Mechanics of epithelial layers subjected to controlled pressure

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A thesis submitted for the degree of

Doctor of Philosophy
PhD Program in Applied Mathematics

Barcelona, April 2023

"You cannot carry out fundamental change without a certain amount of madness. In this case, it comes from nonconformity, the courage to turn your back on the old formulas, the courage to invent the future. It took the madmen of yesterday for us to be able to act with extreme clarity today. I want to be one of those madmen. We must dare to **invent the future**."
- Thomas Sankara

Acknowledgements

I would like to thank all the working people of the world

Abstract

Epithelial sheets are active viscoelastic materials that form specialized 3D structures suited to their physiological roles, such as branched alveoli in the lungs, tubes in the kidney, and villi in the intestine. The shape of these structures depends on active stresses generated by the actomyosin cytoskeleton, active viscoelastic properties of the epithelium, and hydraulics of the luminal fluid. How these active stress and material properties are linked to give rise to epithelial shape remains largely unknown. Here we developed a new experimental and computational approach to probe active epithelial viscoelasticity and then harness the resulting constitutive relation to sculpt epithelia of controlled 3D shape. We developed a microfluidic setup to engineer 3D epithelial tissues with controlled shape and pressure. In this setup, an epithelial monolayer is grown on a porous surface with circular low adhesion zones (footprint). On applying hydrostatic pressure, the monolayer delaminates into a spherical cap (dome) from the circular footprint. Through this approach, we subject MDCK epithelial cells to a range of lumen pressures at different rates and hence probe the relation between strain and tension in different regimes. Slow pressure changes relative to the timescales of actin dynamics allow the tissue to accommodate large strain variations. However, under sudden pressure reductions, the tissue develops buckling patterns and folds with different degrees of symmetry-breaking to store excess tissue area. This behavior is well captured by a 3D computational model that incorporates the turnover, viscoelasticity and contractility of the actomyosin cortex. Informed by this model, we harness the active behavior of the cell cortex to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach for engineering epithelial morphogenetic events.

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Part I

Introduction

The center focus of this thesis is epithelial monolayers. From the materialist perspective, these monolayers are endlessly fascinating. They are shape changing, self healing, continuously deform or jam depending on the requirement. They are the simplest system conceptually to understand the wider world of morphogenesis. This introduction is a primer to all the topics relevant to the thesis. First I will give a brief introduction to the epithelial tissue itself and its key components along side a snippet of its role in disease and development. Then I will give a summary of morphogenesis and how we can think of epithelia as an active material and what are the historical ways of modelling it. Finally, I will conclude with the emerging field of bottom up morphogenesis, where researchers are reconstructing the biological systems from scratch.

Chapter 1

Epithelial tissue

Epithelial tissues are cell sheets with strong intercellular bonds that form physical barriers for major organs such as the lungs, skin, and intestine. It protects the organs from external physical, chemical, and microbial onslaughts. Besides protection, the main functions of epithelial cells include secretion, selective absorption, transcellular transport, and detection of sensation (Powell, D.W., 1981). It also plays a key role in developmental stages by supporting growth and driving critical shape changes.

Epithelial cells are polarized, i.e., their apical side, faces the lumen of the organ, differs in shape and composition from the basolateral side. Its polar organization is reflected in the vectoral functions like transporting epithelia such as those of the renal tubule, absorptive epithelia of the intestine, and secretory epithelial cells like hepatocytes, which are typical examples of epithelia that create and maintain concentration gradients between the separated compartments (Simons, K. and Fuller, S.D., 1985). In addition, polarized epithelia guide the developmental process by determining the fate of cells (Kim, E.J.Y., Korotkevich, E. and Hiiragi, T., 2018).

Epithelial cells have different shapes and may be arranged in single or multiple layers. They are usually classified according to two features: the number of cell layers and the shape of the cells. Simple epithelia are single-cell layers where all the cells contact the underlying basal lamina and have an apical free surface. The shape of the cells can be flat (wider than high), cuboidal (as wide as high), or columnar (higher than wide). However, stratified epithelium contains two or more layers of cells.

