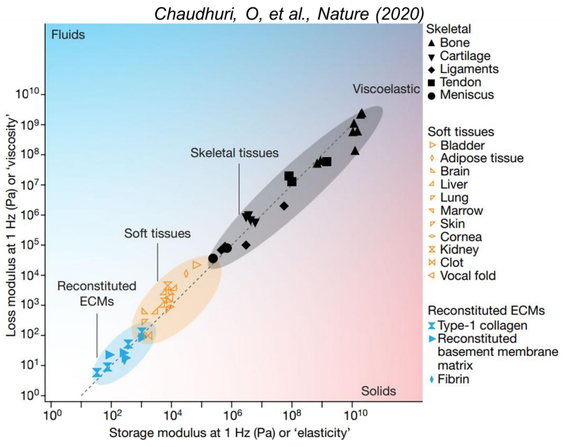
Macrophage polarization in viscoelastic microenvironments

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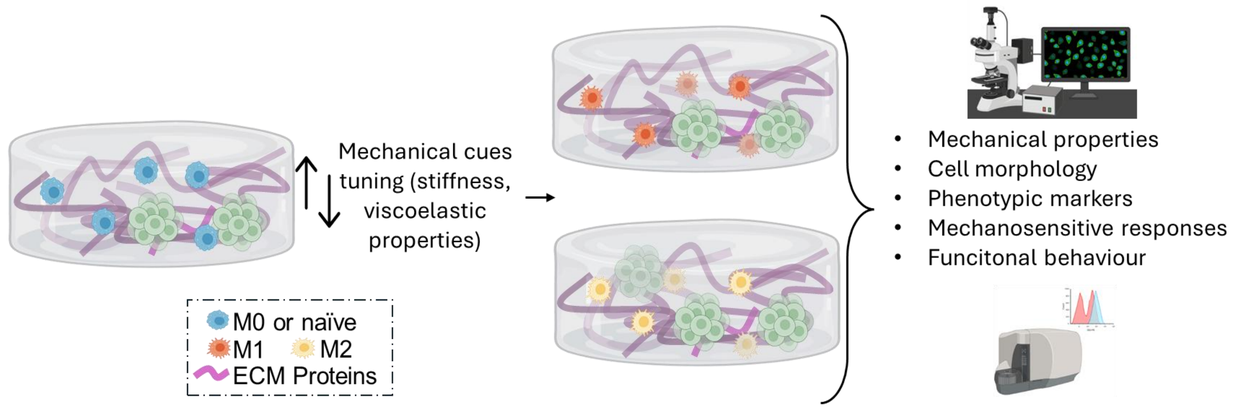
Introduction

- Extracellular matrix (ECM) mechanical properties are known to regulate **macrophages** and **mesenchymal stem cells** (MSCs) [1].
- It is known that biological tissues and the ECMs are **viscoelastic** rather than purely elastic, showing time-dependent mechanical behavior with energy dissipation under applied stress [2].
- Studies have mainly explored how matrix **stiffness** affects macrophages and MSC behaviour [3]. However, the impact of viscoelasticity rather than matrix stiffness on **macrophages polarization** to pro-inflammatory (M1) or pro-regenerative (M2) phenotypes remains largely unexplored.



Methodology overview

- 3D **hydrogel** platform with independently tuned **elastic** and **viscoelastic** properties functionalised with **full-length FN proteins.**
- **Platform enables the** co-culture of naïve macrophages and MSC to investigate mechanically triggered crosstalk and **macrophage polarization** in physiologically relevant microenvironments.



Hydrogel

- **Families of 3D Polyethylene glycol-maleimide (PEG-Mal) hydrogels functionalized with fibronectin (FN)** [4]. Each family with similar stiffnesses, both elastic (V-) or viscoelastic (V+).

