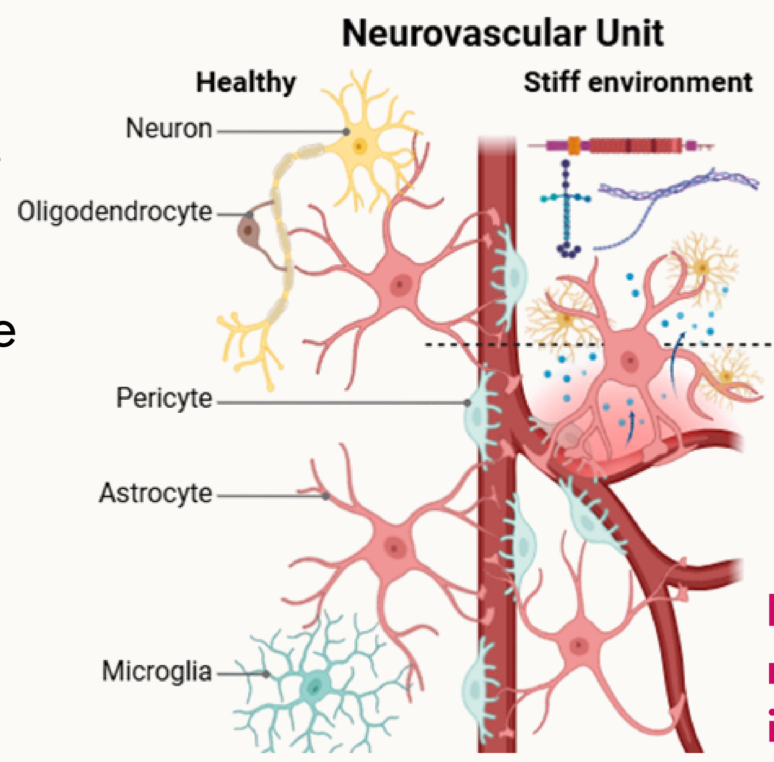
Mechanically defined microenvironments regulate neurovascular organisation in blood–brain barrier organoids

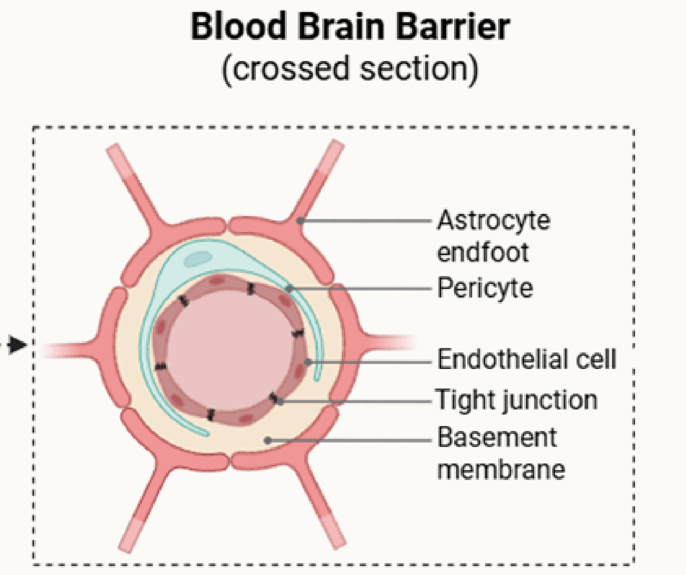
Nerea Caro Guerrero, Amy Charlotte Carnegie, Kimia Witte

Department of Biomedical Engineering, Faculty of Engineering, University of Strathclyde, Glasgow, United Kingdom

INTRODUCTION

- The blood–brain barrier is a specialised neurovascular interface essential for maintaining central nervous system homeostasis [1].
- BBB dysfunction contributes to neurological diseases such as vascular dementia, while glioblastoma is associated with extensive ECM remodelling of the brain microenvironment [1–3] (Fig.1).
- Current *in vitro* models fail to replicate the mechanical complexity of the native brain microenvironment [4–5].
- ECM-functionalised hydrogels provide biomimetic 3D platforms with tunable mechanical properties to investigate disease-associated mechanobiology.





How do disease-associated changes in matrix stiffness and viscoelasticity influence neurovascular spheroid organisation?

Fig 1. Blood-brain barrier structure and disease-associated brain microenvironment.

