Mechanical Modulation of Macrophage Phenotype and Function

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Introduction

Macrophages are highly plastic immune sentinels found across various mammalian tissues. These tissue resident macrophages (TRMs) reside in specific niches where they maintain local homeostasis. These tissue niches confer them their identity via a process referred to as “niche imprinting” [1]. Macrophages are highly mechanosensitive [2], however the role of tissue mechanics on niche imprinting and TRM specification remains elusive.

Moreover, perturbations in tissue stiffness, viscoelasticity, etc; during various pathologies and aging are often overlooked in favour of biochemical changes under these conditions. Hence, it is of interest to study the effects of mechanics on macrophage phenotypes in both health and disease.

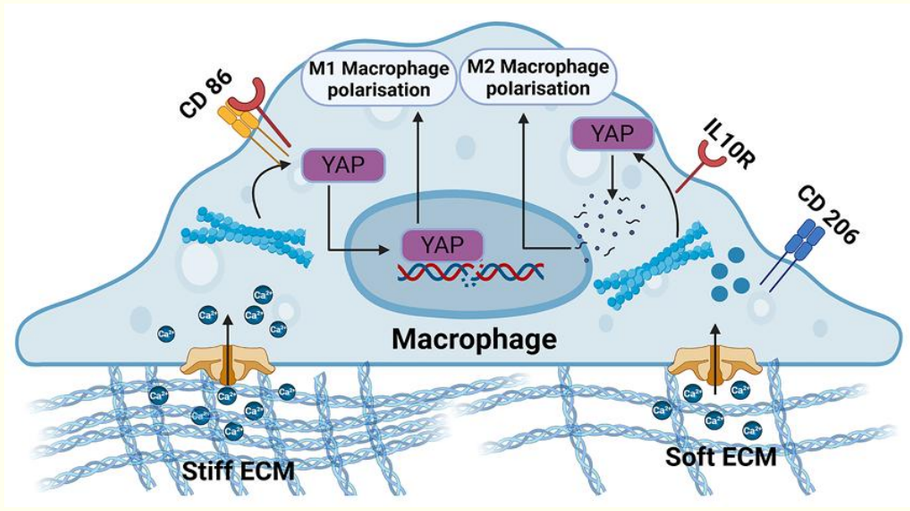

Figure 1. Stiffness-dependent mechanotransduction driven by Piezo1-YAP signalling promotes macrophage polarisation. Taken from [2].

Objectives

This work aims to develop new strategies for directing immune cell identity by engineering physical microenvironments. The core objectives here include:

- Develop a hydrogel system with tuneable Young’s Modulus to mimic the mechanical diversity found in soft tissues
- Elucidate how changes in mechanical properties affects human blood-monocyte derived macrophage phenotypes using FLOW cytometry and immuno-assays