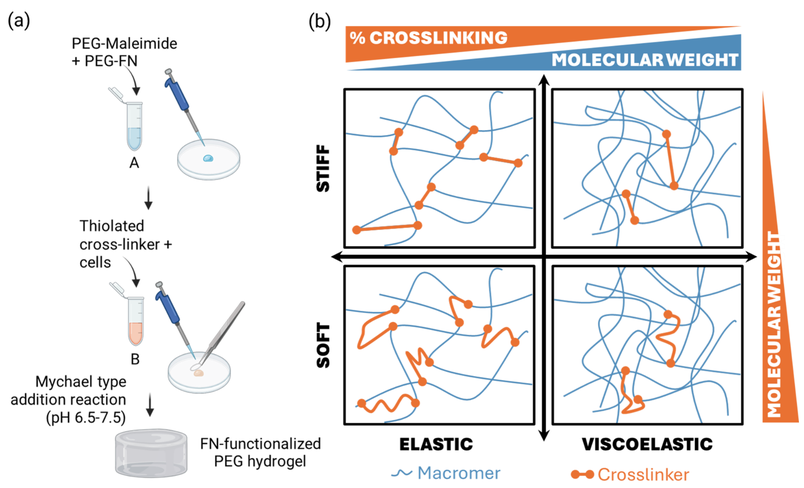


Fig. 1: (a) Hydrogel preparation: two pre-gel droplets are mixed on a hydrophobic surface and crosslinked at 37ºC for 15 min. (b) Hydrogel mechanical properties tuning strategy: stiffness is controlled by crosslinking density (via crosslinker molecular weight) and viscoelastic properties by adjusting the maleimide-thiol crosslinking ratio.

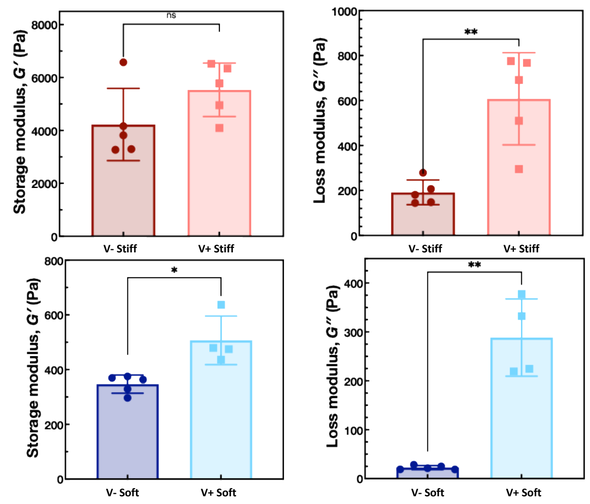


Fig. 2: Frequency dependant behaviour of PEG hydrogels (1% strain). Storage (G') and loss (G'') moduli of hydrogel pairs are shown. One-way ANOVA (ns: p-value ≥0.05, *p-value < 0.05, **p-value < 0.01).

3D Platform (hydrogel + cells)

- **Encapsulated MSC spheroids and THP-1**

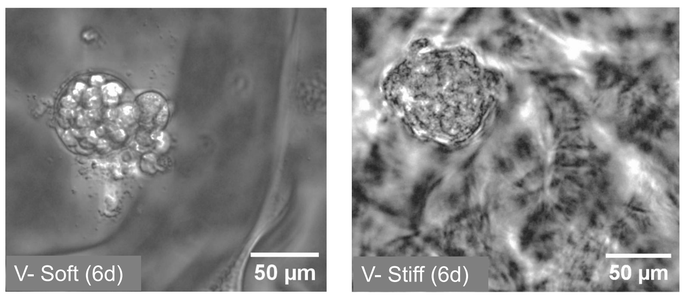


Fig. 6: BF images of MSC spheroids embedded in PEG-Mal soft and stiff elastic (V-) gels.

