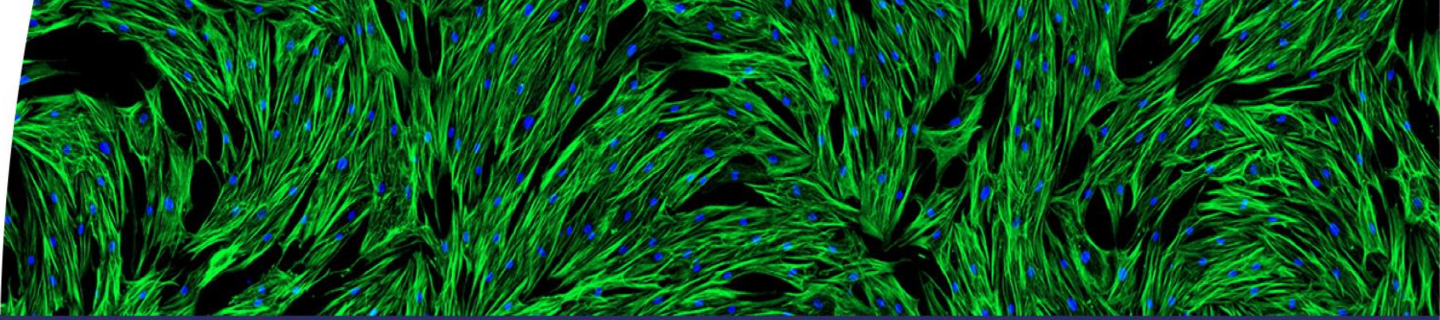


stemNICHE
Engineering Bone Marrow for Discovery

Remodelling bone marrow niche: A 2.5D *in vitro* platform for discovery

Savvas Ioannou[1], Graham J. Day[1], Martín Gómez Redondo[3], Monica P. Tsimbouri[1], Manuel Salmeron-Sanchez[1,2], Hannah Donnelly[1], Matthew J. Dalby[1]

[1]Centre for the Cellular Microenvironment, Advanced Research Centre (ARC), 11 Chapen Lane, University of Glasgow, Glasgow, United Kingdom.
[2]Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.
[3]Institute of Biomedical Research of Salamanca (IBSAL)

1. INTRODUCTION AND BACKGROUND

- The bone marrow (BM) niche complex promotes the quiescence and activation of hematopoietic stem cells (HSCs).
- Mechanical cues (matrix stiffness) and biochemical signals (MSC secretome, ECM deposition) shape the niche's function.
- Understanding this dynamic environment is essential for improving the outcome of various diseases like leukaemia **(Figure 1).**

Hypothesis: *In vitro* remodelling of the BM niche using functional hydrogels enhances mechanosensing and supports the maintenance of primitive HSCs.

Objectives: 1: Characterise MSCs' niche phenotype in 2.5D.
2: Measure cell mechanosensing via focal adhesions (FA).
3: Evaluation of HSC phenotype.

