Mechanoresponse of Curved Epithelial Monolayers Lining Bowl-Shaped 3D Microwells

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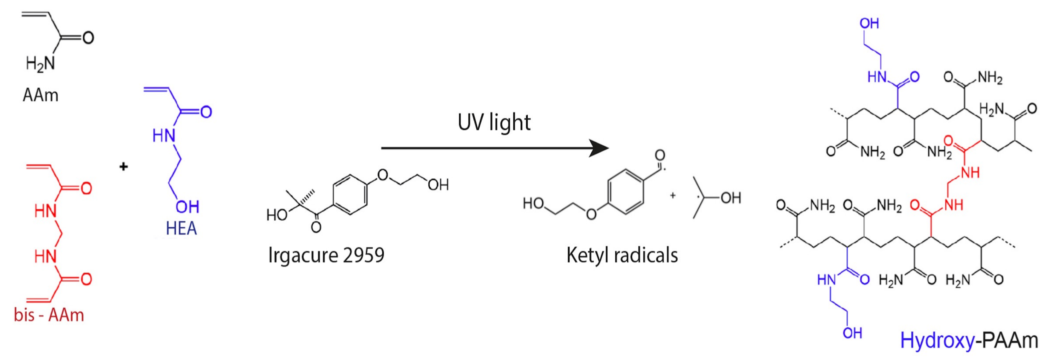
Marine Luciano#, Marie Versaevel#, Yohalie Kalukula and Sylvain Gabriele*

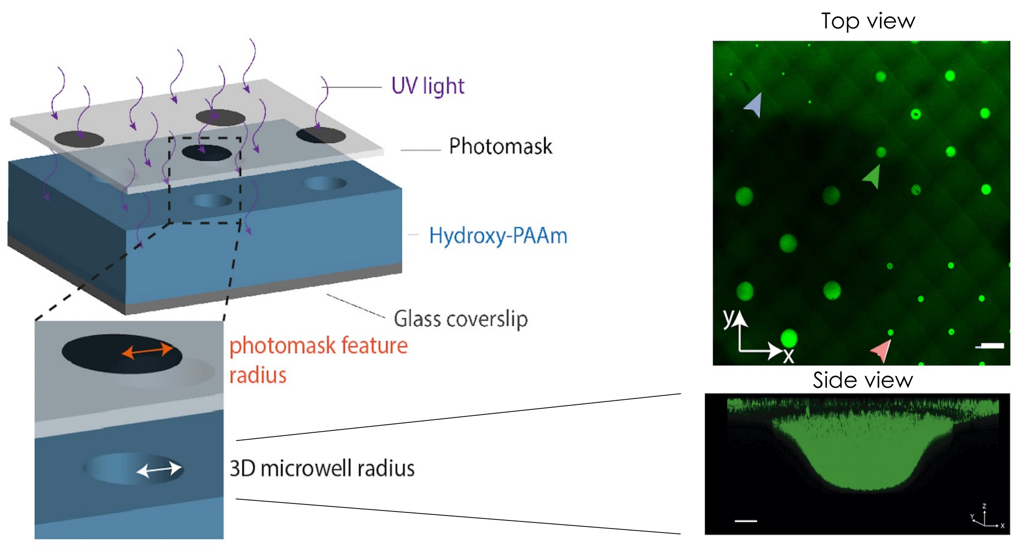
The optimal functioning of many organs relies on the curved architecture of their epithelial tissues. However, the impact of in-plane and Gaussian curvatures on multicellular organization remains poorly understood due to the lack of engineering methods capable of mimicking anatomically relevant 3D complex morphologies, such as those found in lobular structures lining human organs.
To address this gap, here we present a simple method for creating bowl-shaped 3D microwells in hydrogels and engineering curved epithelial monolayers with morphologies resembling those of epithelial tissues in lobular structures. Leveraging this approach, we investigated the distinct roles of in-plane and Gaussian curvatures on the mechanoresponse of curved epithelial monolayers.

