Epithelia can fluidise despite crowding due to curvature changes and osmotic imbalance

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INTRODUCTION

Hemicysts, or fluid filled domes, commonly arise in crowded epithelial cells of the breast, lung and kidney and rely on apicobasal fluid transport to achieve distinct curved architecture. Although these have been documented for several decades, the cytoskeletal and tissue dynamics involved during hemicysts formation has been underexplored. Interestingly, specific agents that influence fluid flux in tissues such as sodium acetate (SA) have shown to induce increased levels of domes. Here, we explored the role of **cell density** and **altered osmotic balance (SA)** on **A)** epithelial tissue dynamics, **B)** expression levels of cell adhesion and tight junction markers, in 3D hemicysts (Fig 1). These findings provide key insights with respect to diseases such as asthma, cystic kidney disease, and cancer metastasis

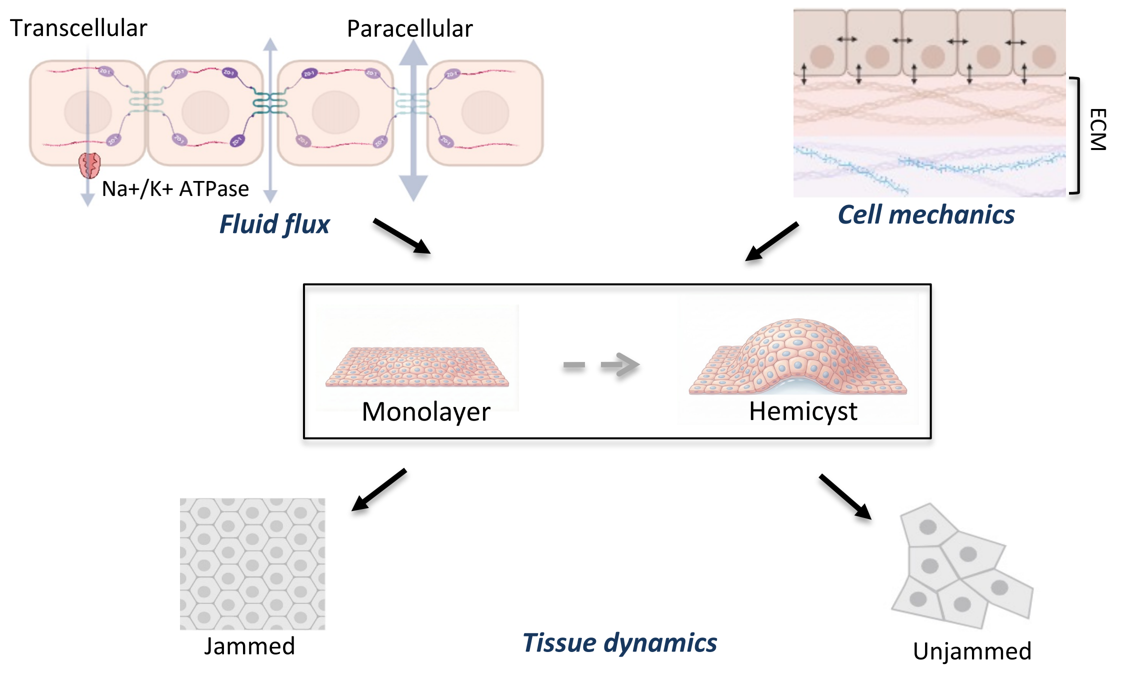
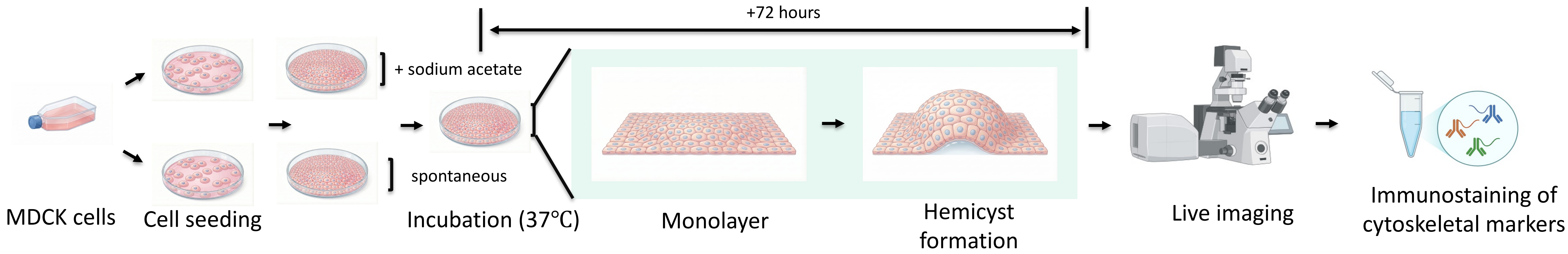


