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Ahearne Lab

Mechanical stimulation driven cellular responses in a 3D corneal model cultured in a custom made bioreactor system

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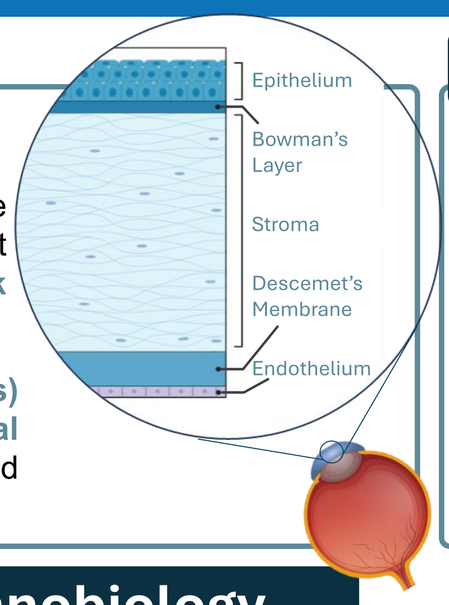


Introduction

Cornea is the outermost layer of the eye, divided into 5 different layers.

Mechanical stimulation, particularly shear stress resulting from the eyeblink impacts cell behaviour in the cornea, but little is known about stromal response. **The response of the anterior cornea to the eyeblink is still an area of active research**.

Emerging strategies are exploring **adipose-derived stem cells (ASCs)** differentiated into corneal stroma cells (keratocytes, CSCs) for **corneal tissue engineering**; however, their functional behaviour beyond differentiation remains largely unexplored.



Objectives

1. **Engineering:** Design and optimisation of a bioreactor system to stimulate a stratified corneal model
2. **Mechanobiology**: Evaluation of keratocytes response to different magnitudes of shear stress
3. **Corneal Tissue Engineering**: Evaluate the potential of the system in corneal bioengineering using ASCs derived keratocytes

1. Engineering

1. Quantification of the shear stress and the Turbulent Re in 400µm-high and 800µm-high flow channels

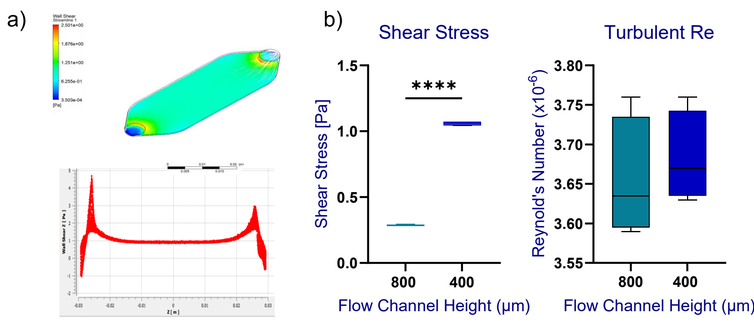


Figure 1. a) Representation of fluid flow in the chip and flow chart. b) Results of CFD analysis with values of shear stress and turbulent Re

2. Nuclear YAP confirms effective epithelial cells stimulation, TRPV4 upregulation suggests stromal cell-mediated changes in mechanosensitive pathways

