Prelights application 2022

Applicant: Yohalie Kalukula (Yohalie.kalukula@umons.ac.be)

Preprint:

Mechanical stress driven by rigidity sensing governs epithelial stability

Surabhi Sonam[1] et al.

Correspondings : Jean-François Rupprecht[2] and Benoît Ladoux[1]

1. Université de Paris, CNRS, Institut Jacques Monod, F-75006 Paris, France
2. Aix Marseille Univ, Université de Toulon, CNRS, CPT, Turing Center for Living Systems, Marseille, France

Short engaging and 'tweetable' summary of the preprint:
Wonder how rigidity sensing contributes to mechanical failure in epithelial tissues? Check this out.
@ JFRupprecht_OM and @BLadoux.

Prelight (Max 1000 words without figure legend):

Background of the preprint:

Epithelia act as permeable protective barriers lining organs and covering most surfaces of the human body[1]. The mechanical feedback between a tissue and its environment, coordinating tissue scale stresses (local compression, tensile stress) and external mechanical stresses (stiffness) contribute to epithelial integrity, and it is becoming increasingly clear that substrate stiffness plays an important role in regulating collective migration and permeability of epithelial cells[1,2]. However, the mechanism underlying rigidity sensing by epithelia in different environments and how this process contributes to mechanical failure and impaired permeability remains unclear.

In this preprint, Sonam et al. identified the need for fundamental understanding of these processes. They used micropatterned epithelial tissues to investigate the influence of substrate rigidity on hole formation through active nematic modelling.

Key findings of the preprint:

To investigate the role of the matrix stiffness on the maintenance of epithelia integrity, the authors cultured renal and colorectal adenocarcinoma epithelial cells on polyacrylamide (PAAm) hydrogels of distinct stiffnesses ranging from 2.3 (soft) to 55 (stiff) kPa. PAAm hydrogels were functionalized with fibronectin circular patterns to standardize the size and density of microtissues of epithelial cells (fig. 1a).

The authors first observed the formation of holes in epithelial monolayers that impair the monolayer integrity and demonstrated that the number of holes is regulated by the matrix rigidity (fig. 1b). The frequency of holes monotonically decreased with the gel stiffening. Considering that stiffness mechanosensing relies on the crosstalk between cell-cell and cell-substrate adhesions, the authors studied both cellular interfaces to explain their observations.

Spontaneous hole formation is reminiscent of an active dewetting process* due to low interactions at the cell-substrate interface. Indeed, previous observations showed that cells exert larger contractile stress on stiff substrates compared to soft substrates[3]. By modulating the fibronectin density to interrogate the role of substrate adhesion on hole formation, the authors confirmed that stiffness sensing is the major contributory factor for mechanical failure.

*Dewetting is opposite of the spreading of liquid on a substrate. It occurs when a liquid film retracts from a solid surface to form a bead-shaped drop. The liquid tends to minimize its surface tension by minimizing interactions with the surface.

To gain further insights on the mechanism of hole formation, the authors focused their attention on cell-cell interfaces, where friction originating from cell-cell adhesive interactions resists cell motion. However, how these forces are influenced by substrate stiffness is unclear. Interestingly, the authors observed a redistribution of vinculin from focal adhesions on stiff substrates to cellular junctions on soft substrates. When focal adhesions fail to form due to a lack of built-up basal tension, vinculin shuttles to cell-cell junctions, leading to a build-up of junctional tension.