3D microwells in hydrogels are produced by UV photopolymerization

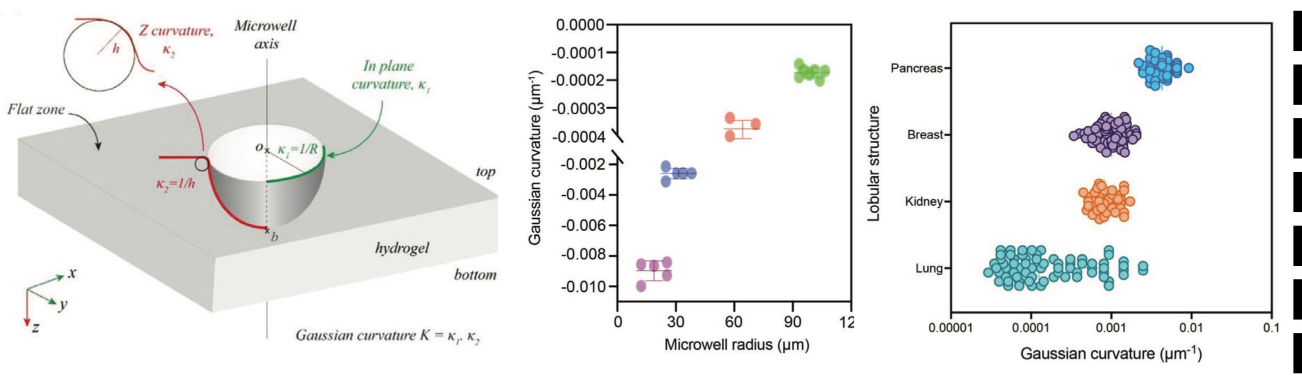
We use the photoinitiator Irgacure 2959 to photopolymerize hydroxy-polyacrylamide (hydroxy-PAAm) hydrogels with UV illumination.

