Mechanoregulation of Microvascular Spatial Patterns

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The "Doughnut" Spatial Pattern

Vasculature is **critical** for **model scaling** & **physiological replication**. However, **recapitulating tissue-specific vascular organisation *in vitro*** remains a **major challenge**[1].

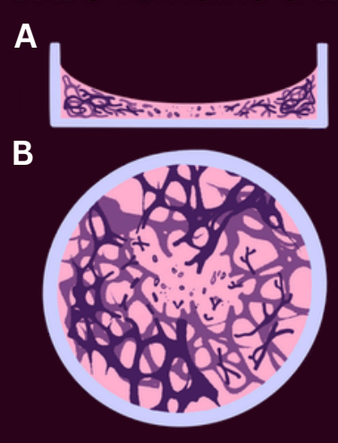


Fig. 1. Vasculogenesis Model Schematic. (**A**) side view, (**B**) top-down view, across 2-6 mg/mL collagen.

Dense, lumenised microvascular networks form at the well boundaries, which are absent in the well centre. **Meniscus formation** (**Fig. 1**) during collagen cross-linking is hypothesised to drive this distinct **"doughnut" pattern** (**Fig. 2**).

This study reports the **potential mechanoregulation of the spatial patterns** observed in this 3D vasculogenesis model.

Elucidating the mechanisms responsible for this cell behaviour will pave the way towards engineering **organ-specific vascular models**.

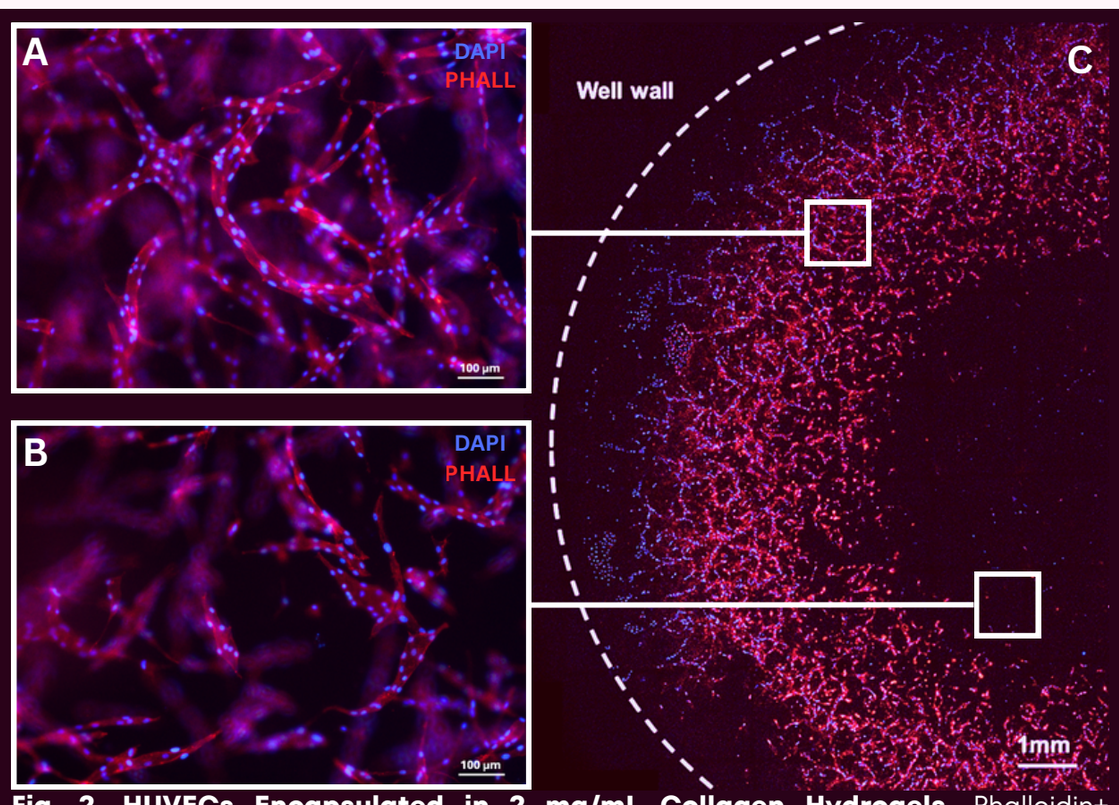
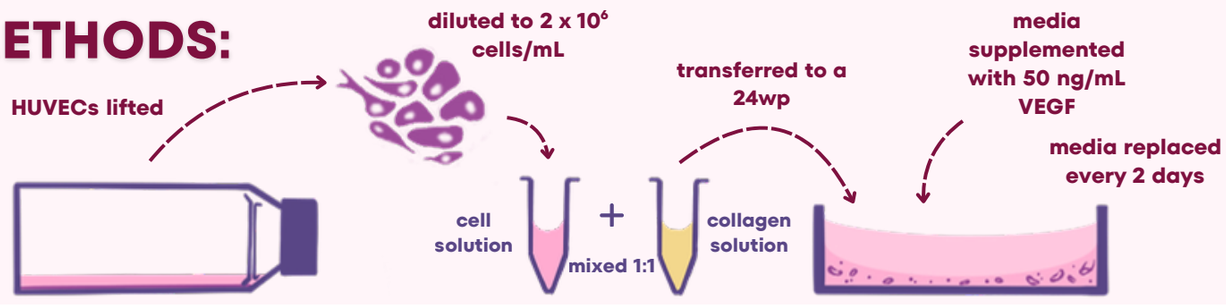