Figure: Art work of quilling and then showing different epithelial types then showing apical basal polarity of epithelial and monolayer.

Epithelial function primarily depends on the tissue's structure and microenvironment. In essence, it can be described completely in three parts: first, cell structure; second, cell-substrate connection; and lastly, microenvironment.

In general, cell structure helps cells maintain their shape along with providing mechanical support to perform vital functions like division and migration. This structure is known as the cell cytoskeleton. It includes different components, playing various roles together. Eukaryotic cells are constructed out of filamentous proteins to support the cell and its cytoplasmic constituents. There are three major filaments, which differ in size and protein content. Microtubules are the largest type of filament of the protein tubulin, with a diameter of about 25 nm. Actin filaments are the smallest type, with a diameter of only about 6 nm. Finally, intermediate filaments are medium-sized, with a diameter of about 10 nm. Unlike actin filaments and microtubules, intermediate filaments are constructed from several different subunit proteins. These filaments dynamically alter themselves in reaction to signals from microenvironments and cell networks (Alberts, B., et al., 2013; Fletcher, D.A., and Mullins, R.D., 2010s). Mechanically, actin filaments are stiffer than microtubules in extension, but they rupture at lower extension. It is also reported that the intermediate filaments exhibit an intermediate extensional stiffness at lower extensions, but that the intermediate filament can sustain much larger extensions than the other two types of filaments while exhibiting a nonlinear stiffening response (Janmey et al., 1991; Mofrad, M.R., 2009).

In the case of epithelial layers, the actin cytoskeleton and intercellular junctions make cell-cell contacts stronger and provide integrity (Braga V. 2016). The perfect example of these tissue-level structures can be seen in wound healing assays: cells surrounding the wound create a ring of actin to close it (Brugués, A., et al., 2014). One must keep in mind that these structures tend to self-organize as well. It can be seen when cells are confined in a specific shape, like in the case of circular islands of epithelial cells that showed radial patterns in the actin organization (Jalal S., et al., 2019).

Multiple membrane molecules can mediate adhesion between cells. One of these are cadherins, critical for epithelial cell cohesion through the formation of adherens junctions. In these junctions, cadherins are coupled to the cell cytoskeleton enabling force transmission between cells. It is finely regulated by both internal and external mechanisms. Desmosomes are another type of intercellular junction. They are coupled with intermediate filaments, and the resulting supracellular network confers mechanical resilience on cell layers (Hatzfeld, M., Keil, R., & Magin,

T. M. 2017; Latorre, E., et al., 2018). Tight junctions (TJ) perform a barrier function and enable the transport of ions across epithelial layers to be actively regulated. This plays an important role in the control of fluid pressure in tissues. Together, adherens junctions, desmosomes, and tight junctions are the major mediators of epithelial cell–cell adhesion, and their regulation enables emergent behaviors in cell sheets that are not observed in single-cell systems (Trepat, X., and Sahai, E., 2018; Ladoux, B., and Mège, R.M., 2017).

Figure: all the cytoskeleton and cell junctions and their mechanics

Extracellular matrix (ECM) is the cell environment or substrate to which cells adhere; it is also known as the matrix or cellular microenvironment. ECM serves many functions: it endows a tissue with strength and thereby maintains its shape; it serves as a biologically active scaffolding on which cells can migrate or adhere; it helps to regulate the phenotype of the cells; it serves as an anchor for many substances, including growth factors, proteases, and inhibitors of such; and finally, it provides an aqueous environment for the diffusion of nutrients, ions, hormones, and metabolites between the cell and the capillary network. On top of that, it is subjected to mechanical forces such as blood flow in endothelia, air flow in respiratory epithelia, or hydrostatic pressure in the mammary gland and bladder (Roca-Cusachs, P., Conte, V., & Trepat, X. 2017; Humphrey, J. D., et al., 2015; Waters, C. M., Roan, E., & Navajas, D., 2012; Paszek, M. J., & Weaver, V. M., 2004; Bross, S., et al., 2003). It is shown that the ECM regulates cell shape, orientation, movement, and overall function in response to these forces.

