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Architecture and migration of an epithelium on a cylindrical wire

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In a wide range of epithelial tissues such as kidney tubules or breast acini, cells organize into bidimensional monolayers experiencing an out-of-plane curvature. Cancer cells can also migrate collectively from epithelial tumors by wrapping around vessels or muscle fibers. However, *in vitro* experiments dealing with epithelia are mostly performed on flat substrates, neglecting this out-of-plane component. In this paper, we study the development and migration of epithelial tissues on glass wires of well-defined radii varying from less than 1 μm up to 85 μm . To uncouple the effect of out-of-plane curvature from the lateral confinement experienced by the cells in these geometries, we compare our results to experiments performed on narrow adhesive tracks. Because of lateral confinement, the velocity of collective migration increases for radii smaller than typically 20 μm . The monolayer dynamics is then controlled by front-edge protrusions. Conversely, high curvature is identified as the inducer of frequent cell detachments at the front edge, a phenotype reminiscent of the Epithelial–Mesenchymal Transition. High curvature also induces a circumferential alignment of the actin cytoskeleton, stabilized by multiple focal adhesions. This organization of the cytoskeleton is reminiscent of *in vivo* situations such as the development of the trachea of the *Drosophila* embryo. Finally, submicron radii halt the monolayer, which then reconfigures into hollow cysts.

collective cell behaviors | out-of-plane curvature | epithelial tubes

In the last years, the physical microenvironment has been repeatedly demonstrated to be crucial in defining cellular behaviors and phenotypes. For example, the rigidity of a substrate on which a cell adheres can dictate its morphology, phenotype, and even fate (1, 2). Also, the substrate's microtopography or nanotopography can change cell adhesion and orientation (3, 4). Few studies, however, have focused on quantifying the role of out-of-plane curvature on cells or cell assemblies. This is surprising because epithelial sheets are often naturally curved, organizing into tubes, cysts, crypts, or villi whose radius of curvature is typically on the order of a few cells or tens of microns (5). Tubules can even be formed out of a cohesive chain of single cells, each enclosing a central lumen (6), as has been observed for the *Drosophila* trachea tip cells (7) and in some parts of the *Caenorhabditis elegans* digestive tract (8).

In vitro, curved substrates provide a controlled way to study the role of an out-of-plane curvature on a system otherwise identical to the classic 2D culture assay on flat substrates. Because multicellular assemblies are largely controlled by the interactions between cells, the influence of curvature on epithelial tissues must be explored on monolayers. In that case, most of the reported experiments have been performed in negative curvature conditions (i.e., tubes or channels) under perfusion (9, 10). In contrast, experiments on positively curved substrates (i.e., wires) have been mostly conducted on single cells. In particular, Schwann cells (11) or fibroblasts (12–15) seeded on glass wires of radii of the order of 10 μm have been shown to align with the wire long axis. More recently, it has been observed that the response of cells to positive curvature depends strongly on the cell type: Epithelial single cells spread in all directions and favor a circumferential alignment of their actin cytoskeleton (16), while

fibroblasts align their cell body and their actin fibers longitudinally (16, 17). Some of these differences are captured by a theoretical model in which cell adhesion and active contractility balance the anisotropic bending stiffness of the stress fibers (18). Very recently, monolayers formed from different endothelial cell types cultured on wires were shown to exhibit cell type-dependent orientations (19).

Since growing monolayers on cylindrical wires intrinsically induces a lateral, although borderless, confinement, it is possible that confinement rather than curvature dictates the behavior of these epithelia. To uncouple these two contributions, we grew cells on smooth cylindrical rods of varying radii and systematically compared our results with experiments conducted on confining tracks of equivalent confinement. We note that, here, the basal side of the cell faces the wire and is inside the tube. The cells therefore have an inverted polarity compared with tubule formation during development. Conversely, the *in vivo* situation of collective migration on blood vessels, nerves, or myofibers (20–22), in particular originating from tumors, is directly comparable to our experiments.

We show that below a radius of the order of 40 μm , the curvature imposes a transverse actin alignment. This cell architecture is coupled with an increase in the number of focal adhesions (FAs) and with a curvature-induced breaking off of the cells at the front edge. In contrast, confinement rather than curvature controls the dynamics of collective migration. Moreover, we show that there exists a critical submicrometric radius under which cells are unable to migrate collectively, resulting in the formation of hollow cysts.

