Novel growth regime of MDCK II model tissues on soft substrates

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MATERIALS AND METHODS

MDCK II cells (#00062107) were obtained from ECACC (UK) and cultured in MEM with Earle´s Salts (# F0325, Biochrom) supplemented with 2mM L-glutamin (# G7513, Sigma-Aldrich), 5% FBS (#F0804, Sigma-Aldrich) and 1% Penn/Strep (#15070-063, Gibco, LifeTechnologies) at 37° C and 5 % CO_2. Cells were split and passaged every other day to keep them sub-confluent (< 80%).

Collagen-I (BD Biosciences) coated elastic polyacrylamide (PA) gels were prepared as described earlier [Ref 15 in the main text] adapted from the original protocol by Pelham and Wang [Ref 11 in the main text]. The Young's elastic modulus E was measured by macro rheology (MCR 501, Anton Paar, Austria) using a cone and plate geometry (Ø = 25 mm, 2°) A typical gelation curve of PA yielding a shear storage modulus G' = after 1h (3,600 s), the usual polymerization time for PA gels is shown in Fig S1A. This is converted to the Young's modulus E using a Poisson's ratio of $\nu = 0.45$ (Engler et al., Meth Cell Biol, 83 (2007) 521). To ensure a homogeneous elastic hydrogel, the macroscopic rheology measurements were complemented with microrheology using atomic force microscopy (AFM, MFP-3D, Asylum Research, Santa Barbara, USA). The resulting force-indentation curves were fitted using a modified Hertz model

$$E = \frac{\pi \left(1-\nu^2\right) F}{\delta^2 \; 2 \tan \alpha}$$

as described in (Engler et al., Meth Cell Biol, 83 (2007) 521) and shown in Fig S1B (black line depicts experimental data, dotted red line shows the fitted Hertz model).

To assure similar collagen-I coating, glass slides were cleaned and treated with the same cross-linker (Sulfo-SANPAH, Pierce, Thermo Scientific) before collagen incubation as described.

MDCK II cells were seeded in two different ways on substrates. First, 30,000 cells were dispersed over the whole area of the substrate to investigate single cell behaviour and the onset of cluster formation. Second, cells were seeded as a highly concentrated (90,000 cells in 7 µL) drop in the middle of the sample, subsequently incubated for one hour before gently adding 2 mL of medium.

Filamentous actin was stained using phalloidin–tetramethylrhodamine B isothiocyanate (#77418-100UG, Sigma-Aldrich) and the nucleus was labelled with Hoechst 33342 trihydrochloride trihydrate (Molecular Probes, Life Technologies). Fluorescence microscopy was performed on a Zeiss Cell Obsever Z1 using 5 x, 20 x and 32 x objectives and images were recorded with a Zeiss AxioCam M3 and the Zeiss AxioVision software package (all Zeiss, Göttingen).

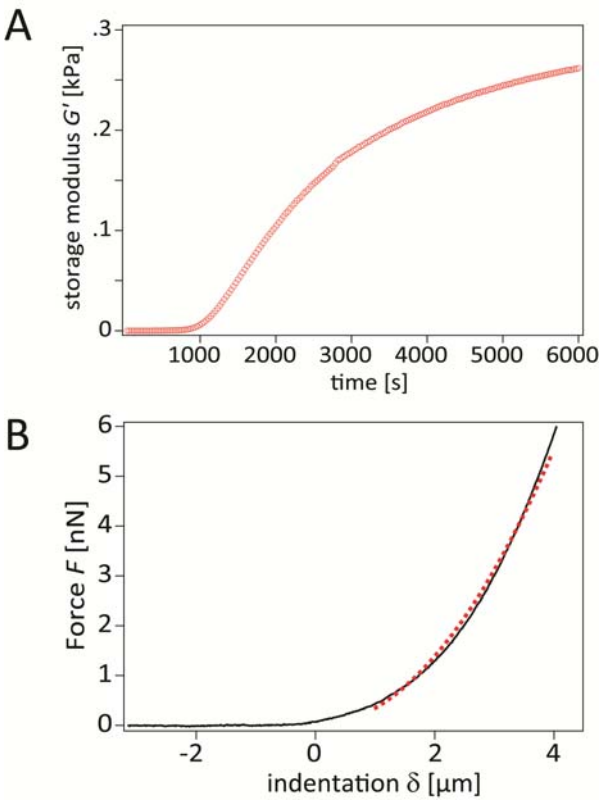

Fig. S1. *A*) A typical polymerization curve of PA yielding a shear storage modulus. *B*) Atomic force microscopy force-indentation curves. The black line depicts experimental data, dotted red line shows the fitted Hertz model.