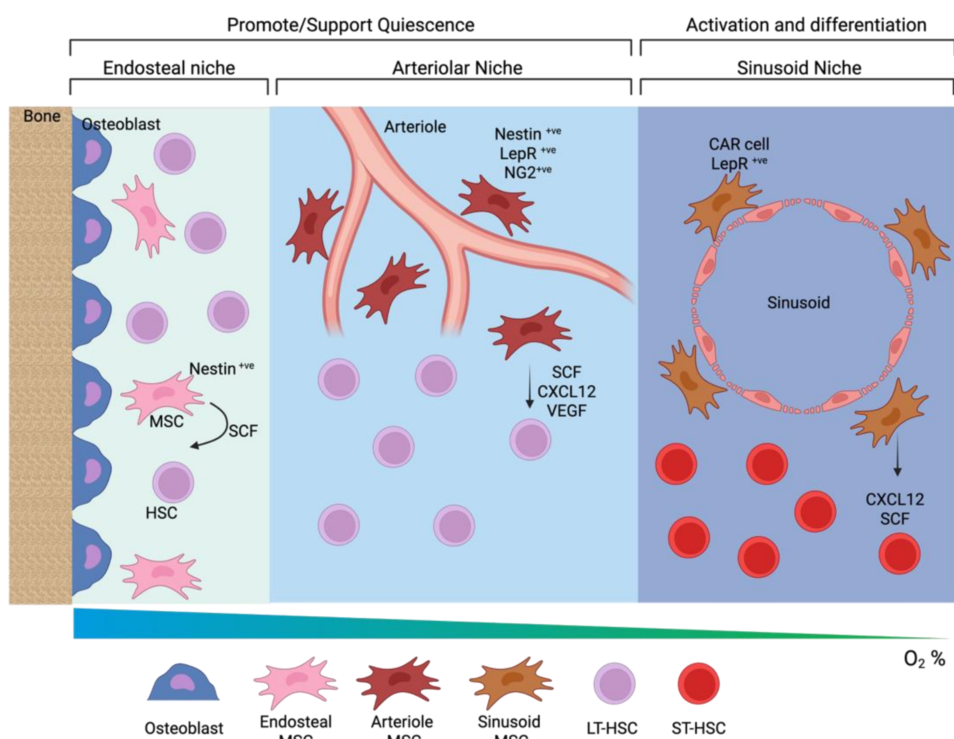


Figure 1. Schematic representation of the BM niche. The BM niche comprises numerous cell types. Here, we focus on the MSCs and their supportive role towards HSCs at the various subtypes of the niche.

2. METHODS

- PEG maleimide hydrogels were selected as the biomaterial.
- The inert PEG hydrogels were functionalised with FIBRONECTIN (FN).
- The degradability of the gels was achieved with the addition of the VPM peptide[2] **(Figure 2).**

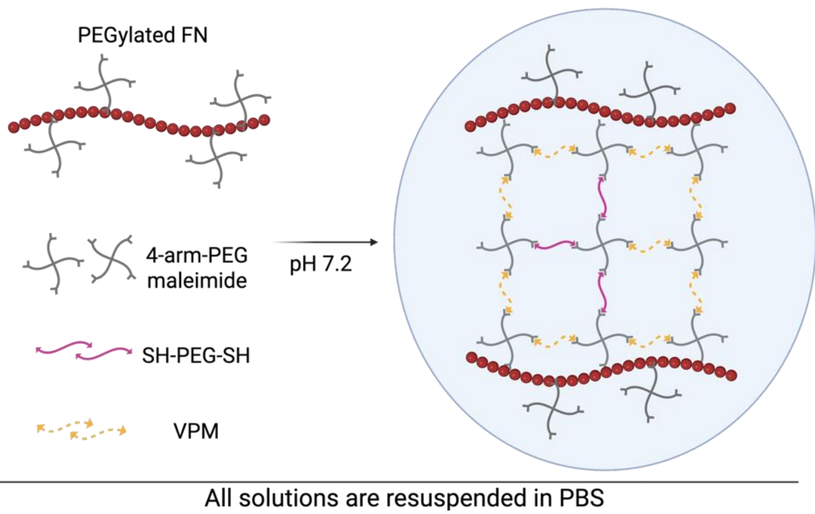


Figure 2. Formation of FN-PEG hydrogels through thiol-Michael addition. SH-PEG-SH crosslinking with functionalised FN and VPM, forming a degradable network.

- MSCs adhere basally to stiff plastic and a soft FN-PEG gel partially embeds on their apical surface, combining 2D adhesion with a 3D-like confinement, creating the 2.5D system shifting the MSC behaviour.
- The resulting phenotype intends to mimic features of the *in vivo* marrow niche **(Figure 3)**.
- Cells are maintained in this system under low-serum conditions.