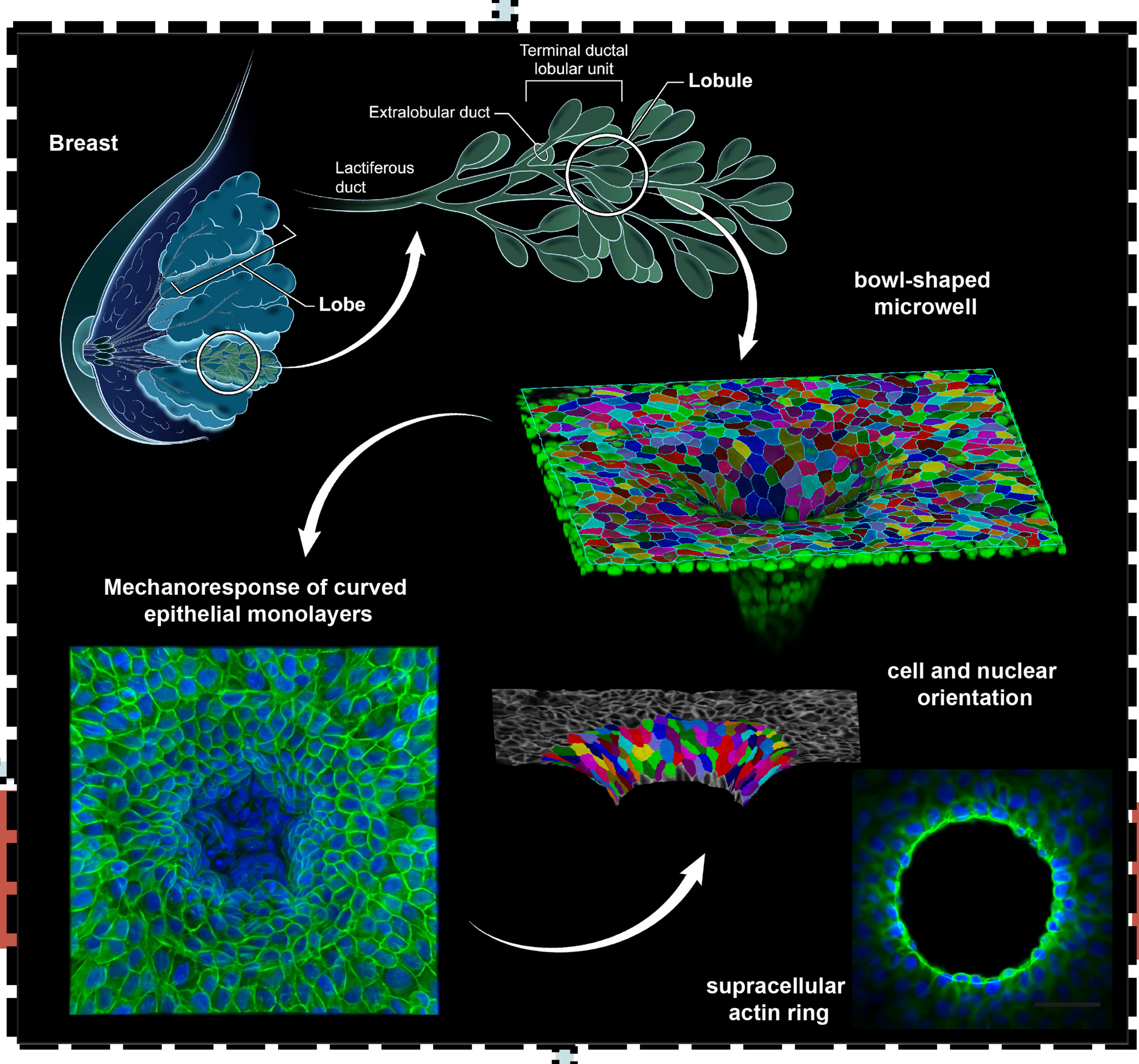


By illuminating the polymer solution through a chromium photomask, we formed 3D microwells of various diameters.



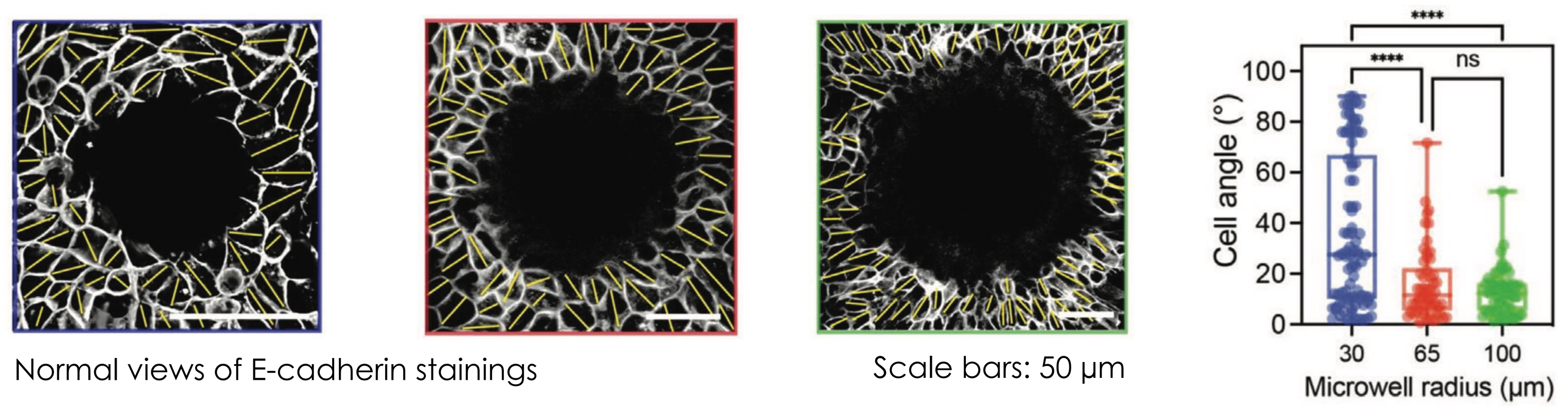
The Gaussian curvature of these 3D microwells matches the curvature range in anatomical lobular structures.