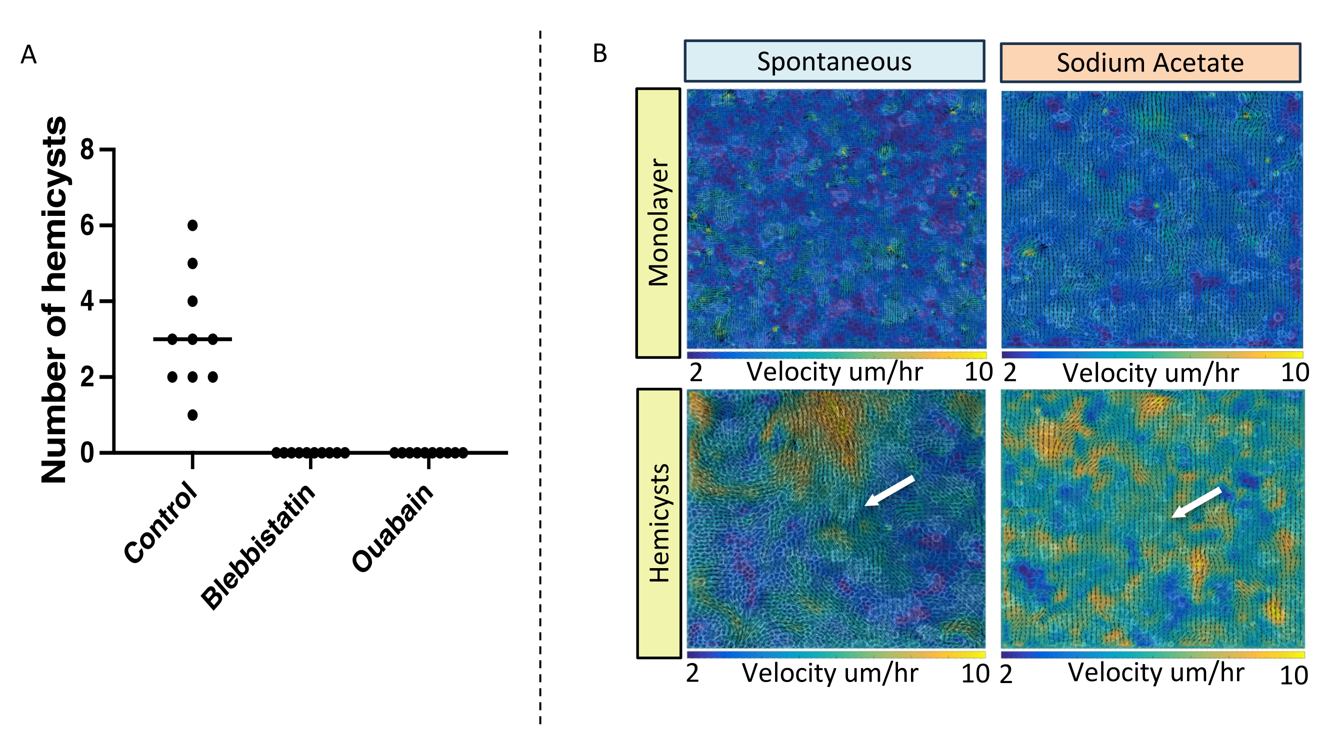
Figure 1: Role of cytoskeletal dynamics and fluid flux in promoting hemicysts induced fluidisation of epithelial tissue