Cells and ECM have a symbiotic relationship with each other from signaling cues to various sensors on the cell surface. These cues are primarily sensed using integrins and focal adhesion complexes in cell-substrate adhesion (Kechagia, J.Z., Ivaska, J. and Roca-Cusachs, P., 2019). Thus, triggering complex molecular processes that are required to maintain homeostasis and strongly affect processes in development or tumorigenesis (DuFort, C. C., Paszek, M. J., & Weaver, V. M. 2011; Northey, J. J., Przybyla, L., & Weaver, V. M. 2017).

ECM is a fibrous network of proteins; from a mechanical perspective, the three primary structural constituents of the ECM are typically collagen (the most abundant protein in the body), elastin (the most elastic and chemically stable protein), and proteoglycans (which often sequester significant water as well as growth factors, proteases, etc.). Due to its water content, the deformation of ECM can produce cracks in epithelial layers. ECM acts as a poroelastic material, soaking up water

upon stretching (like a sponge) and releasing it under compression, causing a hydraulic fracture effect (Casares, L., et al., 2015). Moreover, collagen remodels itself under the influence of cells aiding in migration or under stress (Shields, M. A., et al., 2012; Humphrey, J. D., 2003). Like most cytoskeletal proteins, most extracellular components turnover continuously, albeit some very slowly. For example, collagen in the peridontal ligament appears to have a half-life of a few days, whereas that in the vasculature may have a normal half-life of several months. In response to altered loads, disease, or injury, however, the rates of synthesis and degradation of collagen can increase many folds to have a rapid response (Humphrey J. D., 2003).

Epithelial integrity and homeostasis are of central importance to survival, and mechanisms have evolved to ensure these processes are maintained during growth and in response to damage (Macara, I.G., et al., 2014). For example, epithelial cells have one of the fastest turnover rates in the body. The entire gut cell lining turns over in 3–4 days. This turnover implies constant cell division and death. The excessive rate of division and death may give rise to tumors. It is known that 90% of cancers emerge in simple epithelia (Torras, N. et al. 2018; Eisenhoffer, G.T., and Rosenblatt, J., 2013). Not only this, but it could easily disrupt the barrier function, as no gaps should emerge around dying or dividing cells. There is a range of distinct disease states that all have the effect of compromising epithelial and/or endothelial barrier function.

If the fluid compartmentalization goes awry, it has profound implications for epithelial and stromal homeostasis, fluid and/or electrolyte balance, the generation of inflammatory states, and even the tumor microenvironment. Several bacterial toxins are known to target junctions and cause changes in the TJ protein ZO1, resulting in compromised barrier function and pathologies such as diarrhea and colitis (Fasano, A. et al., 1991). Cancer cells tend to spread and disperse metastatically by using their very high rate of cell motility and a diminished sense of cell adhesion. This elimination and/or reduction of TJ barriers in cancer is essential to allowing metastatic cells to break into and out of blood vessels. The leaky barrier also allows a growing epithelial tumor to access luminal fluids as an additional source of nutrients (Mullin, J.M., et al., 2005).

Epithelia undergo changes in shape with deformation and reorganization from the embryonic to the adult stage. Unsurprisingly, any improper function would lead to damage and disorder. Like in the case of epithelial–mesenchymal transition (EMT), a developmental process when epithelial cells gradually transform into

mesenchymal-like cells by losing their epithelial functionality. EMT is involved in the pathogenesis of numerous lung diseases ranging from developmental disorders to fibrotic tissue remodeling to lung cancer. Another example is bronchopulmonary dysplasia (BPD), a chronic lung disease that occurs in very premature infants and is characterized by impaired alveologenesis and vascular development. BPD develops because of injury or infection in a very immature lung (Bartis, D. et al., 2014). Give better and more prominent examples then to conclude the chapter with telling what follows.