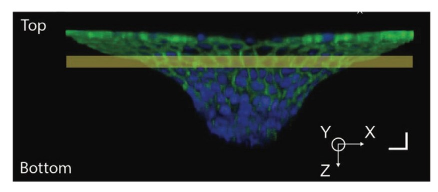


The curvature affects the spatial organization of epithelial cells around the microwell edge

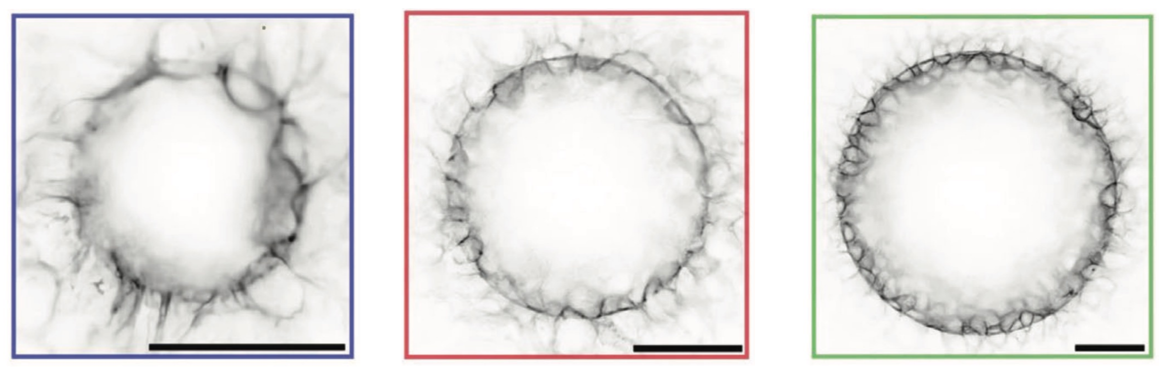
Low in-plane curvature promotes centripetal cell orientation at the microwell edge.



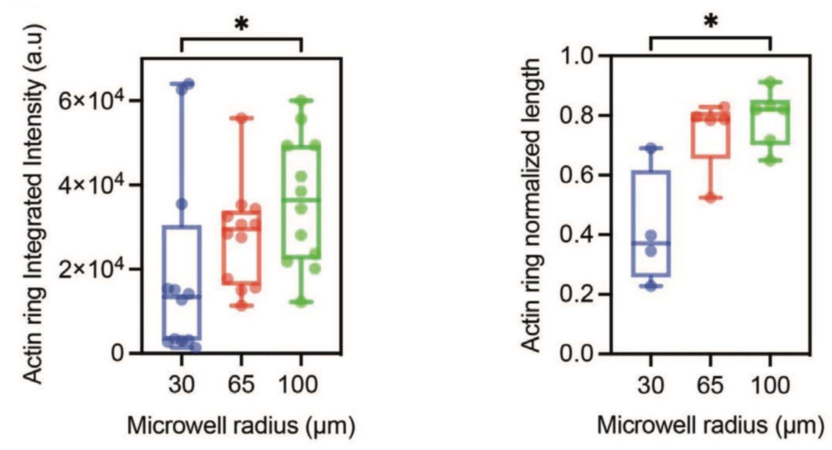
Gaussian curvature at the microwell entrance favors the maturation of a supracellular actin cable.



Confocal images of the actin cytoskeleton at the maximal Z curvature (see the yellow line in the 3D view on the left).