DATA ANALYSIS

SINGLE CELLS

Single cell area was determined after segmentation of actin images as the area of white objects. Error in threshold procedure is negligible compared to variation of the cell size. There are 50 cells in statistics for glass substrate and 90 cells for 0.6 kPa PA gels.

GLASS AND HARD SUBSTRATES

Image analysis was performed with self-developed routines in MALAB (The MathWorks, Natick, MA, USA). The density of large colonies was obtained from an automated procedure whereby the number of objects in a given area was determined from segmented fluorescence images of cell nuclei (Fig SI2*A*). The reported values are spatial averages from the bulk and the edge parts of the cluster performed independently at a fixed instance of time (days 4-12). The averaging is performed over images of a colony on a given day (4, 6,...12) and then over all days.

The error in determination of density emerges when two nuclei are recognized as one (green ellipse in Fig. S2A), or when one nuclei is recognized as two (yellow ellipses in Fig. S2A). The relative error in cell density arising from this effects amounts to 2%, which is calculated after determining the density from images with corrected nuclei recognition step. Since this induces a small error, the reported standard deviation entirely reflects the fluctuations of the cell density within the respective colony compartments. Another reason for a large deviation is that the data set includes colonies of different age. On a level of a single colony at a particular day, the bulk density is at least fifty percent larger than the density in the edge of the cluster.

The area of a large colony is determined by approximating the shape of the cluster by an ellipse, and measuring the minor and major axis. The reported error corresponds to the standard deviation of cluster sizes from several experiments, which is again significantly larger than the error in determining the area of a single colony.

SOFT SUBSTRATES

The density and the monolayer area in small clusters was determined with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012). Thereby, the number of cell nuclei was counted within area of interest, the latter being determined with a freehand selection tool (Fig S2B). Deviation arising from repeatedly selecting the same area is less than 1%. The main contribution to the reported deviation comes from the variation in density when changing the area of interest. Examples of areas of interest are shown in different colours in Fig. S2B. Nevertheless, the relative uncertainly in density remains below 10%, and naturally becomes smaller as the size of the monolayer increases. For example, the area of the monolayer of the colony shown in Fig S2B is determined with the 6% relative uncertainty.

For bigger clusters the same procedure to determine the number of cells in a given image was used as for colonies on hard substrates. On the other hand, the size of the colony was determined after segmenting the images using the Otsu's method (for details see Otsu, N. 1979. A Threshold Selection Method from Gray-Level Histograms. *IEEE Transactions on Systems, Man, and Cybernetics*. 9:62-66). For very large colonies a collage of images was made prior to segmentation. In total, 130 colonies were analysed with sizes differing over five orders of magnitude.

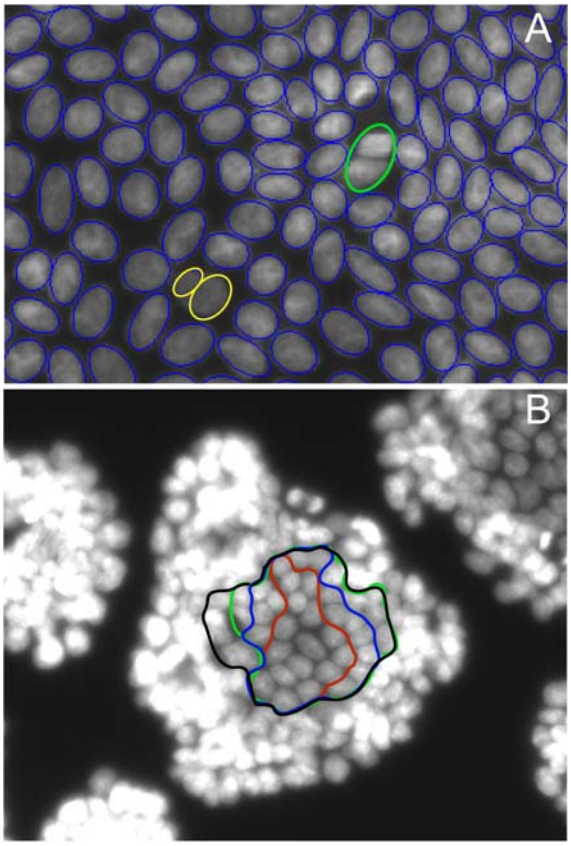

FIGURE S2 Sources of errors in determining the cell density. (A) The MATLAB routine may recognize two nuclei as one object (green) or divide one nucleus into two objects (yellow). This leads to total error of 2%. (B) Freehand selection tool in ImageJ and four possible monolayer areas of interest. The four selections lead to a 6% relative uncertainty in determining the cell density.