Results

Identical smooth glass wires of radius R varying from 2 μm to 85 μm were aligned on the edge of a piece of silicone gel, fixed in place with cross-linked polydimethylsiloxane (PDMS) (Fig. 1A

Significance

Cell sheets often organize in tubular structures, for example, in the kidney. Also, cells from epithelial tumors are known to wrap around vessels or muscle fibers as they migrate collectively. By plating cells on thin glass wires, we mimic these physiological conditions *in vitro* and show that high curvature favors cell detachment at the front edge. This switch from collective to individual migration may reproduce features often observed in cancer invasion. High curvature also induces a circumferential organization of the actin cytoskeleton reminiscent of *in vivo* embryonic morphogenesis situations where tissues develop on a cylindrical template. Finally, monolayer migration is halted at submicron radii, and the tissue reconfigures into hollow cysts at its leading tip.

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and *SI Appendix, Fig. S1*). Unless otherwise specified, the wires were coated with fibronectin. Madin Darby Canine Kidney (MDCK) epithelial cells were then seeded on the fibronectin-coated PDMS supports. We note that these kidney-derived cells assemble into tubes in 3D environments when stimulated by growth factors (23).

A monolayer grew on the supports to confluence and then progressed onto the wires, which were suspended in the medium. The cells wrapped fully around the wires and moved collectively along them while maintaining cell–cell adhesions (Fig. 1 *B–D*). Cell migration was monitored for typically 2 days. To uncouple curvature and lateral confinement, cells were also cultured in 2D micropatterned adhesive tracks of varying widths (24, 25). In the following, confinement is defined as the tracks' width (w) or the wires' perimeter ($2\pi R$).

Fiber Radius Controls the Migratory Phenotype. Cells on relatively thick wires ($R > 40 \mu\text{m}$) remained cohesive and did not escape the monolayer (*Movie S1* and Fig. 2*A*). In contrast, when the wire radius was smaller than $40 \mu\text{m}$, cells at the front edge occasionally broke off and moved as single cells. Such events were more frequent at small radii (*Movie S2* and Fig. 2*A*). After detaching from the monolayer, these cells moved back and forth at very high speeds (up to $100 \mu\text{m}\cdot\text{h}^{-1}$) along the wire and/or around it. Because of this erratic movement, the progressing monolayer eventually caught up with these maverick cells, which then readily reincorporated in the monolayer (Fig. 2*B*). These individually migrating cells switched between elongated and retracted morphologies. In particular, they displaced the fastest

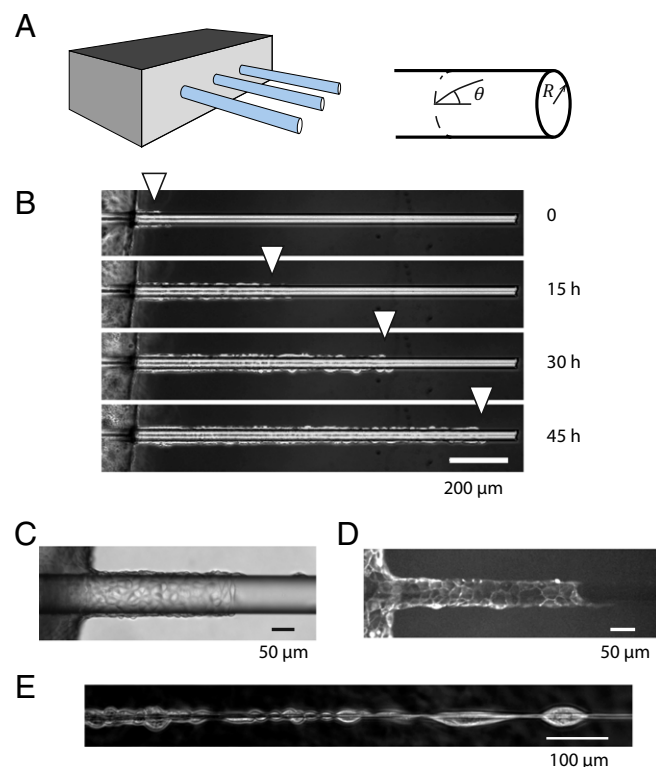


Fig. 1. (A) Schematic of the experimental device with the glass wires aligned on the edge of a PDMS support. (B) The cells grow to confluence on the support at the left of the pictures before invading the wire collectively ($R = 20 \mu\text{m}$) (C and D) Phase contrast (C) and fluorescence image (D) of cells migrating on wires (distinct experiments, $R \approx 50 \mu\text{m}$). (E) For small radii ($R < 5 \mu\text{m}$), there is room for one cell only around the wire. Cells then arrange in the form of chains of cells. Note that they still adhere together at their front end and back end. (B and E) MDCK wild-type cells; (C and D) MDCK LifeAct cells.