Chapter 2

Morphogenesis

During embryonic development, epithelia forms transient structures, such as the neural tube, somites, and the precardiac epithelium, that serve as progenitors for the development of more complex organs. Different epithelia acquire diverse morphological forms and performs their specific functions, such as the thyroid follicles, the kidney tubules, the interconnected bile canaliculi and sinusoids in the liver, and the complex branching structures found in the lung and salivary glands (Gumbiner, B.M., 1992). Owing to its multifaceted regulation and hierarchical organization, epithelial morphogenesis is a complex phenomenon dependent on factors at multi spatial-temporal scales.

It can be fast at cellular level like the change in cell shape driven by apical constrictions, which is required for epithelial remodeling during tube formation of ventral furrow cells in Drosophila gastrulation (Miller CJ, Davidson LA. 2013). Or it could be a slower self-organization at embryo level like a cluster of dissociated mouse embryonic stem cells (ESCs) cultured in vitro spontaneously form an optic cup, exhibiting all layers of the neural retina, when cultured in appropriate medium (Eiraku et al., 2011; Bedzhov, I. & Zernicka-Goetz, M. 2014). This structure underwent similar changes to the in vivo tissue like invaginating to form the characteristic morphology of the optical cup without external scaffolding or original growth environment.

> Figure: slow vs fast process; programmed vs self-organizing structure; function and form logic!

At the end of the day, all cells come from cells ('omnis cellula e cellula') (Virchow R.L.K., 1858), all tissues come from cells that contain essentially the same genetic information. Nonetheless, every tissue exhibits a distinct architecture and

function. One could ask many questions from here, as how form-function work in synchrony? How organization is triggered physically? Reductionists would ask whether function follows form, or it is other way around.

As per the twentieth century architecture principle of "Form Follows Function"; where the organization of a structure should be based upon its intended function. In developmental biology there are many examples of indicating that the same principle is at work in self-assembling systems like intestinal organoids, cancerous spheroids, and functional kidney tissues (Gjorevski, N, et al. 2016; Ishiguro, T, et al. 2017; Morizane, R. and Bonventre, J.V., 2017). Each emerging out of a set of cells in appropriate environment changing and adapting itself to perform the biological function. However, exactly the opposite design principle is at work in numerous in vitro experiments with controlled cellular environment; illustrating geometric constraints drives biological function. For instance, in a micropatterned collagen scaffold (with structures of intestine) a human small intestinal epithelium was generated that replicates key features of the in vivo small intestine: a crypt-villus architecture with appropriate cell-lineage compartmentalization and an accessible luminal surface (Wang, Y et al., 2017). Or cell reprogramming like in case of fibroblasts turning into induced neurons when supported by specific substrate topography (Kulangara et al. 2014).

> Figure: images from D'arcy thompson book and connecting it to the new work of forces.

One could easily reach a conclusion that there are more things involved in understanding dialectics of form and function. This was a subject of D'Arcy Wentworth Thompson's classical text "On Growth and Form" (Thompson, 1917). Thompson tries to explore biological forms during development and across evolution with considering geometric and physical constraints. (*Here talk more about specific examples from the book*) After more than 100 years of its publishing we can answer more specific questions about shape and function using advances in bioengineering and microscopy.

> Structure without function is a corpse, function without structure is a ghost (Wainwright, S.A., 1988.)

It is quite apparent after reading till here that there is a specter is haunting this subject—the specter of force. In last couple of decades, there has been a resurgence

of interest in physical forces as regulator of development, homeostasis, and disease (Ingber, D. 2005; Barnes, J.M. et al., 2017). This has led researchers across the disciplines to examine the physical mechanisms of tissue formation and its regulation. Unravelling mechanism of Thompson's mysteriously generated 'Diagram of the forces' which governs biological processes (Thompson, 1917; Heer, N.C. and Martin, A.C., 2017).