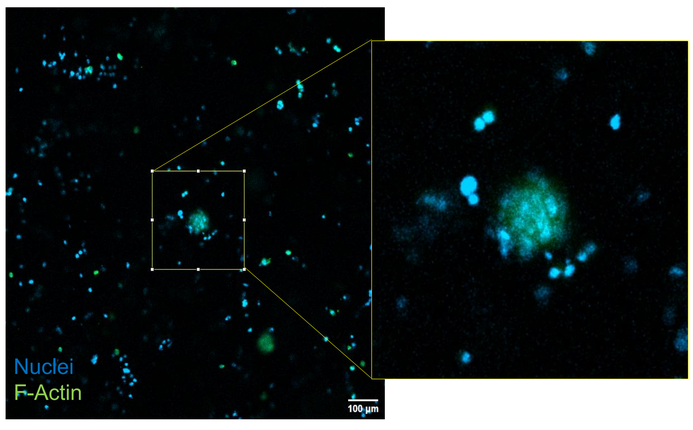


Fig. 7: Confocal images of THP-1 + MSC spheroids in V- PEG-Mal hydrogels stained for nuclei (DAPI, blue) and F-Actin (Phalloidin AF488, green).

- **Brillouin microscopy**: non-destructive, label- and contact-free technique for real-time viscoelasticity monitoring.

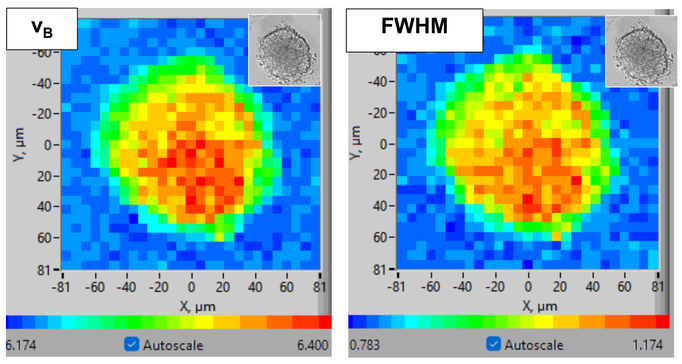
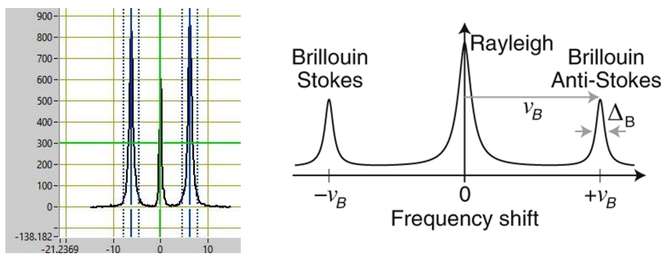


Fig. 8: Brillouin heatmaps showing the Brillouin shift (vB) and full-width at half maximum (FWHM), related to hydrogel and spheroid mechanical properties.



Cells

- **MSC spheroids**

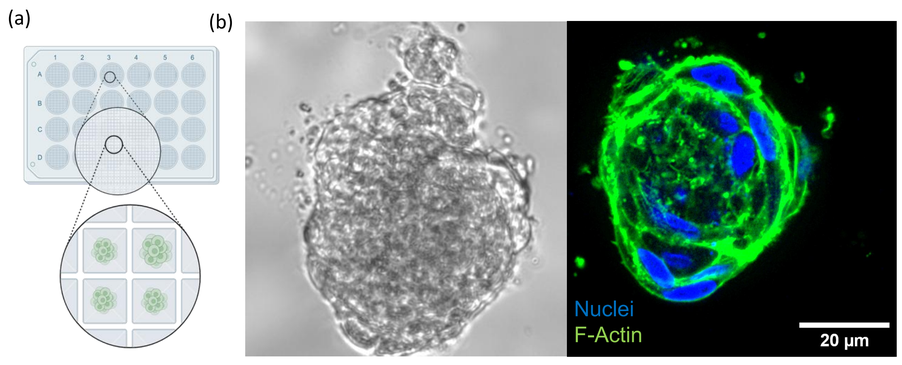


Fig. 3: (a) MSC spheroids are formed by aggregation on microwells. (b) Brightfield (BF) (x20) and confocal (x40) images stained for nuclei (DAPI, blue) and F-Actin (Phalloidin AF488, green).