Hydrogel System

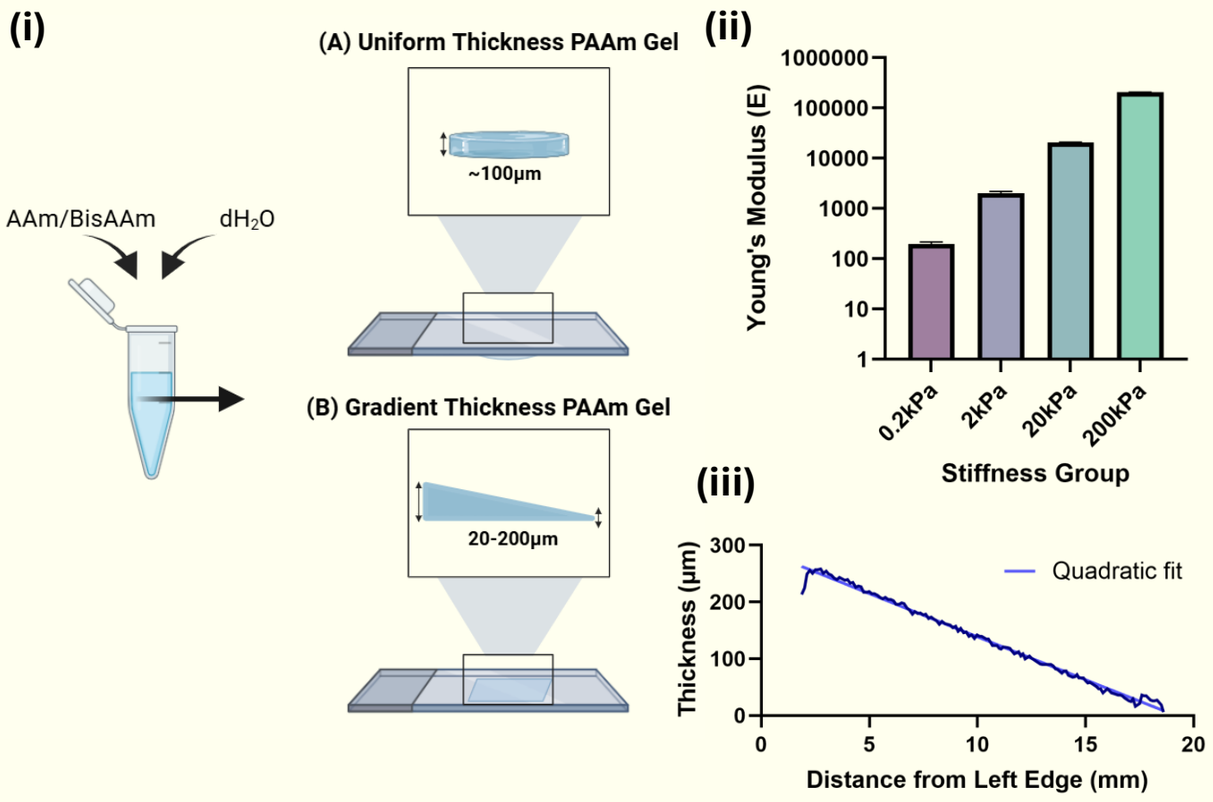

Figure 2. (i) Fabrication of 2D polyacrylamide (PAAm) gels with uniform and gradient thickness. (ii) Measurement of Young’s modulus (E) using nanoindentation. (iii) Derivation of the quadratic relationship describing gel thickness as a function of distance from the thick end (E = 0.2 kPa).