Sonam et al. used a cell-based vertex model to simulate the balance of forces in the epithelial sheet. The vertex model considers the cell sheet as a polygonal network in which cells are connected individual polygons. In this framework, the dynamic organization of the cell sheet, in terms of changes in cell shape and position over time, is dictated by the motion of the vertices. Each vertex moves in response to mechanical forces originating from growth, interfacial tension, and pressure within each cell. Using a simple viscoelastic model, the authors describe how these forces are balanced to dictate vertices dynamic. They state that the total stress load is shared between a cell-cell elastic modulus (spring) and the viscous drag on the substrate (damper). Interestingly, the viscous drag translates into a frictional force at the cell-substrate interface which is known to increase with substrate stiffness[4]. Keeping that in mind, lower substrate stiffness leads to stress loads balanced between a weaker damper and cell-cell spring. When the substrate fails to provide sufficient mechanical feedback, this causes mechanical failure at cell-cell junctions

Further supporting their model, they also revealed that stiffness sensing contributes to hole formation via a switch from a tensile state on soft substrates to compressive state on stiff substrates. This supports the wetting transition observed when switching from a soft to a stiff substrate.

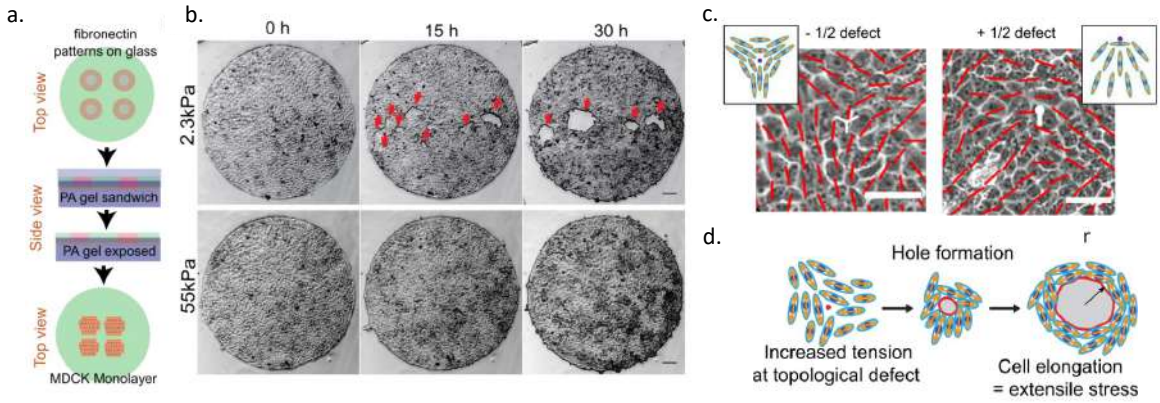

Figure 1- a. Schematic of the process of fibronectin patterning on PA gels; top: fibronectin coating on coverslips patterned through deep UV; middle: PA gel is placed between patterned and silanized coverslips; bottom: MDCK cells are seeded on patterned PA gel. **b.** Time based montage of MDCK cells forming holes on 2.3kPa PA gels (top panel) and intact monolayers on 55kPa gels (bottom panel). Scale bare: 100 µm. **c.** Orientation field overlaid over phase contrast images obtained from experiments on soft (2.3kPa) substrates representing -1/2 defect (left column) and +1/2 defect (right column). Sb: 50µm. **e.** Schematic representing the process of hole opening. Adapted from Sonam *et al.*

Finally, an originality of their approach is to consider the contribution of spatial organization and orientational alignment in the order within a cell sheet. To this aim, the authors used an elegant active nematic model - making the analogy with particle organization in liquid crystal - to understand the origin of the increase in local stress prior to hole formation. They identified the presence of active topological defects (fig. 1c) on both soft and stiff substrates, suggesting a strong correlation between the persistence of tensile regions and the lifetime of defects on soft substrates. Upon correlating the location of hole formation with the defect location, they found that holes form at specific locations around topological defects on soft substrates (fig 1 c and d).

Context of the study

Epithelia are very cohesive collective systems and understanding the mechanisms that lead to their mechanical failure is crucial. To address this exciting open question, Sonam et al. combined an original experimental approach with a cell-based vertex model[5,6] to show that topological defects could sustain the large amounts of stresses required to trigger hole opening when cell-substrate interactions are weak. Their elegant approach exploits a timely hot topic, the modelling of epithelia monolayer as nematic liquid crystal, and brings important insights into the understanding of the mechanical integrity of epithelial monolayers.