Chapter 3

Active tissue mechanics

Chapter 4

Bottom-up biology

Part II

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Chapter 5

Novel microfluidic system for generating 3D epithelia with controlled pressure and shape

Chapter 6

Epithelial stretching is driven by viscoelasticity of actomyosin cortex

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Part III

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Chapter 8

Conclusions and Discussion

8.1 Developed a novel device to create 3D pressure controlled epithelia

8.2 Actin cortex drives epithelial tissue stretching

8.3 Rapid deflation leads to buckling

8.4 Controlling 3D patterns of epithelial sheets

Appendices

Appendix A

Methods

A.1 Creating pressurized epithelia

A.1.1 Fabrication of microfluidic devices.

Polydimethylsiloxane (PDMS) gels (Sylgard PDMS kit, Dow Corning) were used to make the microfluidic devices. PDMS was synthesized by mixing the curing agent and elastomer in 1:9 weight ratio. This mixture was centrifuged for 2 min at 900 rpm to remove air bubbles. The unpolymerized PDMS was poured into a mold or spun to obtain the desired shape. There are four parts to the device (fig S1X showing device scheme). First is the top block, a thick PDMS block with four inlets and one channel for the application of hydraulic pressure. The second is a 200 um thin PDMS layer with a 1.2 mm diameter hole in the center with a 400 nm porous membrane (Polycarbonate filtration membrane 0.4 um, Whatman membranes) attached to it. The third is another 200 um thin PDMS layer with a channel for seeding the cells. Lastly, all these PDMS parts are attached to, the fourth part, a glass-bottomed 35 mm dish (35 mm, no. 0 coverslip thickness, Cellvis). The top block was made using replica molding in a 3D printed mold. This mold was 3D printed with vat polymerization and a digital light processing 3D printer (Solus DLP 3D Printer with SolusProto resin). The mold's surface was then silanized using Trichlorosilane (Trichloro(1H,1H,2H,2H-perfluorooctyl) silane, Merck) for preventing adhesion with unpolymerized PDMS. PDMS was poured into the mold and degassed for one hour. PDMS is cured with a hot plate at 100 C for 30 min. Once cured, PDMS is removed, cut into devices, and punched with 1.5 mm. 200 um thin PDMS layers were made by spin coating 4.5 ml unpolymerized PDMS on a 15 cm dish at 500 rpm for 1 min. These dishes were incubated in an oven at 80 C to polymerize for 12 hr. These thin sheets were cut into the parts of devices using a Silhouette cutting machine (Silhouette Cameo 4, Silhouette America). The sheets were attached to a Silhouette cutting mat and then Silhouette software was fed with the pattern of the device layers. A sharp cutting tool in the machine cut the PDMS along the pattern. These cut PDMS were peeled off with help of 70% ethanol. These devices are assembled with the aid of ozone plasma cleaner (PCD-002-CE, Harrick Plasma). Glass bottomed dishes and thin PDMS layers with cell channels were treated for 1 min under plasma. Then bonded together by placing the layers in contact for 2 hr at 80 C. Similarly, the top block and thin membrane with porous membrane were also bonded. These layers were later bonded together again using plasma cleaner.

A.1.2 Patterning protein on the device

The devices were filled with 96% ethanol for removing air bubbles. Then, devices are treated with 5% v/v (3-aminopropyl) triethoxysilane (Merck) diluted in 96% ethanol for 3 min and rinse three times with 96% ethanol. Later the devices were filled with MilliQ water to remove ethanol traces. PRIMO (Alveole Lab) was used to pattern adhesion-promoting protein. For this setup, devices were incubated with PLL (Poly-L-lysine solution, Merck) for 1 hr, subsequently with SVA PEG (50mg/ml in 8.24 pH HEPES) for 30 min, and rinsed with HEPES. Before using PRIMO, devices were filled with a photoinitiator. Desired protein pattern was loaded into the PRIMO software (Leonardo, Alveole Lab). PRIMO uses a microscope to shine the laser in the specific region according to the loaded pattern to cut PEG chains. Samples were rinsed with phosphate-buffered saline (PBS, Merck). Then the samples were filled with fibronectin and fibrinogen (100 ug/ml Fibronectin in 2% Far-red fibrinogen solution in 1X PBS) solution for 5 min. Then samples were rinsed again with 1X PBS. Fibrinogen labels the fibronectin with Far-red signal to image the coated protein pattern.