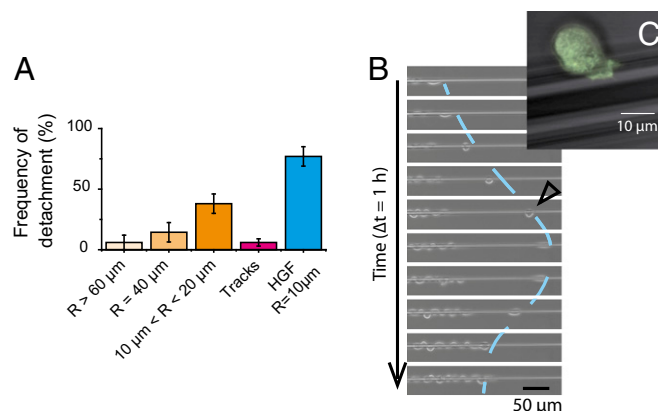


Fig. 2. (A) Frequencies of detachment of a cell at the front edge. The “frequency of detachment” is the proportion of experiments for which detachment was observed in 22 h. Track measurements were concatenated for $50 \mu\text{m} < w < 200 \mu\text{m}$. Measurements in presence of HGF were conducted on $10\text{-}\mu\text{m}$ wires. Error bars are SDs ($n > 17$ for each box). (B) Sequence illustrating the detachment of a cell at the front edge. Note the change of direction and the reintegration of this cell in the epithelium. $R = 5.3 \mu\text{m}$. The line is a guide for the eye. (C) Close-up of a detached cell in its fastest migration mode (similar to the cell marked with an empty triangle on B). Note the rounded shape. Superimposition of phase contrast and fluorescence images (LifeAct cells). $R = 10 \mu\text{m}$.

when they took a characteristic “rounded up” shape by which they minimized their contact area with the wire (Fig. 2*C*).

Experiments performed on the adhesive tracks showed that, at equivalent confinement, cell detachment was seldom observed in tracks of widths larger than $50 \mu\text{m}$ (equivalent radius $R = w/2\pi \approx 8 \mu\text{m}$) (Fig. 2*A*). In the few outlying cases where cells did break off on tracks, they remained close to the front or stretched along the edge of the track before being reincorporated into the monolayer. Therefore, we conclude that the unique phenotype of the detached cells seen on wires is due to curvature and not to confinement.

As long as the wire radius was larger than $5 \mu\text{m}$, several cells were needed to circle the wire. Above this radius, cells maintained the classical 2D arrangement observed on flat surfaces, and on the largest radii, migration fingers preceded by leader cells were present (26, 27). In contrast, single cells could wrap around thin wires ($R < 5 \mu\text{m}$) and formed chains in which adhesions were maintained between the back edge of a cell and the front edge of its follower (Fig. 1*E* and *Movie S2*).

Cell Architecture on Cylindrical Fibers. Since we observed a change of behavior of the cells as a function of the wire radius, we turned toward their polarity and their cytoskeleton organization. The cells were fixed and imaged after the front had progressed for 48 h. Staining the monolayer on the wire for ezrin, an apical marker (28), confirmed that the apical surface of the cells was facing the medium and their basal plane contacted the wire (see *SI Appendix, Fig. S3*). Thus, the tissue polarity seen on flat substrates was conserved on the curved surfaces.

The cell architecture was probed by labeling the actin cytoskeleton. Actin at the basal plane in contact with the wire was primarily in the form of fibers (see *SI Appendix, Fig. S4*). We found that the actin orientation across the tissue was random for large radii, as it is for flat substrates (Fig. 3*A*; see *SI Appendix, Fig. S5*). In contrast, the actin fibers were highly oriented perpendicular to the wire's longitudinal axis below $R \approx 40 \mu\text{m}$ (Fig. 3*B* and *C*; see *SI Appendix, Fig. S5*). It is worth noticing that the same transverse actin alignment was observed even for wires whose radius was small enough to be fully wrapped around by a single cell (Fig. 3*C*). The same organization of the actin fibers was also observed on uncoated glass wires (see *SI Appendix, Fig. S6A*) and with another epithelial cell line [retinal pigmented