METHODS



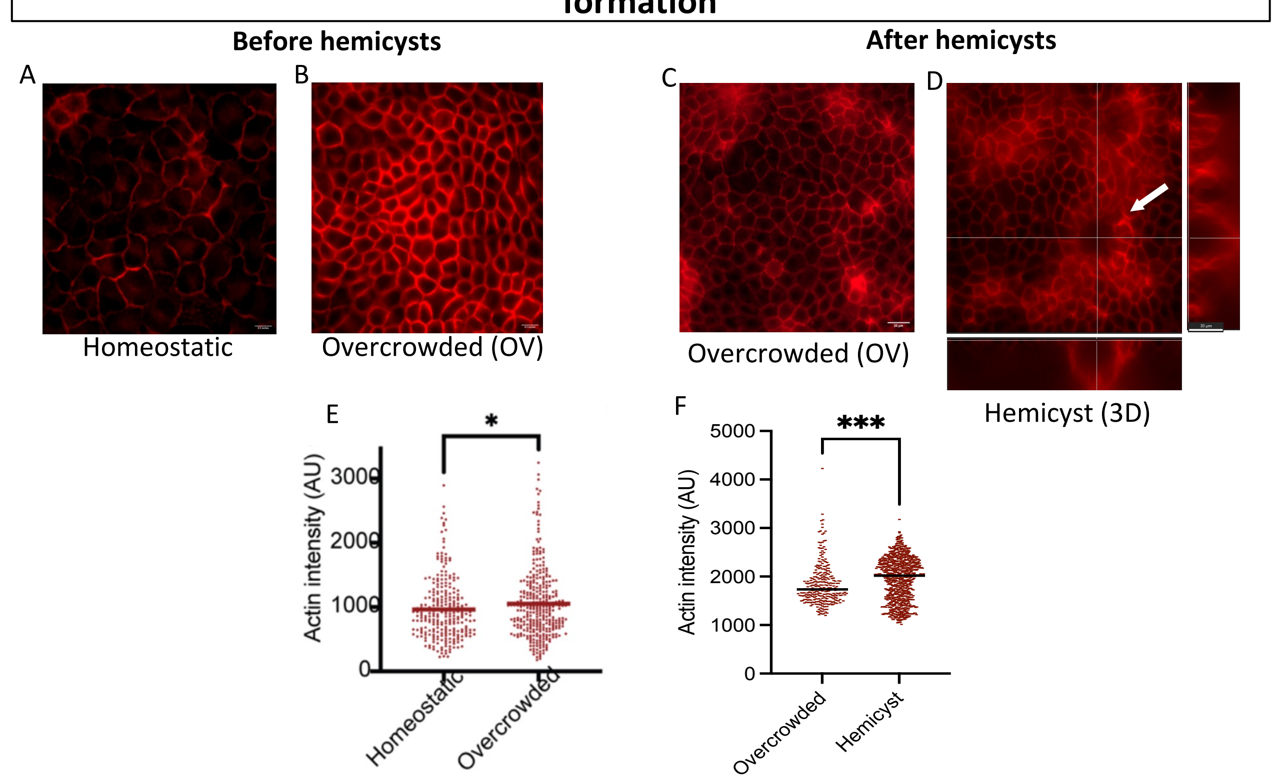
RESULTS

Figure 2: Cell mechanics and fluid flux are required for hemicyst formation



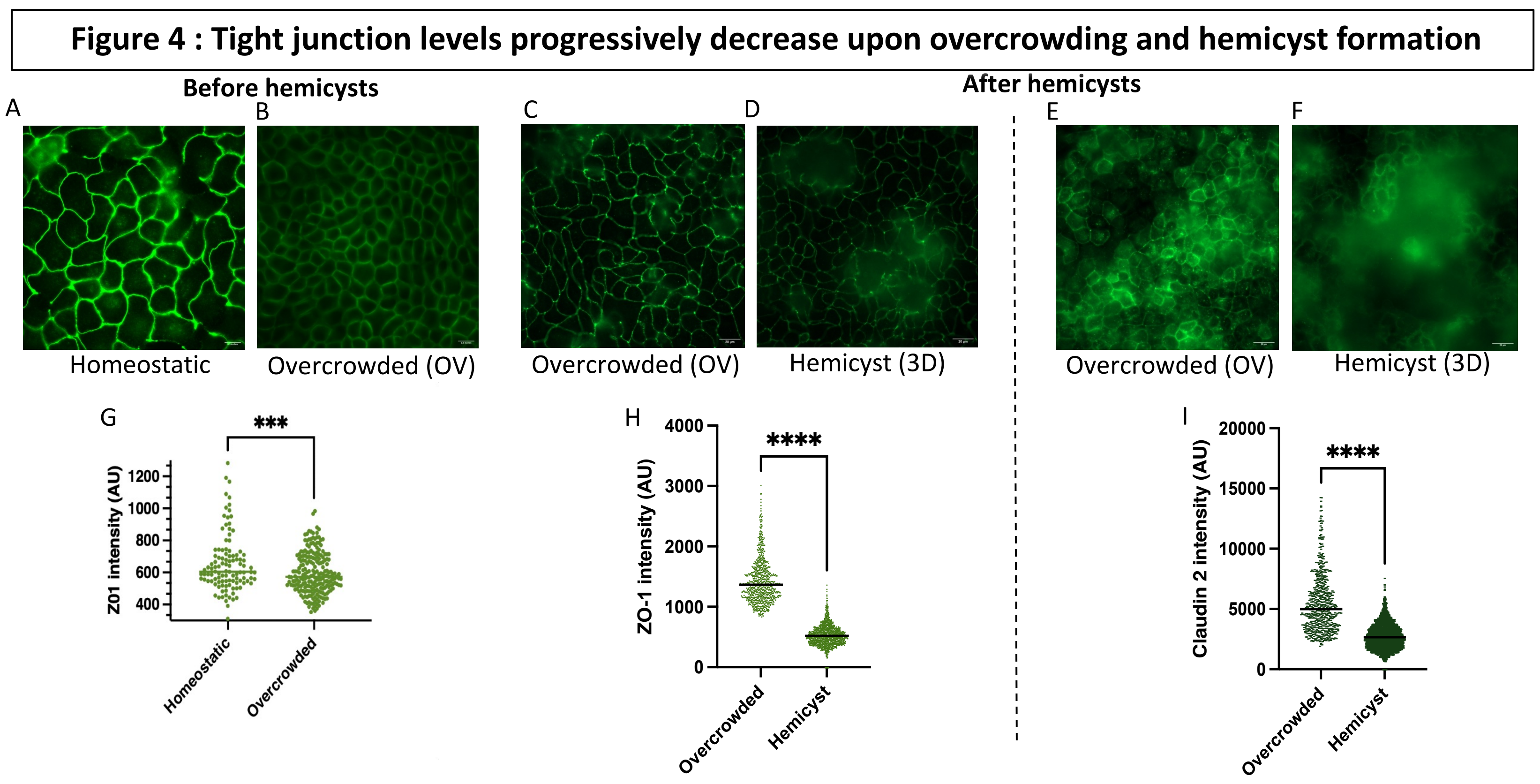
A) Absence of hemicysts upon treatment with Blebbistatin or Ouabain compared to control B) Increased levels of tissue fluidisation upon formation of hemicysts and treatment with sodium acetate

Figure 3 : Actin levels progressively increase upon overcrowding and hemicyst formation