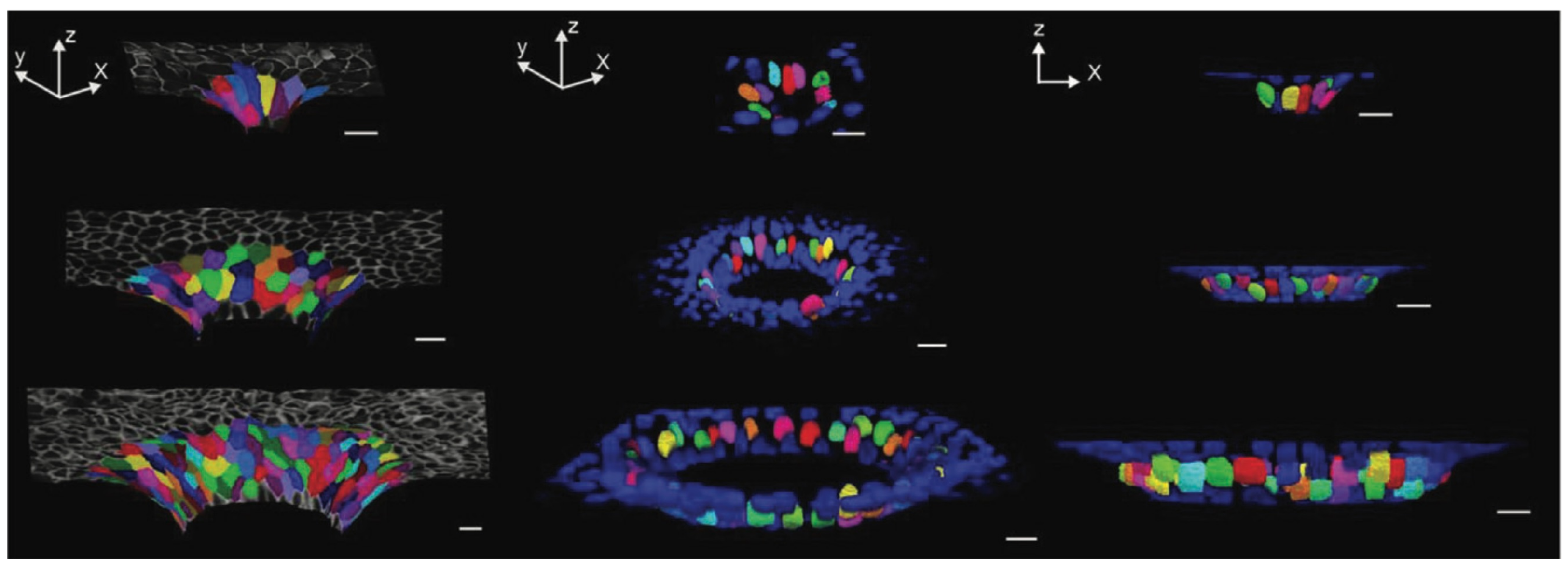


Scale bars: 50 μm

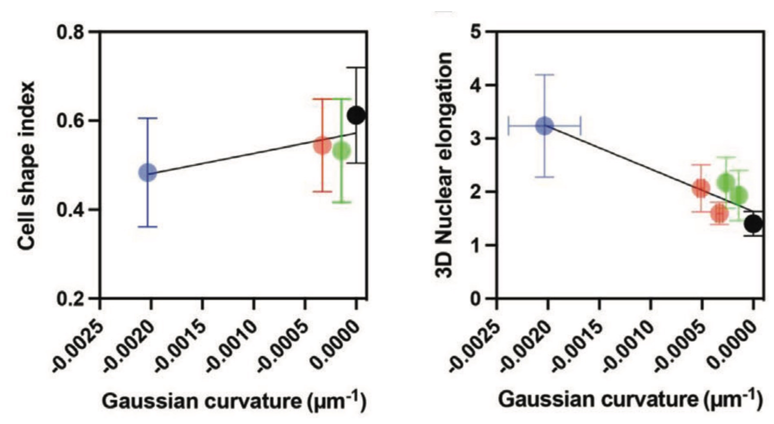


Gaussian curvature induces nuclear deformations, chromatin reorganization …

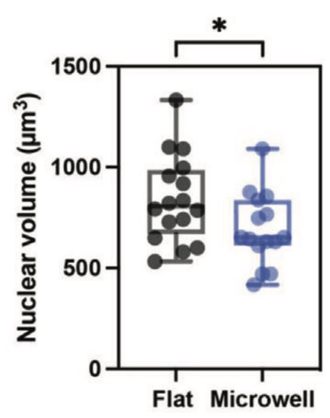
The Gaussian curvature at the microwell entrance induces cellular and nuclear elongation.