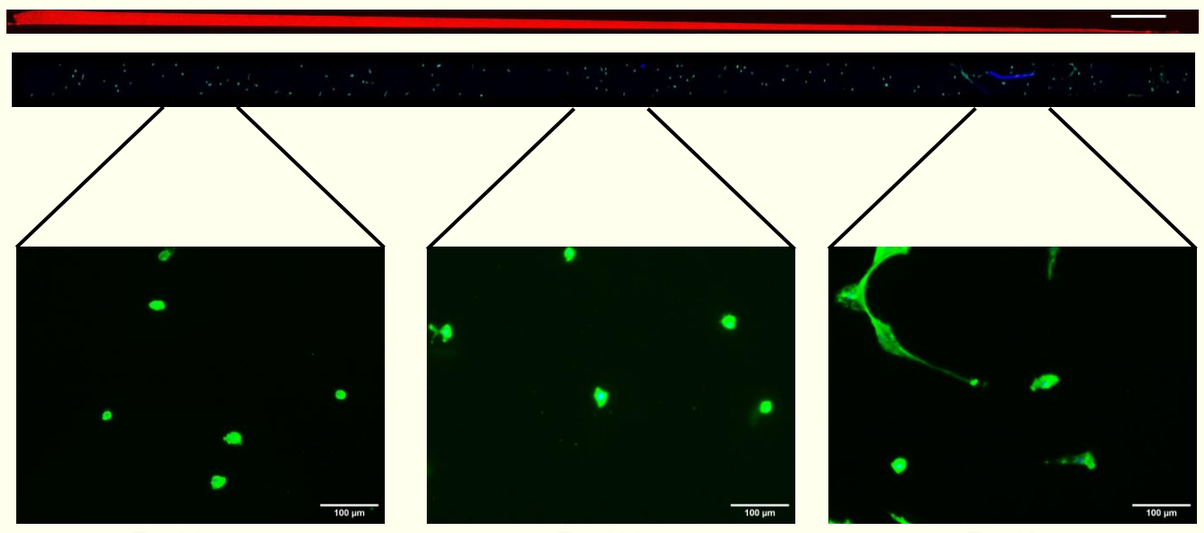

Figure 3. Confocal reconstruction of 2D PAAm gel thickness gradient and cell morphology response. Confocal z-stack reconstruction (top) of fluorescent bead–embedded PAAm gels, used to determine gel thickness as a function of position (scale bar = 1 mm). Below, a corresponding spatial cell map is shown. Y201s were cultured on the gradient gel and stained for phalloidin and DAPI. Local cell morphology at three representative regions corresponding to gel thicknesses (T) of 280 µm, 100 µm, and 43 µm is depicted (scale bar = 100µm) (E =0.2kPa)

References & Acknowledgements

[1] Guilliams, M., Thierry, G.R., Bonnardel, J. and Bajenoff, M. (2020). Establishment and Maintenance of the Macrophage Niche. *Immunity*, [online] 52(3), pp.434–451. doi:https://doi.org/10.1016/j.immuni.2020.02.015.

[2] Khanmohammadi, M., Mirzaalikhan, Y. and Baratchi, S. (2025). Immunity in motion: The role of mechanics in macrophage biology. *Cell Chemical Biology*, [online] 32(12), pp.1442–1457. doi:https://doi.org/10.1016/j.chembiol.2025.11.004.

This work is supported by Engineering and Physical Sciences Research Council (EPSRC) via the Mechanomeds Programme (HT2050)

Macrophage Response

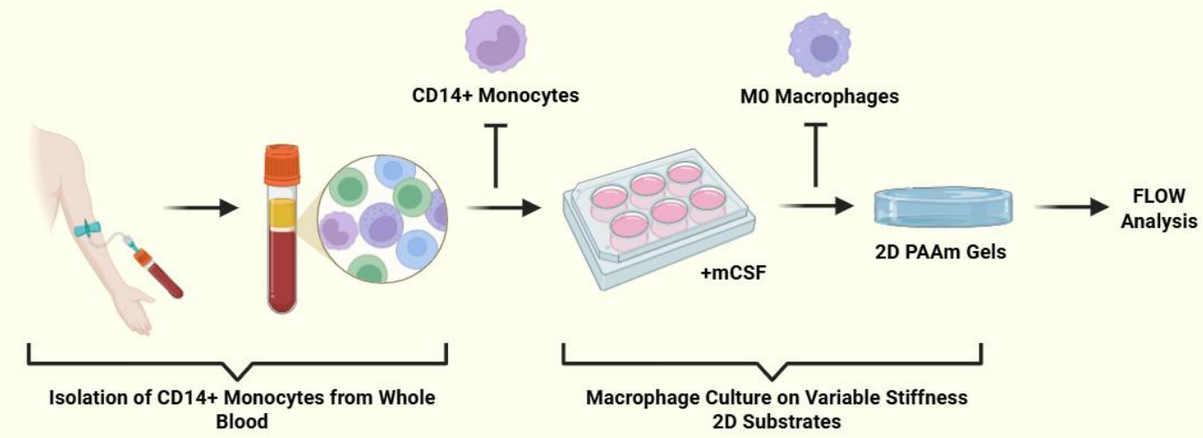

Figure 4. Experimental workflow for human monocyte-derived macrophage differentiation and mechanotransduction assay on PAAm gels. CD14+ monocytes were isolated from human whole blood and differentiated into M0 macrophages using M-CSF. Cells were then seeded onto collagen-functionalised PAAm gels spanning a stiffness range of 0.2–200 kPa. On day 7, flow cytometry was performed to assess macrophage surface marker expression in response to substrate stiffness.