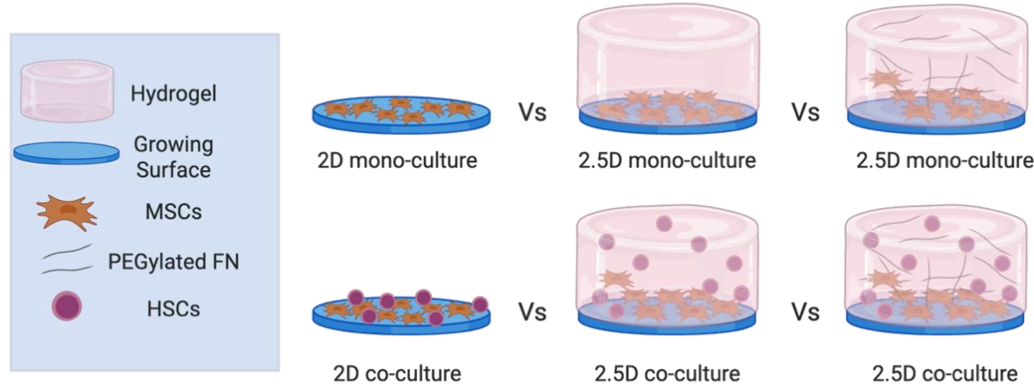
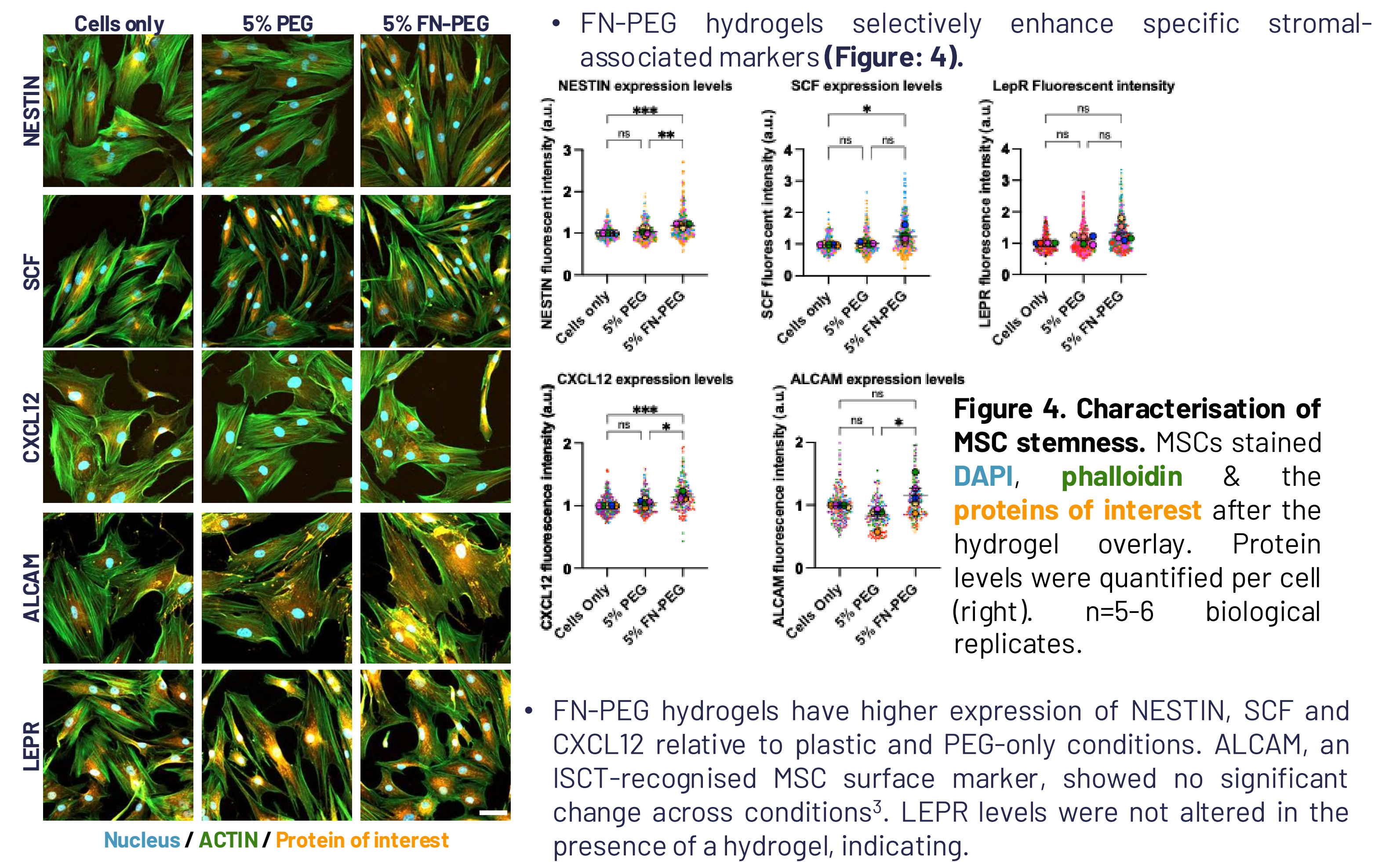


Figure 3. Schematic representation of the 2.5D culture system. MSCs are seeded on plastic to create a stromal layer, and a hydrogel is overlayed. Other cell types such as HSCs can be introduced.