A.1.3 Cell culture in the device

To image cell shape and tissue structure Madin-Darby Canine Kidney (MDCK) cells expressing CIBN-GFP-CAAX were used for the experiments. CIBN-GFP-CAAX labels plasma membrane. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco Thermofisher) with 10% v/v fetal bovine serum (FBS, Gibco, Thermofisher), L-glutamine (Thermofisher), 100 ug/ml streptomycin and penicillin. Cells were incubated at 37 C with a 5% CO2 condition. Before seeding cells in the device, it is filled with a cell culture medium. Cells are trypsinized and diluted at a concentration of 25x106 cells/ml. The cell channel of the device is filled with 30 ul of cell solution and incubated for cell adhesion. After one hour of incubation, devices are rinsed with media to remove unattached cells. Devices were kept 24 hr in the incubation for the growth of a monolayer before the experiment.

A.1.4 Application and measurement of the pressure

The pressure is applied via hydrostatic forces similar to the previous studies [Choudhury2022 Piotrowski2017 Palmer2021]. The two channels in the chip were separated by the porous membrane. Cells are on the bottom side of the membrane. The

pressure in the channel (top side of the membrane) is used to inflate the structures on the top. This channel has one inlet and one outlet for removing bubbles. The inlet is connected to a 35 ml reservoir of cell culture medium (in a 50 ml falcon tube) by tubing (PTFE Tubing 1/16" OD for Microfluidics, Darwin microfluidics) and the outlet is connected to a shutoff valve (Microfluidic Sample Injection / Shut-off Valve, Darwin microfluidics). Once bubbles are removed, closing the valve would apply the pressure on the basal side of the cells according to the difference between the height of the fluid level. All tubings are connected to the chip with a steel insert (Stainless steel 90° Bent PDMS Couplers, Darwin microfluidics). We are able to find zero by matching the height of the device to the liquid and air interface in the reservoir. This is confirmed with the experiments, where on applying pressure domes form but on reduction in pressure to zero domes deflate.

A.1.5 Confocal Microscopy

For timelapse imaging of domes at a larger time interval ($>$ 1 min), an inverted Nikon microscope with a spinning disk confocal unit (CSU-W1, Yokogawa) was used with Nikon 40x, 20x, and 10x air lenses. For shorter time intervals ($<$ 10 s), a Zeiss LSM880 inverted confocal microscope was used with laser scanning mode. Fast imaging was enabled by imaging a single line in the middle of the dome.

A.1.6 Fabrication method for the Light-Sheet device

The devices used with the light-sheet microscope consisted of a single PDMS block bonded to a glass microscope slide (76x26 mm, RS Components BPB016). The blocks were made using a 3D printed mold (Ultimaker 3 with Ultimaker PLA Printer Filament 1616). PDMS was mixed, centrifuged, degassed, and cured as described above for the normal devices. Once cured, the PDMS was removed, cut into individual devices and punched with a 1.5mm biopsy punch. The PDMS blocks were then attached glass slides using a thin layer of unpolymerized PDMS, that was coated onto the glass slides using a spatula. The devices were then kept on a hotplate at 100C for 30mins to allow the PDMS bonding to fully cure. The 400nm porous membranes were then attached to the devices. The edges of the membrane were carefully dipped into unpolymerized PDMS, before being placed flat on the top of the device. Particular care was taken to ensure the centre of the membrane over the punched pressure-application hole remained free of PDMS.