METHODOLOGY

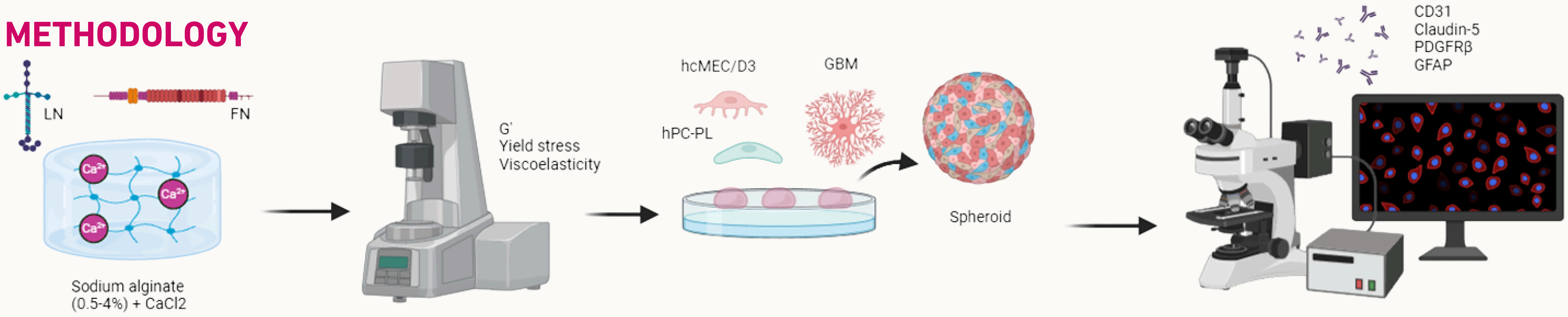


Fig 2 .Experimental workflow. ECM-functionalised alginate hydrogels were mechanically characterised by rheology and used to generate multicellular spheroids comprising hCMEC/D3, hPC-PL, and GBM cells. Spheroid phenotype and organisation were assessed by immunofluorescence staining for DAPI, CD31, Claudin-5, PDGFRβ, and GFAP.