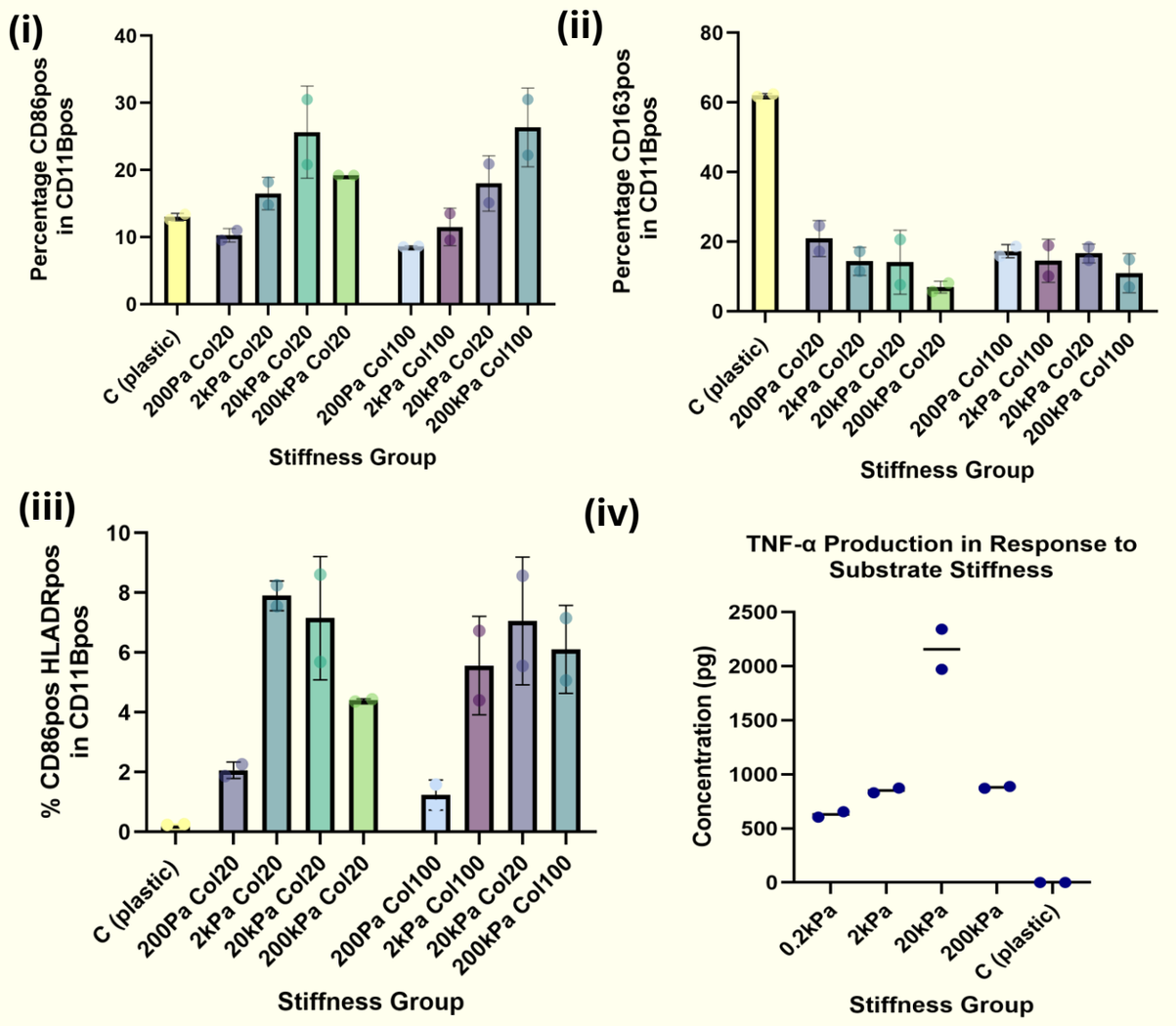

Figure 5. Stiffness-dependent macrophage polarisation and inflammatory response on collagen-functionalised PAAm gels. (i–iii) Flow cytometry analysis of CD11b+ macrophages showing the percentage of CD86+ cells, CD163+ cells, and CD86+HLADR+ double-positive cells across a range of substrate stiffnesses and two collagen coating densities (20 and 100 µg/mL), including a plastic control. (iv) Quantification of TNF-α secretion measured by ELISA under 20 µg/mL collagen conditions across all stiffness groups. Collectively, these data show stiffness-dependent modulation of macrophage activation and inflammatory phenotype.

Future Work

- Incorporate a viscoelastic component to the hydrogel system to mimic stress relaxation properties
- Elucidate the mechanosensing pathways involved
- Transition to 3D encapsulated microenvironments
- Employ label-free microscopy techniques like FLIM to characterise functional macrophage phenotypes in real-time