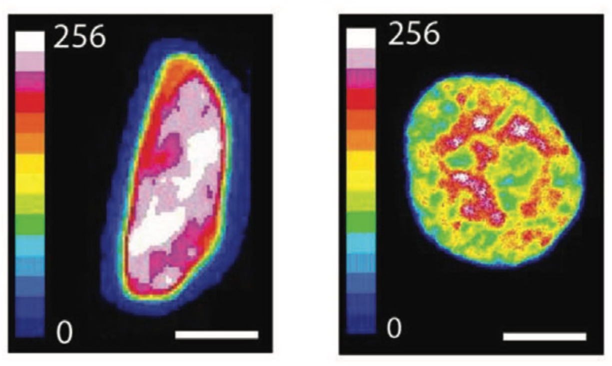
Confocal views of cells (left) and nuclei (middle and right) located at the maximal convex curvature zone in microwells with decreasing gaussian curvature (from top to bottom). Scale bars are 20 μm.



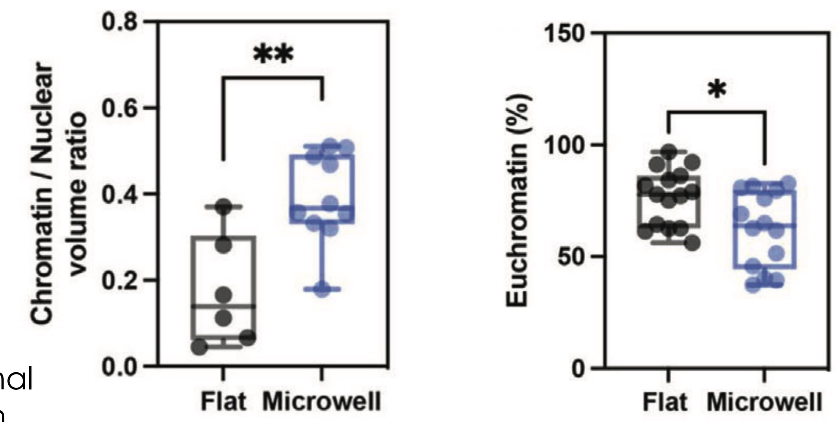
Compared to cells grown on a flat substrate, gaussian curvature at the microwell entrance induces a nuclear volume loss.



Gaussian curvature at the microwell entrance induces an increase in chromatin condensation and a lower level of histone H3 acetylation at lysine 9 (H3K9ac), which corresponds to loosely packed euchromatin transcriptionally accessible.