RESULTS

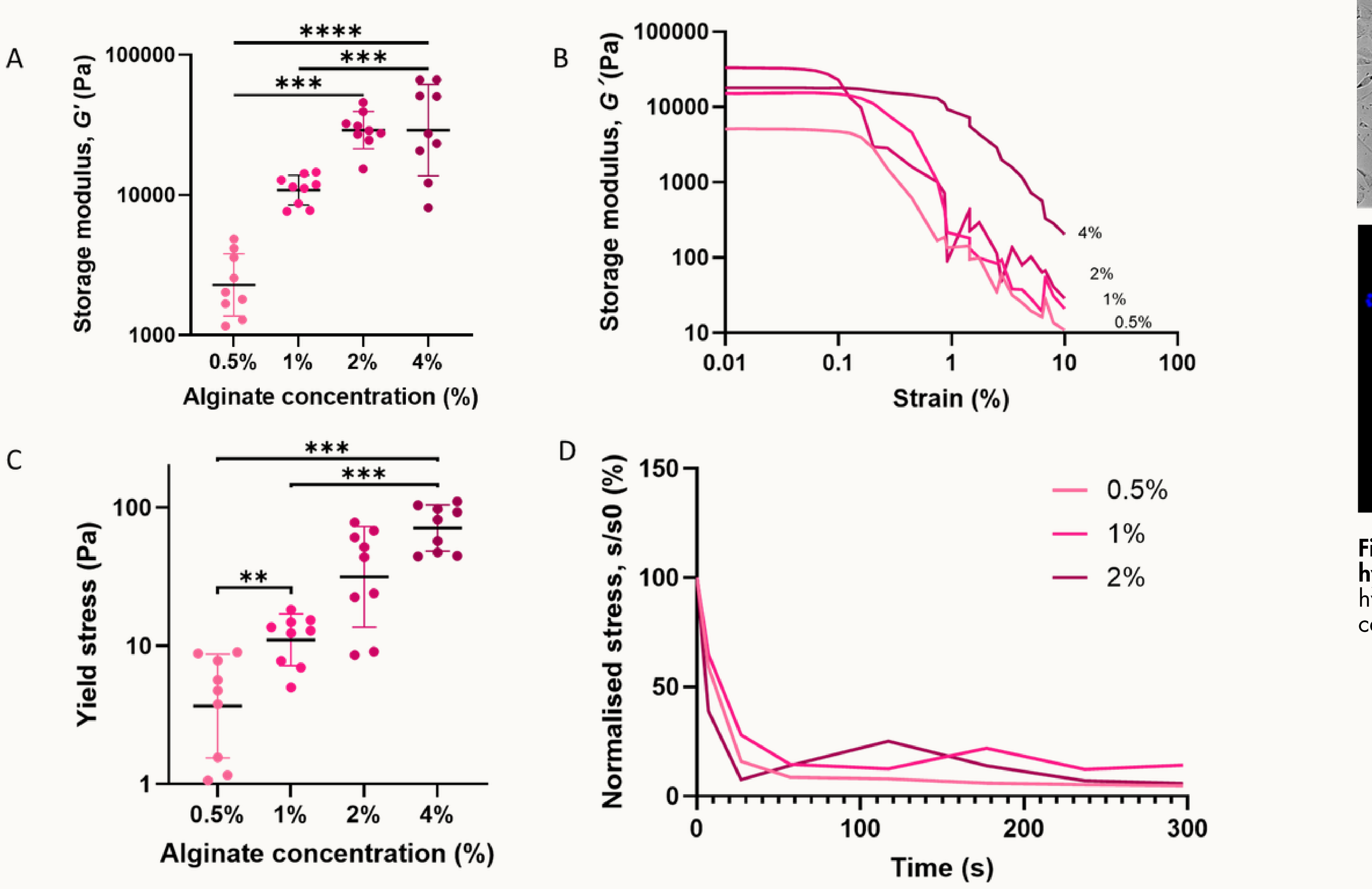


Figure 3. Mechanical tuning of alginate hydrogels by varying polymer concentration. **(A)** Storage modulus (G′) increased significantly with alginate concentration, indicating enhanced hydrogel stiffness. **(B)** Representative amplitude sweep curves showed that higher concentration hydrogels maintained elastic behaviour over a broader strain range before yielding. **(C)** Yield stress increased with alginate concentration, demonstrating improved mechanical robustness. **(D)** Representative stress relaxation profiles confirmed that all formulations retained viscoelastic behaviour. Together, these results show that alginate concentration enables modulation of hydrogel mechanical properties while preserving tissue-relevant viscoelasticity. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA with Tukey's post-hoc test for storage modulus and Games–Howell multiple comparisons test for yield stress (*P < 0.05, **P < 0.01, ***P < 0.001).

