eta_i \rangle = \sigma v_{\text{max}}$, where $v_{\text{max}}$ sets the maximal crawling velocity of boundary cells.

In addition to the continuous changes in cell sizes and attachments, cells may divide. Motivated by our experimental quantification of the cell area growth curve (Fig. 4) we make the average rate for cell division explicitly dependent on cell size $l_i = r_{i+1} - r_i$ by using $p(t) = \max(\gamma(\rho(t) - \rho_{\text{max}}), 0)$ in our simulations, which is also consistent with our finding of a growth size checkpoint. The division process replaces the dividing cell by two equivalent daughter cells with attachment points set to the middle of each cell and the sum of internal length matches the internal length of the mother cell.

We have simulated the dynamics of this model (with parameters $k = 1$, $\alpha = 0.02$, $\sigma = 1$, $\Gamma = 1$, $\gamma = 0.2$) using custom-written Matlab programs implementing matrix inversion for the dynamics of the vertices and the Runge-Kutta method for the dynamics of the attachment points. Programs are available upon request.

SI Materials and Methods Cell culture. Cells were seeded at uniform density (around 600 cells/mm$^2$) on a fibronectin (Sigma-Aldrich, F1141-2MG) coated PDMS membrane (McMaster-Carr, 87315K62) and imaged in phenol red free IMEM (Cellgro, 10-26-CV) supplemented with Penicillin-Streptomycin and 5% FBS. The media was replaced daily and the culture conditions were kept at 37°C and 5% CO$_2$ by means of a custom-made microscope stage enclosure. Single colony experiments were performed by seeding cells at a density of about 1 cell/cm$^2$ in a glass bottom petri dish. Images of the colony spanning 9 × 9 contiguous fields of view were captured and stitched together, making it possible to follow single cell motion as well as tissue-wide dynamics with good resolution.

Image processing. Quantitative data on cell area was obtained with the help of contrast enhancement by Gabor-filtering (7, 8) and successive removal of low contrast regions. Frames taken 10 min apart were compared to remove poorly segmented cells. Cell size, shape, topology, and structure functions were obtained from the segmented images. Mean displacement measurements were made by cell tracking using a PIV-type analysis (9). The position of nuclei in a segmented image was propagated in time by correlation analysis. The trajectory thus obtained was then corrected for stage drift and r.m.s. velocities were calculated as $\sqrt{\langle v^2 \rangle - \langle v \rangle^2}$. To extract the correlation length, the two point correlation function $C_v(R)$ for $\langle v \rangle$ was computed and the length for which $C_v(R) = 0.3$ was used.

Rate analysis. To calculate the division rates shown in fig. 4E, eq. 1 was discretized; an overdetermined linear system was constructed based on measurements of the size distribution at different times, and was subsequently pseudo-inverted (10). The total number of cells per frame was obtained from the average area.

3. Lewis FT (1928) The correlation between cell division and the shapes and sizes of prismatic cells in the epidermis of cucumis Anat Record 38:341.
Fig. S1. (A) Aspect ratio of the Voronoi cells constructed for the segmented nuclei. Aspect ratio is defined as the ratio between the largest and the smallest eigenvalues of the inertia tensor defined by cell vertices. Regular polygons have aspect ratio between 1 and 1.5. The figure presents the fraction of the cell population with aspect ratio $ar < 1.5$ (black), $1.5 < ar < 3$ (blue) and $ar > 3$ (red). As a function of time, the fraction of regular polygons increases and the fraction of deformed cells decreases dramatically. Time zero refers to the morphological transition. (B) Change in the cellular coordination number across the morphological transition. Each histogram (coded by a color) represents the distribution of the number of cell vertices at a given time during the experiment. The last distribution plotted is stationary and is analogous to the one found in other biological tissues (3–6). (C) Cell size heterogeneity across the morphological transition. Different colors represent the coefficient of variation as a function of time for different experiments. The coefficient of variation was obtained by calculating the ratio of the difference of the quartiles 75 and 25 to the median: $(Q_{75} - Q_{25}) / Q_{50}$. Data from different experiments were time aligned referring to the morphological transition. (D) Average cell thickness as a function of cell area. We observe that below 200 μm, cell height does not change appreciably. Cell height is measured by finding the best focal plane at a given time and then tracking the position of the objective during the whole experiment.

Movie S1. Dynamics of a growing epithelial colony. The movie is composed of 9 × 9 patches for a total of 3.02 × 4.05 mm. Six cells were seeded and imaged for 10 d. Media were replaced daily. Scale bar: 1 mm.

Movie S1 (MPG)
Movie S2. Dynamics of epithelial tissue in the bulk. Cells were seeded at uniform density and imaged continuously for a week. Media were replaced daily. Confluency is reached at $t = 0.6$ d. By day two cell motion has completely disappeared, and only small scale vibrations can be observed. Scale bar: 100 μm.

Movie S2 (AVI)