3. RESULTS



- FN-PEG hydrogels selectively enhance specific stromal-associated markers **(Figure: 4).**

Figure 4. Characterisation of MSC stemness. MSCs stained DAPI, phalloidin & the proteins of interest after the hydrogel overlay. Protein levels were quantified per cell (right). n=5-6 biological replicates.

- FN-PEG hydrogels have higher expression of NESTIN, SCF and CXCL12 relative to plastic and PEG-only conditions. ALCAM, an ISCT-recognised MSC surface marker, showed no significant change across conditions[3]. LEPR levels were not altered in the presence of a hydrogel, indicating.

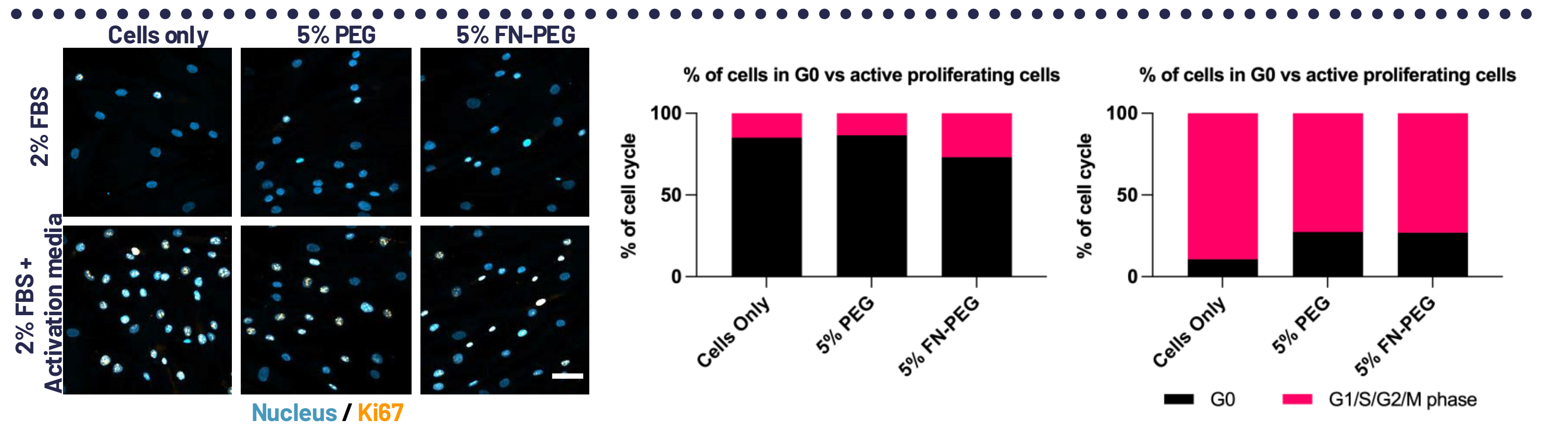


Figure 5. Primary MSCs have the ability to re-enter a proliferation state. Representative images of primary MSCs stained with DAPI (blue) and ki67 (gold) after 14 days of a hydrogel overlay. The levels of ki67 were quantified per cell. n=2 biological replicates, n=6 technical replicates per condition.