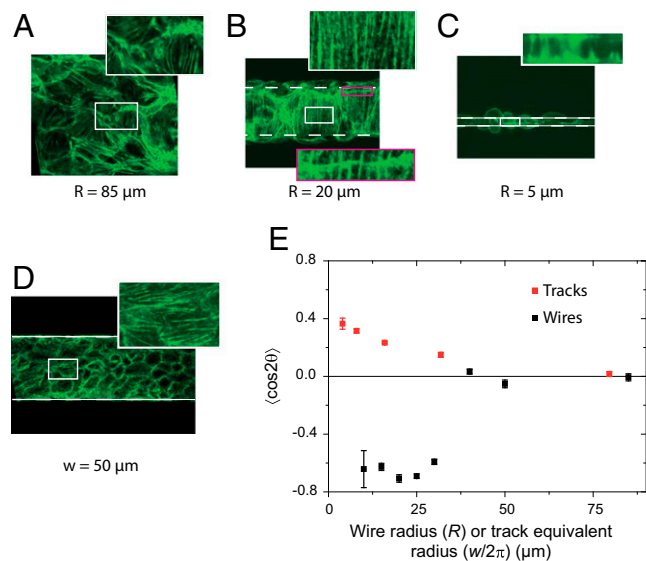


Fig. 3. (A–C) Orientation of the actin stress fibers for wires of various radii. On the 85- μm wire, the actin fibers are disordered. Below $R = 40 \mu\text{m}$, actin fibers take a circumferential orientation. Note the continuity of the actin fibers visible across a cell boundary (B, *Inset*), demonstrating the mechanical continuity in these pluricellular assemblies. C shows circumferential orientation even though the wire is wrapped up by only one cell. (D) The actin fibers on tracks of similar confinement are oriented along the tracks. (E) The order parameter $S = \langle \cos(2\theta) \rangle$, quantifies these two opposite trends (different signs of S) at small radii/widths. Note the common horizontal scale used to compare wires and tracks. On wires, the transition between not ordered ($R > 40 \mu\text{m}$) and ordered ($R < 40 \mu\text{m}$) is very abrupt. Error bars are SDs. (A, B, and D) Phalloidin-Alexa488 labeling. (C) LifeAct cells.

epithelial (RPE1) cells] but not with NIH 3T3 fibroblasts that tended to orient in the direction of the wires (see *SI Appendix, Fig. S6 B and C*). These results indicate that this organization is not caused by surface composition and could be general to epithelial tissues.

Very interestingly, we observed continuous fibers from cell to cell (Fig. 3B, *Inset*). This observation implies a mechanical relay at the cell–cell adhesion sites (29). It is a clear signature of local mechanical continuity.

The orientation of these actin fibers was further quantified by using the apolar order parameter $S = \langle \cos(2\theta) \rangle$, where θ is the angle between the actin fiber and the wire or track axis (Fig. 1A) (30, 31). $S = 0$ corresponds to a random orientation, $S = 1$ corresponds to a perfect alignment with the main axis, and $S = -1$ corresponds to a perfect orientation perpendicular to the main axis. Fig. 3E clearly shows the tendency of the fibers to orient themselves perpendicular to the wire below $R \approx 40 \mu\text{m}$.

Experiments performed on micropatterned tracks showed that the actin fibers tended to orient parallel to the tracks at small widths (24) (Fig. 3D). Of note, the order was more pronounced on wires at small confinement. Further, the order parameter measured in tracks decreased smoothly to zero with decreasing confinement (larger width), unlike the situation on wires that shows a sharp transition around $R = 40 \mu\text{m}$ (Fig. 3E). Note that in this plot, S is plotted against R or $w/2\pi$ to allow comparison between wires and tracks at equivalent confinement. Moreover, on tracks, actin cables on the boundary cells lined the limit of the adhesive track. These boundary effects were not present on the wires where cells were laterally confined but did not experience physical borders. We therefore conclude that the transverse actin orientation on wires is a consequence of curvature and not confinement.

We then characterized the adhesions of the cells on the wires. Fig. 4A shows the density of FAs on the wires for different radii. On wires of radius $R < 10 \mu\text{m}$, we measured an increase in the

density of FAs, as well as a decrease in their size compared with the flat control ($0.7 \pm 0.9 \mu\text{m}^2$ on flat substrates vs. $0.3 \pm 0.5 \mu\text{m}^2$ on wires of radius $10 \mu\text{m}$; $P = 3 \times 10^{-12}$). Furthermore, many FAs were found to be spaced along the same fibers (Fig. 4B). The situation on wires is therefore very different from flat surfaces where stress fibers are anchored to the surface via only two large, well-defined FAs at their extremities (see *SI Appendix, Fig. S7*).