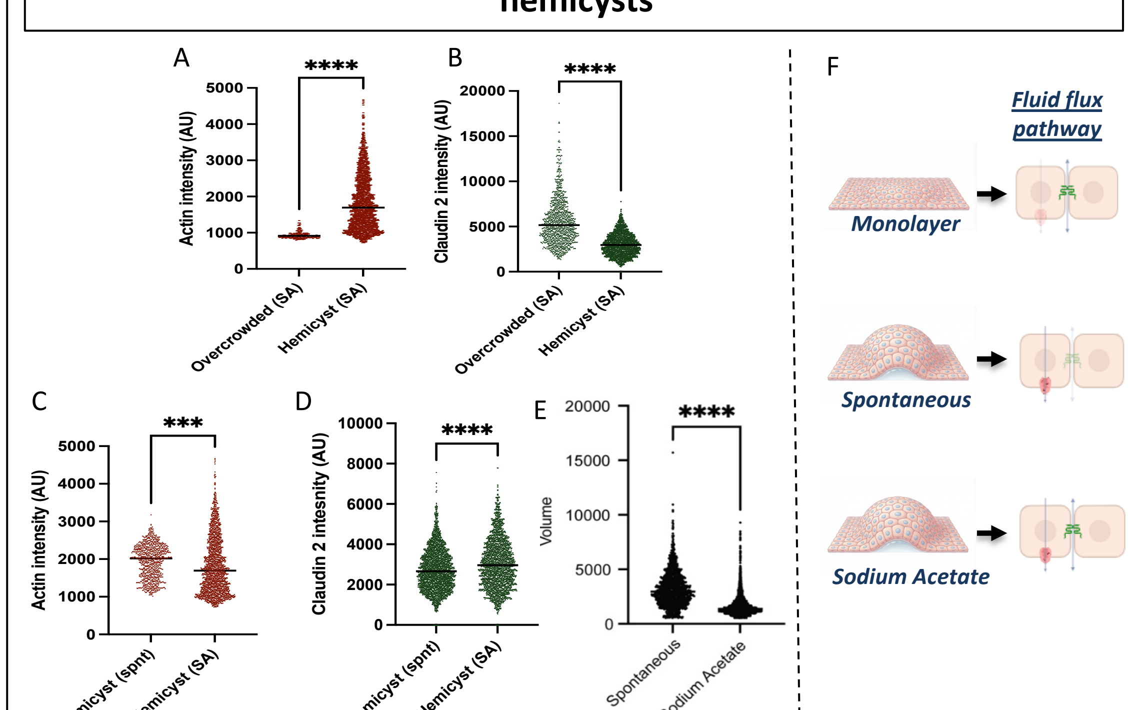
Actin levels in each of A) Homeostatic monolayer, B) OV monolayer before hemicyst, C) OV monolayer after hemicyst formation D) hemicyst E), F) Actin levels before and after domes respectively

Figure 4 : Tight junction levels progressively decrease upon overcrowding and hemicyst formation



ZO-1 in each of A) Homeostatic monolayer, B) OV monolayer, C) OV monolayer after hemicyst formation. D) hemicyst E), F) Claudin 2 levels in overcrowded monolayers after hemicyst formation and hemicysts respectively G), H) ZO1 levels before and after hemicysts I) Claudin 2 levels in monolayers and hemicysts

Figure 5: Altered fluid flux in SA treated compared to spontaneous hemicysts



A), B) Increased actin and decreased claudin 2 in SA hemicysts compared to SA monolayer respectively. C), D) Lower actin, and higher claudin 2 in SA vs spontaneous hemicysts E) Higher volume of cells in spontaneous vs SA hemicysts F) Summary of fluid flux results

CONCLUSIONS AND FUTURE PERSPECTIVES

- Cell density and altered osmotic balance induce fluidisation, with curvature eliciting a greater effect, due to high-density states
- Hemicyst formation involves increase in mechanical activity and reliance on transcellular fluid flux pathways
- Change in tight junction expression upon overcrowding and altered fluid flux could be the result of actin driven mechanical perturbation upon hemicyst formation
- Key factors responsible for increased fluidisation upon SA treatment likely involves less mechanically robust domes and altered fluid flux pathways compared to those in spontaneous hemicysts
- Future perspectives: A) FITC-dextran to differentiate trans/paracellular fluid transport B) Analyse effect of inhibiting alternative ion transporters such as NHE family transporters on hemicyst formation

References

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Meet the lab

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