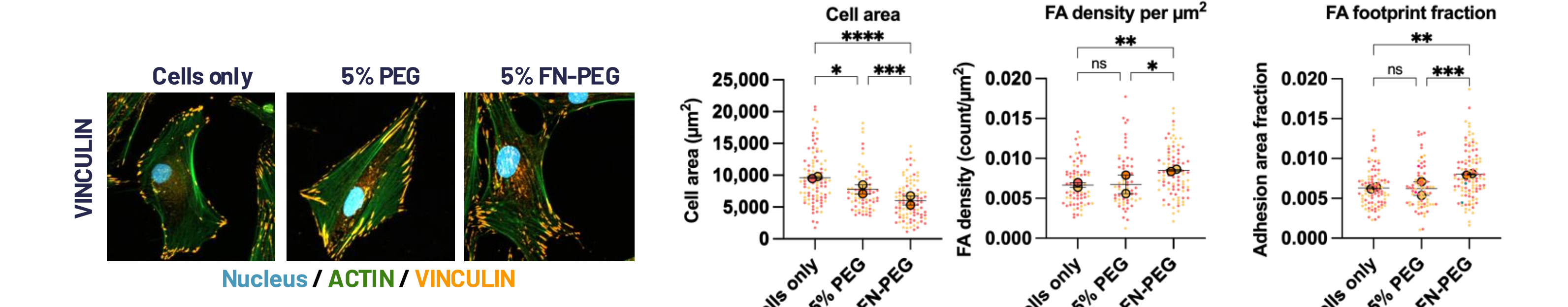


Figure 6. Gel-cell interaction in a 2.5D system. Representative images of MSCs stained with DAPI (blue), phalloidin (green) and Vinculin (gold) after 7 days of a hydrogel overlay. VINCULIN metrics were quantified per cell. n=2 biological replicates.

- Low serum conditions induced a reversible growth arrest. Activation media restored proliferation, confirming that the MSCs preserved the ability to re-enter the cell cycle. This indicates. Quiescent state, not senescence.
- It was demonstrated that MSCs were confined by the PEG and FN-PEG hydrogels.
- Cell-gel interaction was demonstrated by VINCULIN staining; MSCs overlayed by FN-PEG hydrogel demonstrated a higher focal adhesion (FA) density per µm² and a larger FA footprint **(Figure 5-6).**

- A 2.5D MSC set-up maintains a primitive HSC phenotype, supporting niche-relevant haematopoiesis and colony forming ability **(Figure: 7).**

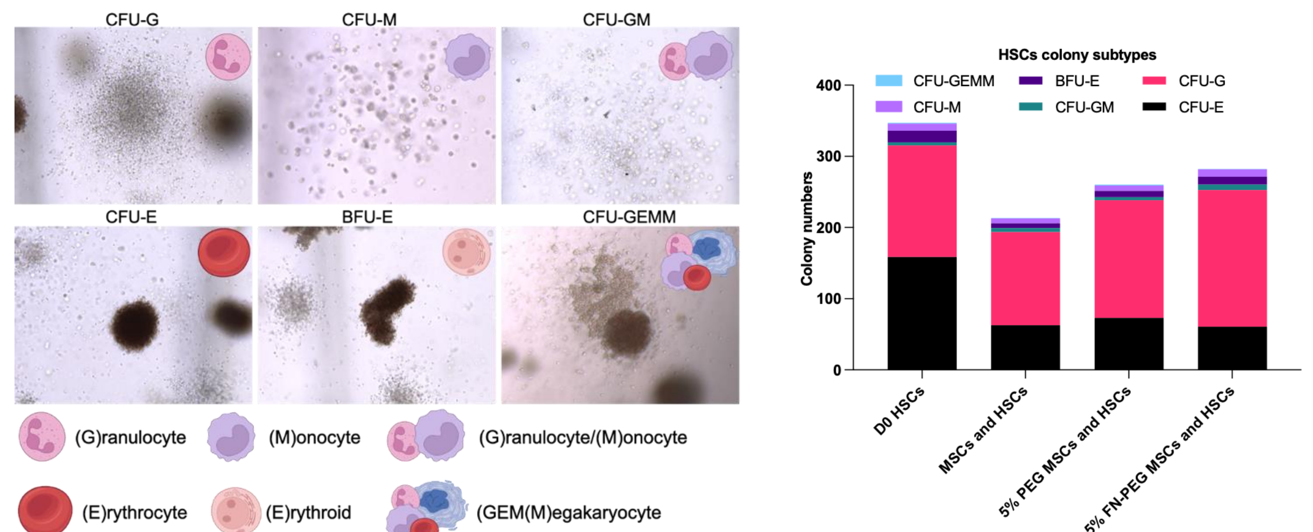


Figure 7. MSCs support HSCs in a 2.5D set-up. MSC and HSC co-culture was took place for 7 days. Flow cytometry analysis showed that HSCs maintain a primitive state in the presence of a PEG and a FN-PEG hydrogel and that was reflected on their ability to form colonies. n=2, 12-technical replicates per condition.

4. DISCUSSION AND CONCLUSIONS

- FN-PEG overlays promote MSC stemness.
- MSCs interact with their overlayed FN-PEG hydrogel.
- FN-PEG-treated-MSCs maintain HSCs in a primitive state.

Next steps:
- ECM remodelling will be explored.
- The regenerative niche will be expanded, and other cell types will be introduced to it.

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Contact: Savvas.Ioannou@glasgow.ac.uk

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