The devices were then kept at 65C for 1 hour to allow the PDMS bonding to fully cure.

A.1.7 Device protein patterning and cell culture in Light-Sheet device

The light-sheet devices were protein patterned and cell cultured using the same methods and steps as outlined above for the normal devices, with the one minor addition of the use of a simple PDMS and glass cap for a few critical steps. The porous membrane for pressure application, and thus the site of protein patterning and cell seeding, for the light-sheet devices is exposed and on the top side of the devices. This mostly allowed for easy application of reagents as a droplet could be applied and aspirated directly, however for the more sensitive steps in the procedure, a simple PDMS and glass device was used to create a temporary covered channel over the porous membrane to regulate the procedure and ensure the treatment of the devices was highly standardised. Specifically, the cap was used for the application of photoinhibitor during PRIMO, and for the application of cell solution during cell attachment. The caps were fabricated using 2cm x 2cm squares of a 400μm thick PDMS layer, with a keyhole shape cut in from the side. Each PDMS piece was then stuck to a 18mm diameter coverslip (18mm, no.1 Cover glasses circular, Marienfeld 0111580) using the innate attraction between the surfaces.

A.1.8 Application and measurement of pressure

The experimental apparatus and measurements for the light-sheet devices were the same as the normal devices as outlined above. Light-sheet microscopy The imaging of the light-sheet devices was done with a dual-illumination inverted Selective Plane Illumination Microscope (diSPIM) (QuVi SPIM, Luxendo, Brucker) with Nikon 40x immersion lenses (Nikon CFI Apo 40x W 0.8 NA NIR water immersion objective). For the buckling experiments, only single objective illumination and detection was used.

A.2 Quantitative analysis of the domes

A.2.1 Quantification of the dome areal strain and tension

As mentioned earlier, the domes were imaged in 3D with confocal microscopy. We used ImageJ to manually section the dome in the middle in the YZ plane, XZ

plane is a plane parallel to the monolayer, with Reslice function along the Z axis. This section was used to calculate the height, radius of curvature, and base radius. Strain was calculated as [Strain = height2/base2] and tension as [Sigma = 0.5PR]. The raw data was extracted in ImageJ and then MATLAB was used to compute and plot the strain and tension.

A.2.2 Analysis of the kymographs

For cyclic pressure or buckling experiments, the domes were imaged at low resolution and high noise levels to capture fast dynamics. The previous method of manually quantifying each time point is not feasible. Thus, we used the ImageJ function of the Reslice function along the time axis. We resliced it along the Y-time axis in the middle of the dome, such that we get a kymograph of height as a function of time. Also, we performed the reslicing along the XT axis at the plane of the monolayer, such that we get the kymograph of the base radius with respect to time. These kymographs were in form of images save manually with ImageJ. A custom-built MATLAB code was used to digitize the kymographs, where maximum intensity along each time was considered as the current dome height position. The first 30 s of the experiment pressure is zero, so the unstretched monolayer position is determined fom those time points. Dome height is calculated with the difference between the current position and the initial position. Base radius is calculated similarly by subtracting two sdes. The radius of curvature is calculated using the relation between the base and height of the dome. [R = h2+a2/ 2h]

A.2.3 Qualitative analysis of the buckling event

Whether domes are buckling or not was determined manually checking every frame during the deflation. If dome maintains the smooth circular geometry in XZ plane during the deflation, we mark the dome as “not buckling”. However, if the dome has a visual discontinuity in the curvature or a kink it is then considered to be “buckling”. Imaging the fast events in XY plane was done in an ad hoc manner. To capture the folds, the dome as imaged closer to the apical surface of the monolayer. The type of fold was determined by carefully observing the way which monolayer makes contact with the imaging plane. If there is one point of contact in the centre and spreads outwards, it is considered as accumulation along the periphery. In case where there are multiple points of contact and they all join in the middle, it is considered as a network of folds.

Appendix B

Detailed protocols

Appendix C

Codes

Appendix D

Funding

Bibliography