Fig. 2. HUVECs Encapsulated in 2 mg/mL Collagen Hydrogels. Phalloidin+ filamentous-actin & DAPI+ nuclei. *Scale bars: 100µm (**A, B**) & 1mm (**C**).*

METHODS:



AIMS:

1. **Visualise collagen fibre** organisation.
2. **Measure & map** the model's **mechanical gradient**.
3. Quantify **gene expression** at the **centre & edge** of the patterned cell-laden collagen hydrogels.

1. Reflectance Confocal Microscopy for Label-Free Collagen Fibre Visualisation

Acellular collagen hydrogels in glass-bottom 24-well microplates were imaged by **reflectance confocal microscopy** (**RCM**).

RCM facilitates **label-free collagen fibre visualisation** by detecting light of illuminated samples with the same wavelength as the laser[2].

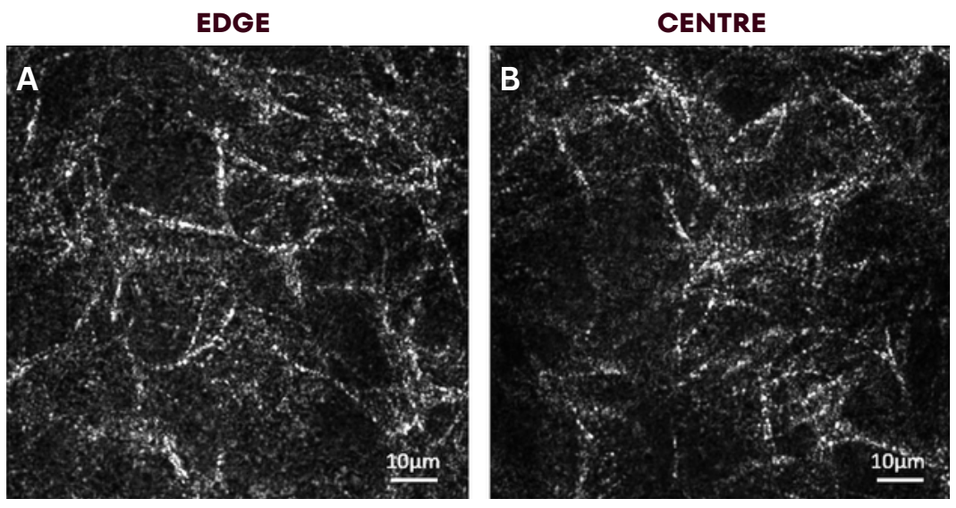


Fig. 3. Reflectance Confocal Micrographs of Acellular Collagen Hydrogels. 2 mg/mL hydrogel (**A**) edge & (**B**) centre taken at 40X magnification. Scale bar: 10µm; collagen fibres in white. **Microscopy performed in the McConnell lab.**

Images acquired by RCM could not be reliably quantified. Future work will image high-concentration collagen hydrogels & adapt gelation conditions (pH/temperature) to produce **thicker fibres for improved imaging quality**[3,4].

If reliable quantification confirms that there is **no directional trend**, this supports the hypothesis that mechanics primarily **drives cell patterning**.

2. MOT & Hydrogel Simulations Indicate Spatial Mechanical Gradients Influence Cell Patterning in Collagen Hydrogels

Compressive mechanics of acellular gels were measured with a **rotational rheometer** & **nanoindentor** (Fig. 4A & 4B, respectively).

Microrheology with optical tweezers (MOT; Fig. 4C) provides **focal viscoelastic** information about the suspending acellular hydrogel by measuring **thermal fluctuations** of embedded polystyrene **microbeads** (2.88µm).