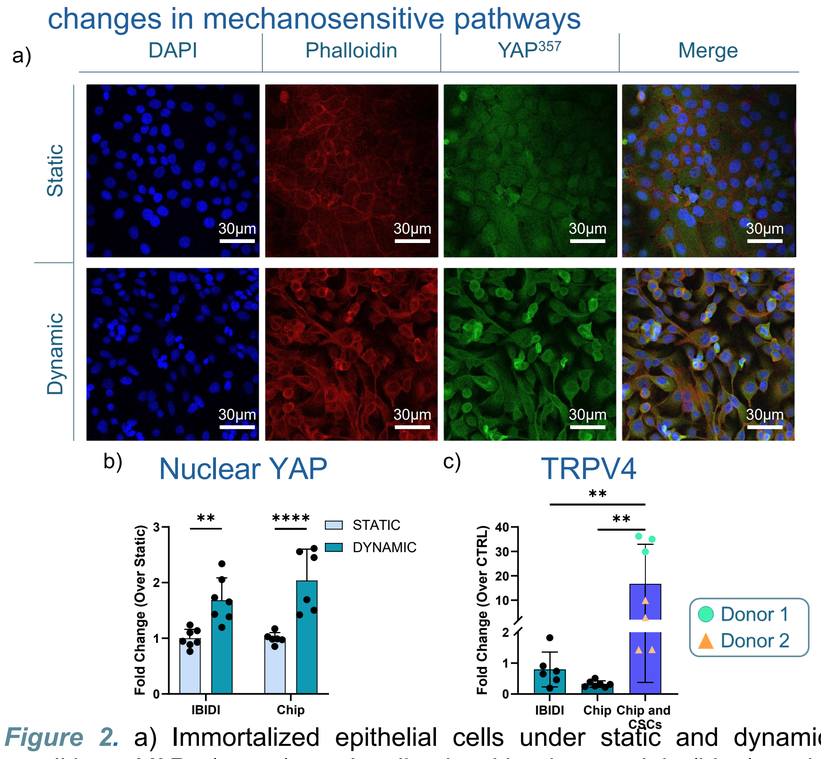


Figure 2. a) Immortalized epithelial cells under static and dynamic condition. YAP (green) co-localized with the nuclei (blue). b) Quantification of nuclear YAP expression. c) gene expression of TRPV4. Donors represent independent primary keratocytes used in the co-culture condition.

2. Mechanobiology

1. Fluid flow reverses the effect of the co-culture reducing α-SMA expression following 1 day of stimulation and upregulating ALDH3A1 after 3 days of stimulation

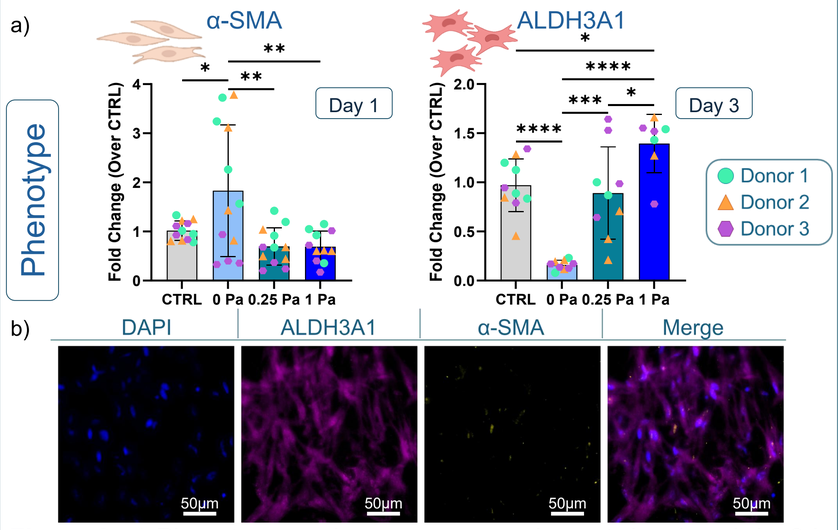


Figure 3. a) Phenotype marker gene expressions at day 1 and day3 in primary CSCs. b) representative protein-level images. α-SMA (fibroblast, yellow) and ALDH3A1 (keratocyte, magenta).

2. Following 3 days of fluid flow stimulation, ECM-related genes are upregulated and the collagen content increases

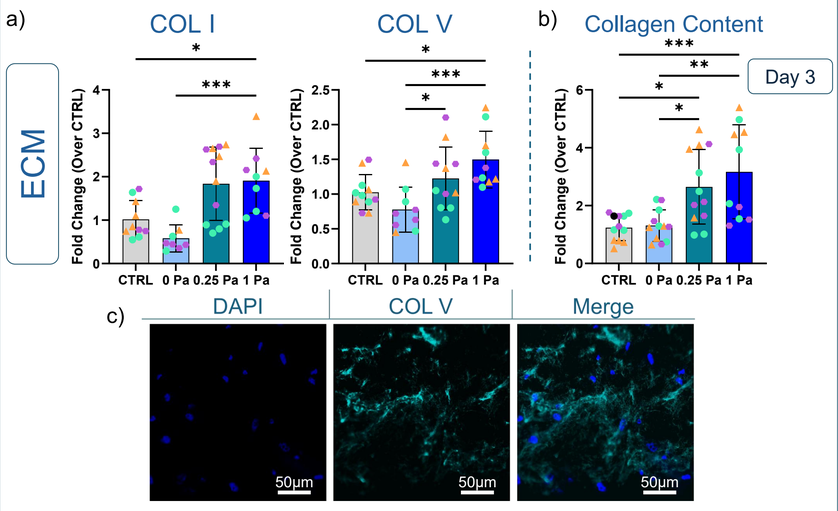


Figure 4. a) ECM –related markers gene expression at day 3 in primary keratocytes, b) Biochemical analysis of collagen content at day 3. c) Representative protein-level images of collagen 5 production (cyan).