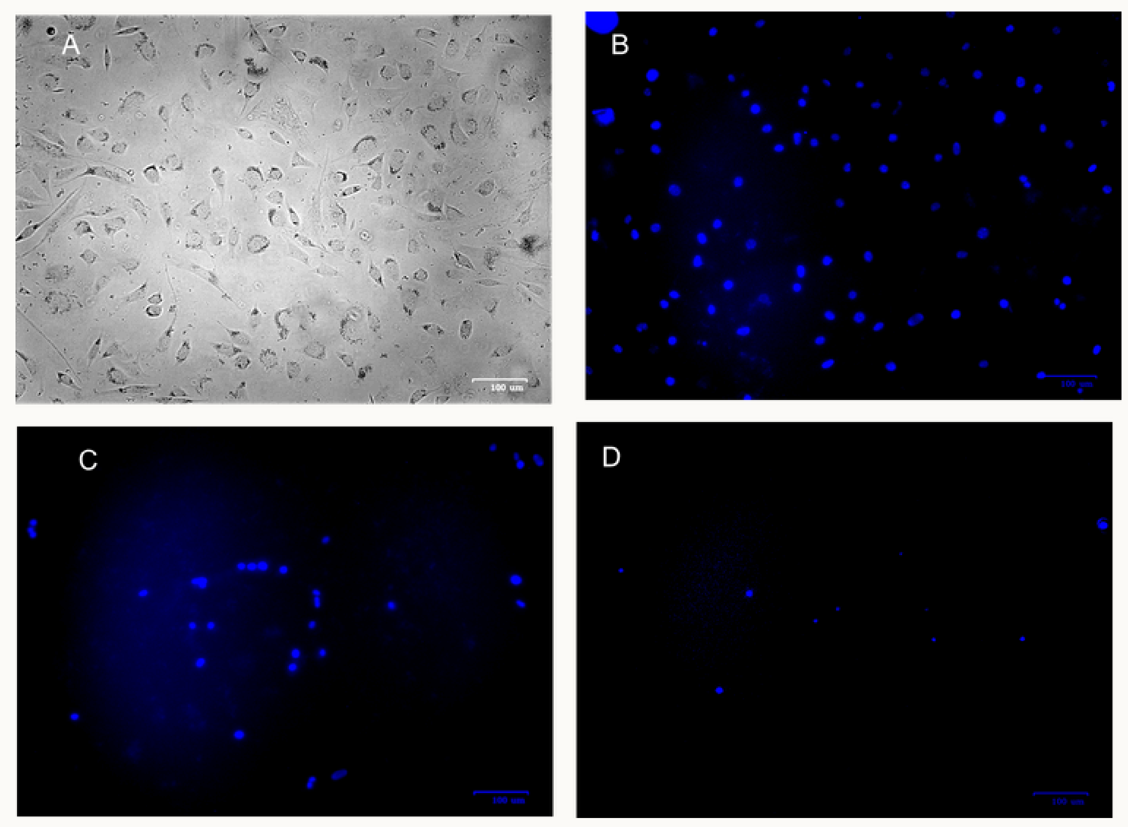


Figure 4. DAPI staining of hCMEC/D3 and HP-PLC co-cultures on alginate hydrogels. **A)** Brightfield fibronectin-coated hydrogel. **B)** Fibronectin-coated hydrogel: high cell density and attachment. **C)** Laminin-coated hydrogel: lower cell density. **(D)** No ECM hydrogel control: minimal cell adhesion.

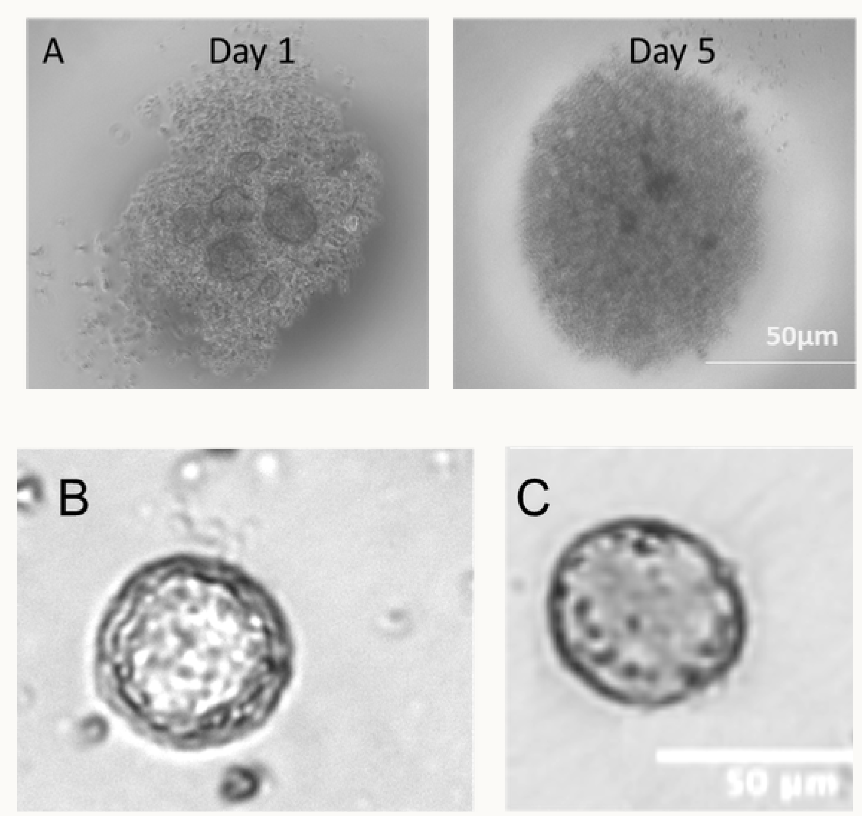


Figure 5. Spheroid formation. **(A)** Brightfield images of hCMEC/D3, hPC-PL, and GBM spheroids at Day 1 and Day 5. **(B-C)** Brightfield images of a self-assembled spheroid formed in a hydrogel during co-culture hCMEC/D3 and hPC-PL, showing a compact, multicellular structure using both fibronectin **(B)** and laminin **(C)**.

CONCLUSION AND FUTURE WORK

Mechanically tunable alginate hydrogels supported neurovascular spheroid formation, with laminin enhancing spheroid growth, establishing a promising platform to investigate disease-associated brain microenvironments.

Goal: Generate BBB organoids using a glass capillary microfluidic device for vascular dementia disease modelling, characterisation, and drug screening.

References:
[1] Shabir O., Berwick J., Francis S.E. (2018). BMC Neurosci, 19:62,
[2] Alahmari A. (2021). Neural Plast, 2021:6564585.
[3] Konig S. et al. (2025). Nat Commun, 16:7233.
[4] Cho C.F. et al. (2017). Nat Commun, 8:15623.
[5] Urich E. et al. (2013). Sci Rep, 3:1500.

For further discussion contact me at: **nerea.caro-guerrero@strath.ac.uk**