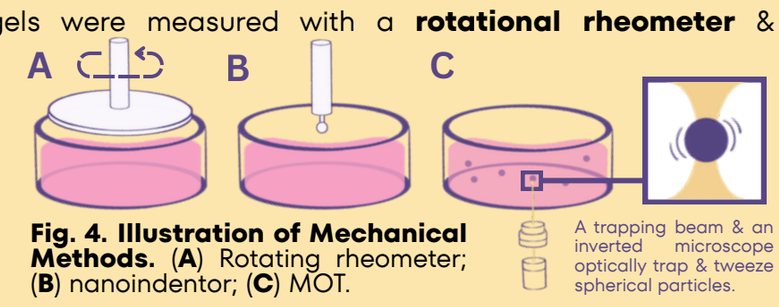


Fig. 4. Illustration of Mechanical Methods. (**A**) Rotating rheometer; (**B**) nanoindentor; (**C**) MOT.

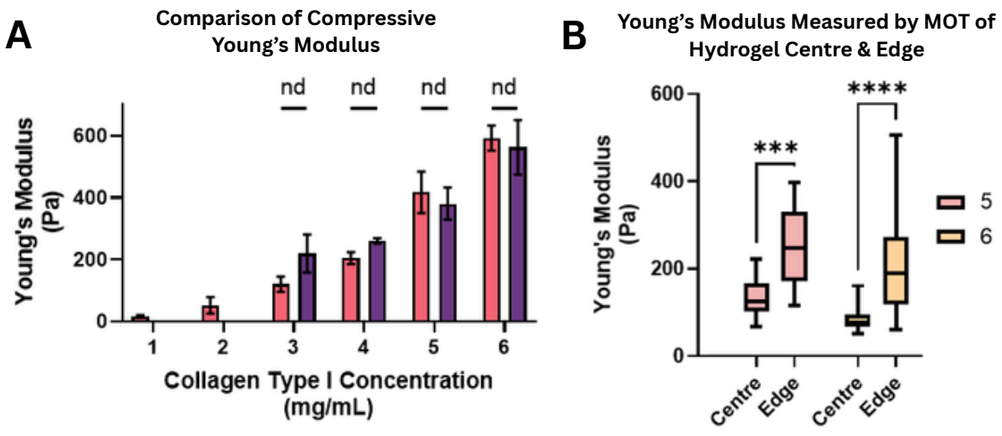


Fig. 5. Mechanical Measurements of Acellular Hydrogels. (**A**) Bulk rheometry (pink) & nanoindentation (purple) measure collagen hydrogels within a ~15 to 500 Pa range across 1-6 mg/mL. (**B**) MOT measurements at the centre & edge of 5 & 6 mg/mL collagen hydrogels. *Data presented as mean ± SE analysed by two-way ANOVA; nd p>0.05; ***p<0.001; ****p<0.0001.* **Nanoindentation performed by the Vassalli lab; MOT by the Tassieri lab.**

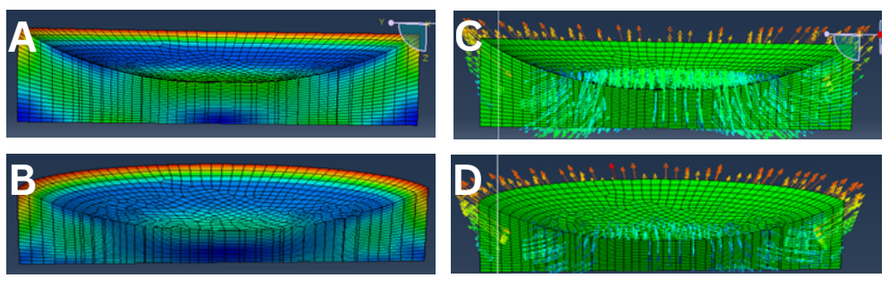
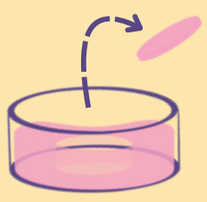