Future directions:

For future experiments, it would be interesting to test the influence of other fundamental physico-chemical properties of the matrix. For instance, the density of ligands, the matrix porosity, or the loss modulus (viscous part) are important parameters influencing tissus that could be taken into account.

Questions for the authors:

- Is there any other physico-chemical properties of the synthetic matrix that should be taken into account instead of the rigidity such as the density of fibronectin, the porosity, or the loss modulus (viscous part) ? Did you confirm your observations by performing similar experiments on natural matrices (i.e. collagen) of similar rigidities?

- Topological defects are related to nematic organization, as observed in liquid crystals. You used MDCK and colorectal adenocarcinoma epithelial cells that display such features. Could you generalize your findings to any other epithelial cell types ? Do you already observe such topological defects in vivo?

- The formation of holes in thin monolayer is a beautiful analogy to the dewetting process in polymer thin films which is mainly driven by capillary forces and often triggered at a topological defect. Could you comment this analogy and give us some insights of the amount of forces required to break adhesive bonds between cells?

- The emergence of holes is attributed to a balance of tensile versus compressive internal forces driven by the matrix rigidity. Do you expect the formation of holes in suspended epithelial monolayers ?

1. Ladoux, B. & Mège, R.-M. Mechanobiology of collective cell behaviours. *Nat Rev Mol Cell Bio* 18, 743–757 (2017).

2. Jones, J. Substrate stiffness regulates extracellular matrix deposition by alveolar epithelial cells. *Res Reports Biology* 1 (2011). doi:10.2147/rrb.s13178

3. Alert, R. & Casademunt, J. Role of Substrate Stiffness in Tissue Spreading: Wetting Transition and Tissue Durotaxis. *Langmuir* 35, 7571–7577 (2019).

4. Vazquez, K., Saraswathibhatla, A. & Notbohm, J. Effect of substrate stiffness on friction in collective cell migration. *Sci Rep-uk* 12, 2474 (2022).

5. Fletcher, A. G., Osterfield, M., Baker, R. E. & Shvartsman, S. Y. Vertex Models of Epithelial Morphogenesis. *Biophys J* 106, 2291–2304 (2014).

6. Luciano, M., Xue, S.-L., Vos, W. H. D., Redondo-Morata, L., Surin, M., Lafont, F., Hannezo, E. & Gabriele, S. Cell monolayers sense curvature by exploiting active mechanics and nuclear mechanoadaptation. *Nat Phys* 17, 1382–1390 (2021).

Réponse de Benoit Ladoux,

- The formation of holes in thin monolayer is a beautiful analogy to the dewetting process in polymer thin films which is mainly driven by capillary forces and often triggered at a topological defect. Could you comment this analogy and give us some insights of the amount of forces required to break adhesive bonds between cells?

We observe that normal stresses are important for hole formation. Stress values required to trigger hole openings are around 100 Pa.µm or (100 $N.m^{-2}$) x µm. Considering one molecule per 10 nm, we obtained a force value of 1pN/molecule. This is below the typical value previously found for a single molecule of cadherin. However, this is a rough estimation. It should be considered that the conformation in cellular systems is completely different from that of single molecule studies.

- The emergence of holes is attributed to a balance of tensile versus compressive internal forces driven by the matrix rigidity. Do you expect the formation of holes in suspended epithelial monolayers?

The emergence of holes appears mostly on soft surfaces where the tissue is under tension. More specifically it appears at areas of large stress fluctuations and tensile stress (for instance in the tensile region of topological defects). Having a monolayer suspended and under tension should lead to hole opening. Looking at this video (Vedula et al. Nature Materials 2014 movie 1) from Vedula et al. showing the formation of suspended multicellular bridges, it is exactly what is happening.