Strikingly, local laser photoablation did not trigger a significant retraction of these fibers (retraction smaller than $0.5 \mu\text{m}$ for $R < 20 \mu\text{m}$). This observation is consistent with the high density of FAs and their localization along the fibers. It is in sharp contrast to the control experiments on flat surfaces where we routinely observed retractions of several microns, as expected (32). Furthermore, the leading edge presented a clearly defined circumferential pluricellular actin “cable” similar to the contractile cable present at the edge of a classical 2D closing wound (27, 33, 34) (see *SI Appendix, Fig. S8*). On the wires, the number of adhesions was much less along this cable than along the cells’ stress fibers and retraction after the cut was larger (up to several micrometers), which is a clear signature of the elastic tension stored in this cable (Movie S3 and Fig. 4C). Upon ablation, the initial retraction speed was significantly smaller on small radii wires compared with flat surfaces ($0.2 \pm 0.1 \mu\text{m}\cdot\text{s}^{-1}$ vs. $0.4 \pm 0.2 \mu\text{m}\cdot\text{s}^{-1}$, $P = 10^{-5}$), this last value being consistent with previous measurements (27, 33) (Fig. 4D).

Collective Migration Speed Is Controlled by Confinement. We then turned our attention to the impact of the wire radius on the speed of collective migration, as cell dynamics is related to cytoskeletal organization and adhesion (35). Cells on wires with a radius below $R = 20 \mu\text{m}$ (corresponding to a confinement $2\pi R = 126 \mu\text{m}$) migrated collectively faster than cells on thicker wires, which had velocities comparable to those on a flat surface (Fig. 5A, black points).

The cell fronts on wires above $R = 10 \mu\text{m}$ (confinement larger than $63 \mu\text{m}$) migrated with a constant speed, as evident from the kymographs (see *SI Appendix, Fig. S9A*). For smaller radii, the average progression was faster (up to $35 \pm 3 \mu\text{m}/\text{h}$), but it was also less regular (see *SI Appendix, Fig. S9B*), because of cell divisions that interfered with the forward motion on the thin wires. This effect, also present on small-width tracks (24), became noticeable when the number of cells around the wire was small.

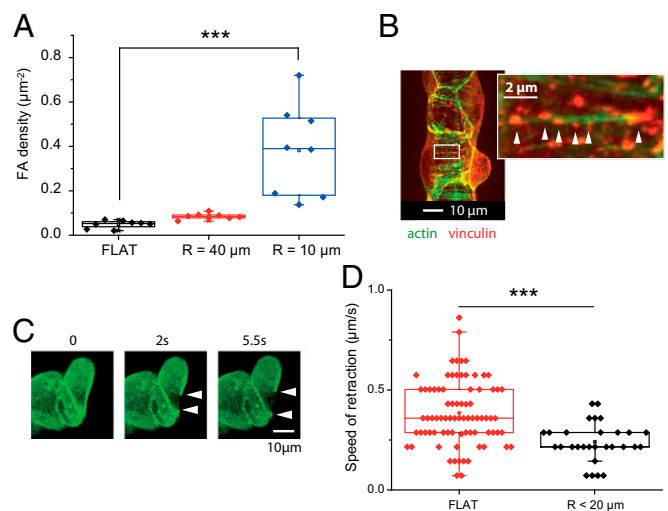


Fig. 4. (A) Influence of the radius of curvature on the density of FAs. FAs are more numerous on thin wires. (B) At a more local scale, the FAs decorate the circumferential actin fibers; vinculin immunofluorescence and phalloidin-Alexa488 staining. (C) Succession of images of the front-edge actomyosin cable after laser ablation ($R = 10 \mu\text{m}$); MDCK-LifeAct cells. (D) Initial retraction speeds of the actin cable after photoablation.

Laser Photoablation. Photoablation experiments were performed on an LSM 710 NLO (Zeiss) microscope equipped with a two-photon MaiTai laser and a 63 \times water objective. The two-photon laser was used at 85% power and at a wavelength of 890 nm. Retraction speed of the fibers was measured over the first 3.8 s after ablation.

Image Analysis. Images were processed with the ImageJ software (53) or with Matlab (MathWorks) routines. Further analysis was occasionally performed on Origin (OriginLab). In particular, local orientation analysis was performed in Matlab by a Fourier Transform technique (54). The order parameter, S , was computed by averaging $\cos 2\theta$ over the images (around 120 subwindows for the smallest radii and up to 10,000 for the largest ones) and wires (at least four) for the same radius.

Statistical Analysis. Unless otherwise specified, error bars in the figures represent the SDs. Two-sample t test was used to compare distributions. The number of replicates was always larger than eight, over at least four independent experiments.

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