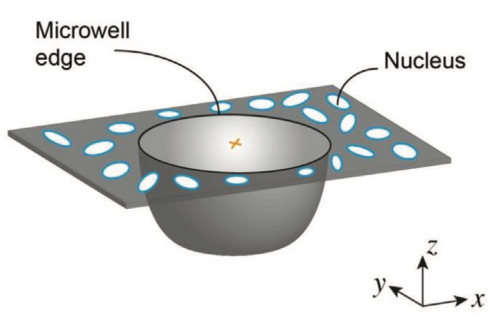


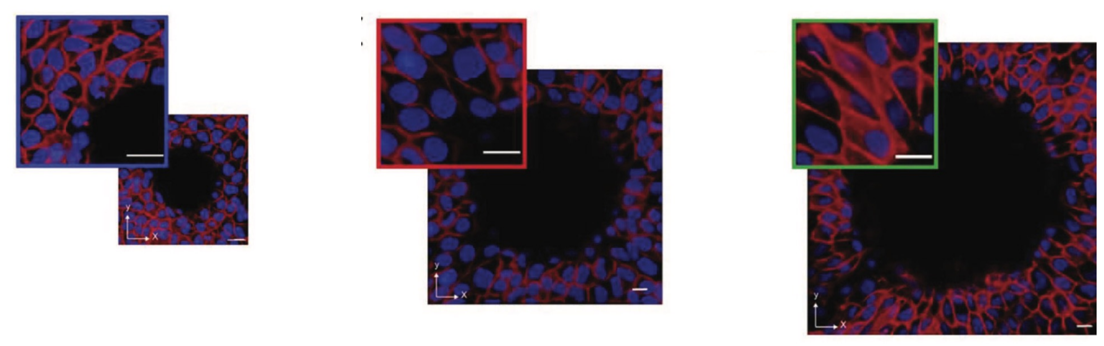
Intensities of DNA staining for a nucleus at the microwell maximal curvature zone (left) or in control flat region (right). SB = 10 μm.



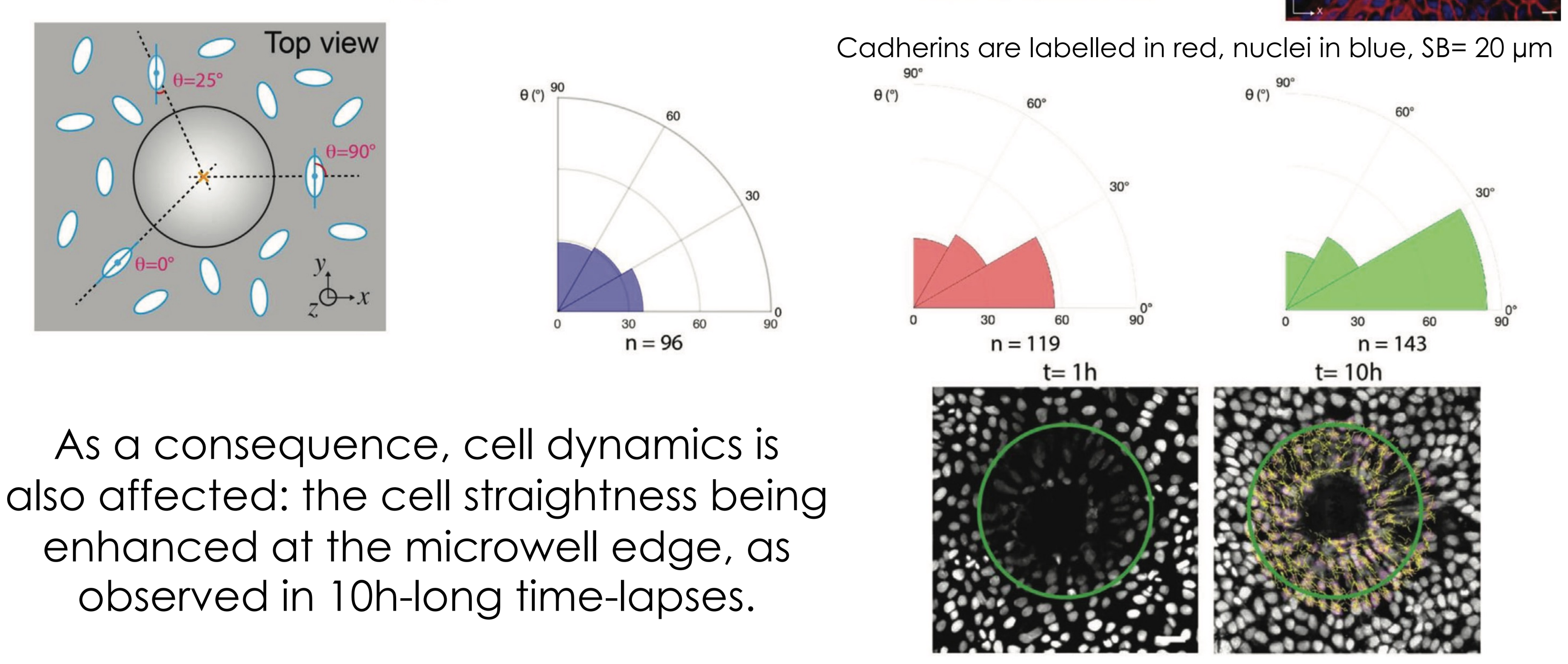
… and nuclear orientation.