3. Tissue Engineering

1. Fluid flow reduces α-SMA expression and upregulates ALDH3A1 expression after 3 days of stimulation

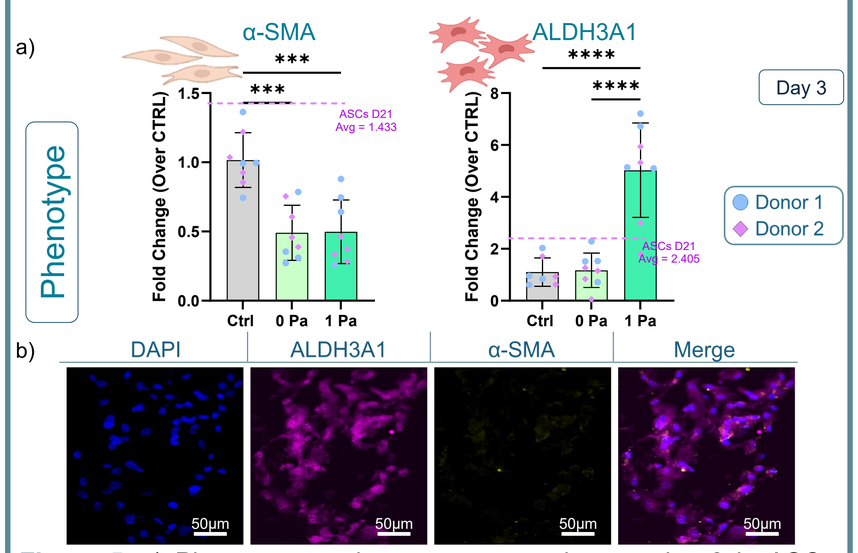


Figure 5. a) Phenotype marker gene expressions at day 3 in ASCs-derived keratocytes. b) Representative protein-level images. α-SMA (fibroblast, yellow) and ALDH3A1 (keratocyte, magenta).

2. Fluid flow reverse the negative effect of the co-culture on ECM-related genes

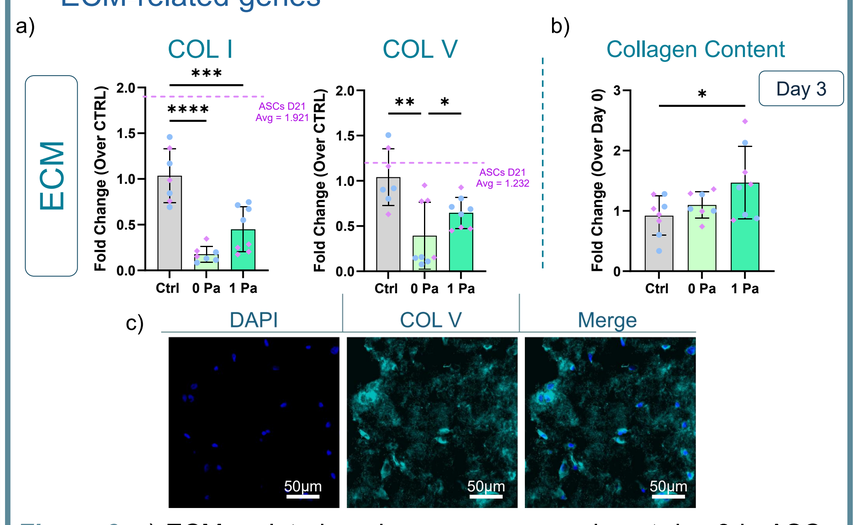


Figure 6. ***a***) ECM –related markers gene expression at day 3 in ASCs-derived keratocytes, b) Biochemical analysis of collagen content. c) Representative protein-level images of collagen 5 production (cyan).