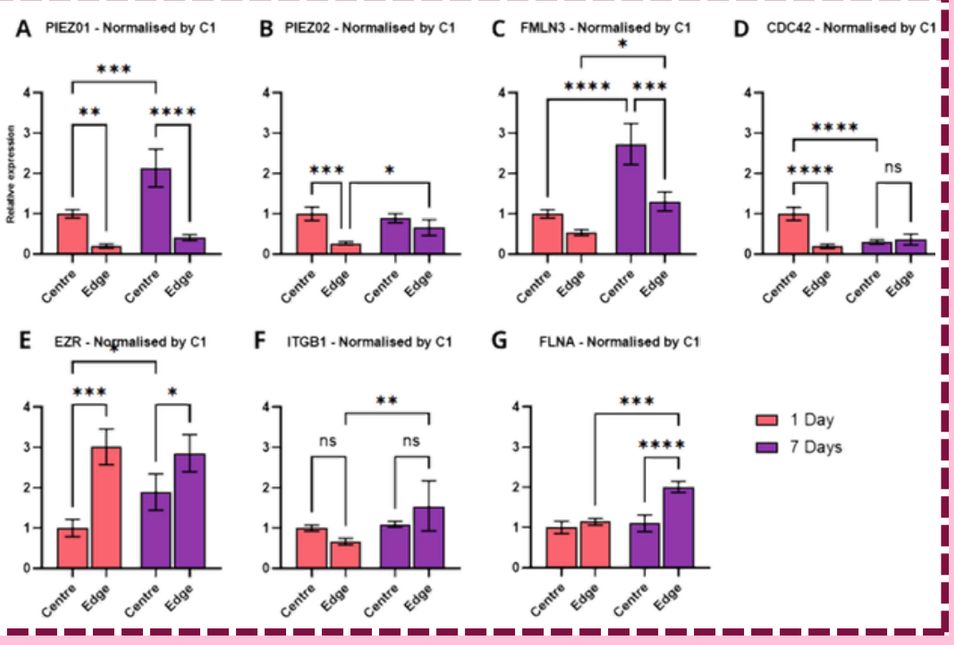
Fig. 6. Gross Acellular Collagen Hydrogel Simulations. Mathematical maps of the hydrogel's max principal stretch (gravity). The boundary is modelled with a fixed bottom face and side surfaces. Bulk mechanics are modelled at 200 Pa (**A, C**) & 500 Pa (**B, D**), & their principal directions are shown by arrows (**C, D**). *Red is low stress; blue is high.* **Performed by the Gao lab.**

3. Mechanotransductive Gene Expression Differs at Centre & Edge



- Cell-laden hydrogel **centres & edges** (Fig. 7) were harvested at **1 & 7 DIV** & flash-frozen.
- To **ensure high-quality RT-qPCR**, 3 samples were pooled for a minimum of 1 x 10^4 cells per sample.
- Subsequently, samples were lysed & RNA extracted using QIAzol, **yielding 16 to 193 ng/µL of RNA**; sufficient for downstream cDNA synthesis.

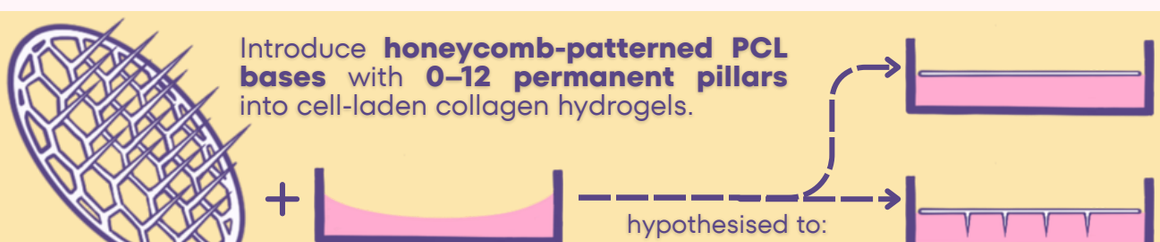
Fig. 7. Hydrogel Extraction Schematic.



PIEZO1/2 is upregulated at the **centre,** indicating an unfavourable mechanical environment, with **overexpression** linked to **maladaptive Ca^{2+} influx** & **oxidative stress**[5].
FMNL3 & **Cdc42** are cytoskeletal organisation genes linked to **angiogenic sprouting.** Their **inverse trend in expression** indicates an **abortive attempt at sprouting** in the centre.
Upregulation of **EZR, ITGB1 & FLNa** at the **periphery** is consistent with **vessel maturation,** facilitating **stronger adhesion & cytoskeletal reinforcement**[6].

Fig. 8. Gene Expression of HUVECs at 1DIV & 7DIV. Data expressed as mean ± SEM analysed by two-way ANOVA. **qPCR performed by the Caporali lab.** *(NS p>0.05; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; **** p<0.00001).*

NEXT STEPS:

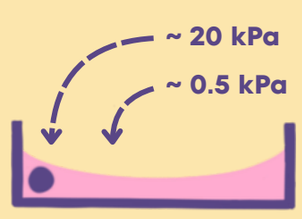


Introduce **honeycomb-patterned PCL bases** with **0–12 permanent pillars** into cell-laden collagen hydrogels.

No pillars: prevent meniscus formation.

Pillars: facilitate focal mechanical patterning control.

Furthermore, **mechanical gradients in disease/injury**, such as stroke (clot: ~50 kPa, neural tissue: ~0.5–2kPa), could be investigated by this strategy to **elucidate changes in angiogenic activity** in the **post-stroke brain.**



CONCLUSIONS:

- **Morphological, biochemical, mechanical, & physical results** gathered thus far indicate the **presence of a spatial gradient.**
- Current gradients are hypothesised to be **introduced** by the **hydrogel's meniscus**.
- Further work is needed to **investigate strategies to customise the spatial gradient** & confirm its impact on HUVEC patterning to achieve **tissue-specific microvascular networks *in vitro***.

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