The nuclear orientation around the microwell edge was assessed for microwells with various curvatures, demonstrating a centripetal nuclear orientation at the edge of the well for low in-plane curvatures.



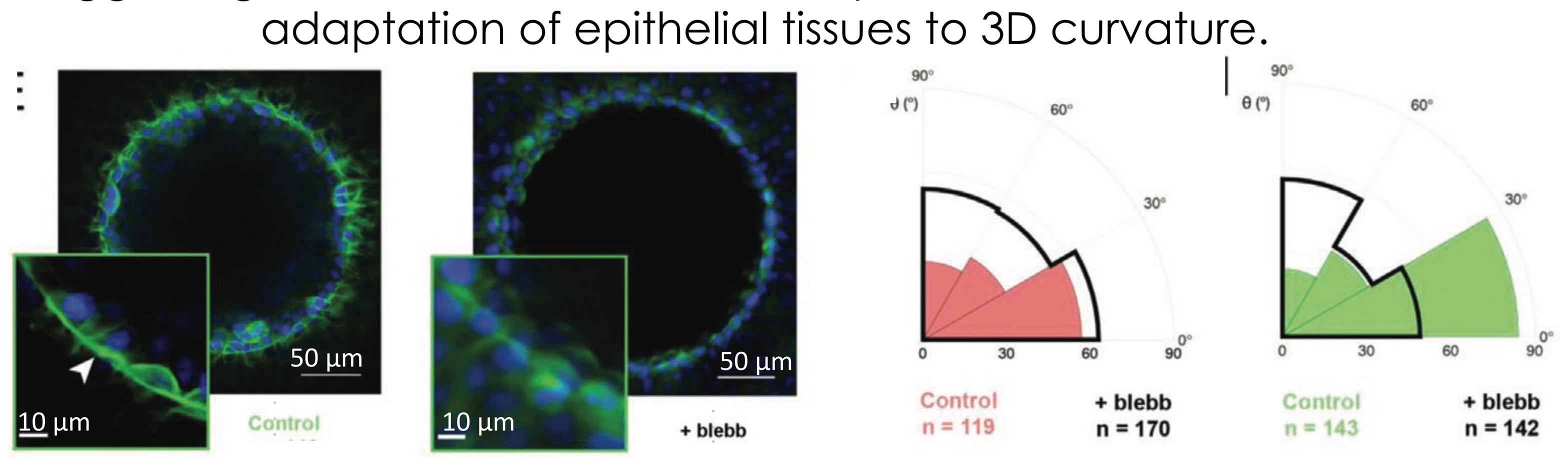


Cadherins are labelled in red, nuclei in blue, SB= 20 μm



As a consequence, cell dynamics is also affected: the cell straightness being enhanced at the microwell edge, as observed in 10h-long time-lapses.

Blebbistatin treatment inhibits the preferential nuclear organization around the microwell entrance and suppresses the supracellular actin ring, suggesting a role of contractile actomyosin forces in the mechanical adaptation of epithelial tissues to 3D curvature.



These novel insights into the influence of curvature in anatomically relevant 3D microstructures on epithelial tissues contribute to a deeper understanding of curvature-responsive mechanical regulation in epithelial tissues that line lobular structures and open up new avenues for creating complex microenvironments.

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UMONS University of Mons
MECHANOBIOLOGY & BIOMATERIALS group