Methods

1. Set-up of the bioreactor system and the 3D co-culture parameters

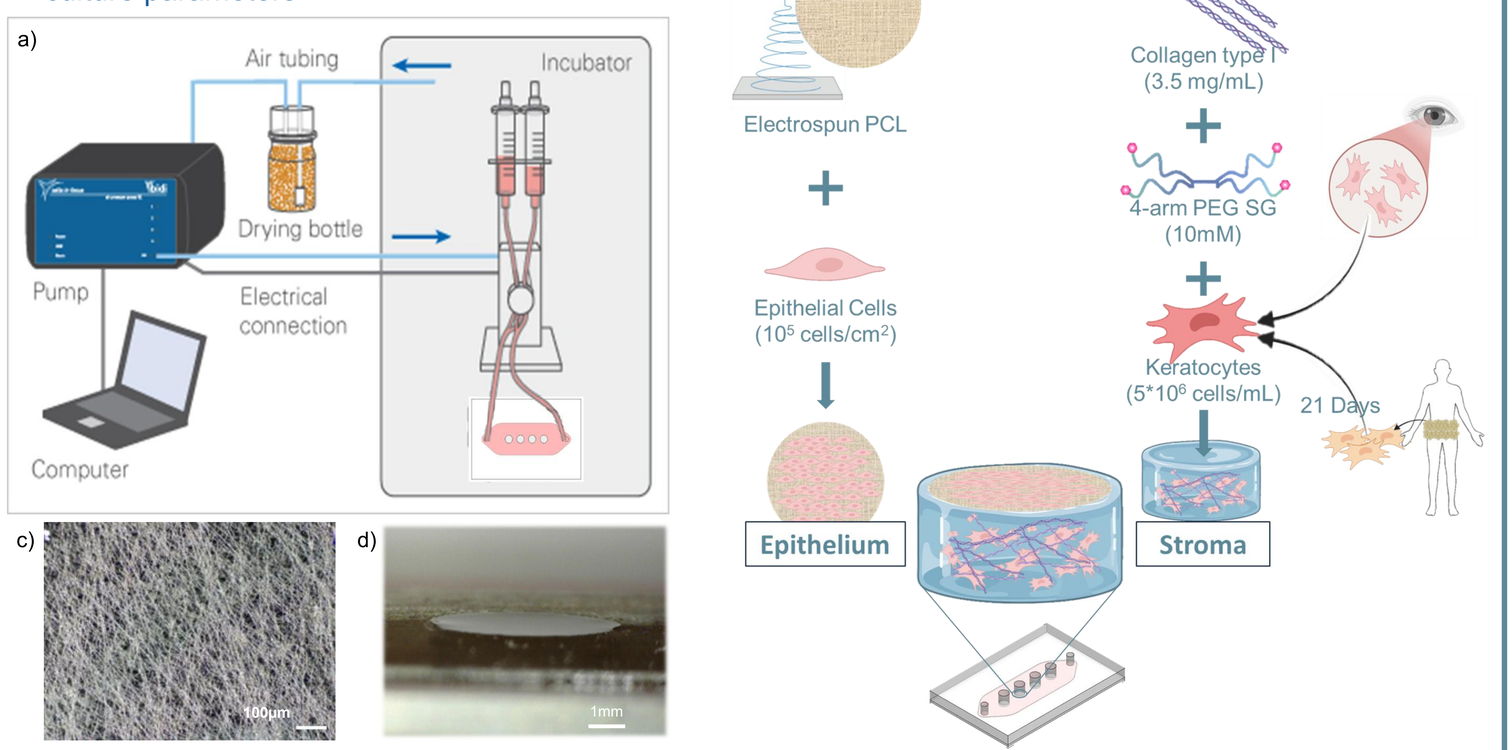


Figure 6. a) Schematic view of the bioreactor system. b) Schematic view of the 3D co-culture model. Immortalized epithelial cells are seeded onto electrospun functionalised PCL membranes, keratocytes are embedded in a collagen-PEG hydrogel. On the right the two sources of keratocytes, primary (objective 2 – mechanobiology) differentiated from ASCs (tissue engineering – objective 3). c) Electrospun membrane coated in Polydopamine. d) Representative image of the corneal model in the well.

Conclusion

Validated the bioreactor function via YAP analysis, established co-culture as key for regulating cells behavior.

Co-culture (0 Pa) induces a fibroblastic shift in corneal stromal cells, while epithelial-mediated shear stress positively affects keratocytes.
Mechanobiology data (3 days, 1 Pa) were selected for subsequent tissue engineering studies.

Co-culture (0 Pa) downregulates ECM-related genes, whereas shear stress upregulates phenotype-related and ECM-related markers. The shear stress might be used to precondition engineered tissue before transplantation.

Acknowledgements

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