- **Macrophages (derived from THP-1)**

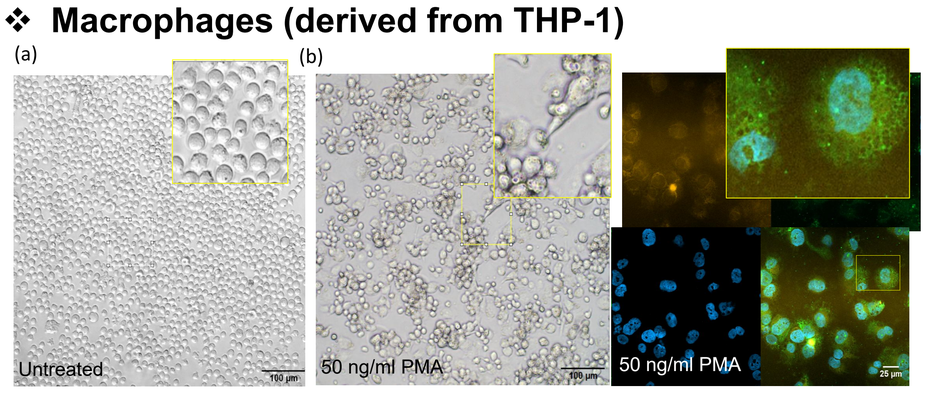


Fig. 4: THP-1 cells. (a) Untreated THP-1 cells on FN (10 ug/mL), BF (x20). (b) THP-1 cells treated with PMA (50 ng/mL, 2 days + 3 days rest) on FN (10 ug/mL), BF (x20) and confocal (x63) images stained for CD14 (monocyte-associated marker, orange) and CD68 (phagocytic macrophage marker, green). Nuclei stained with DAPI (blue).

- **Macrophages (derived from blood monocytes)**

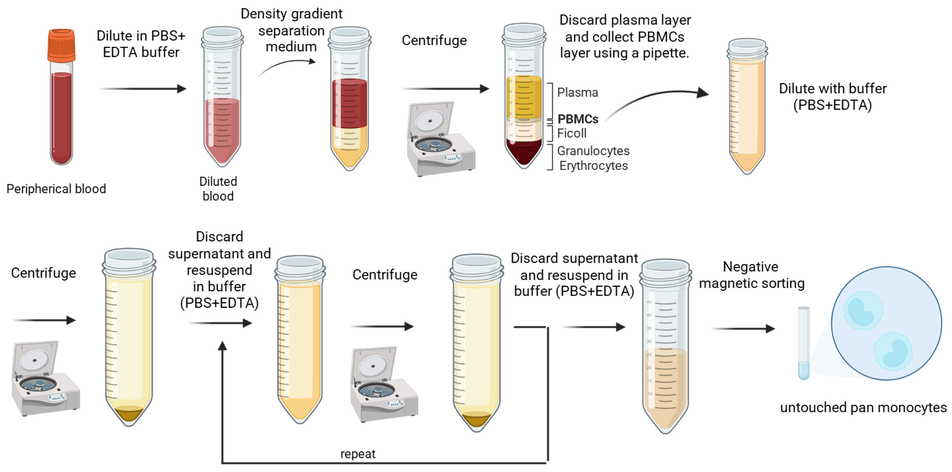


Fig. 5: Protocol for monocyte isolation from whole blood through gradient separation and magnetic sorting.

Outlook

- We have developed a tuneable 3D PEG-MAL hydrogel platform with controlled stiffness and viscoelastic properties in which to study MSC-macrophage interaction.
- This project will allow us to understand the immune microenvironment in a mechanically relevant biomimetic system.
- Ongoing work focuses on further system characterization and optimization, as well as obtaining donor-derived monocytes and differentiating them into M0 macrophages to study this interactions.

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