Polymer-ECM Substrates Regulate Mesenchymal Stromal Cell Phenotype via Stiffness Modulation

Paris Alexandros Kalli[1], Monica P. Tsimbouri[1], Savvas Ioannou[1], Vineetha Jayawarna[1], Melanie Jimenez[2], Manuel Salmeron-Sanchez[1,3], Matthew J. Dalby[1]

[1]University of Glasgow, [2]University of Strathclyde, [3]Institute for Bioengineering of Catalonia

Introduction

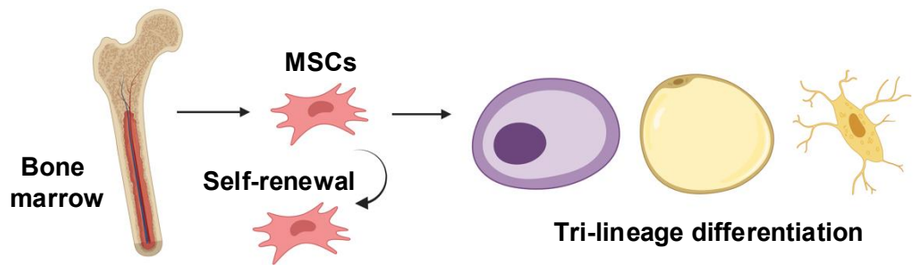


Figure 1: Flowchart explaining how MSCs are derived and the vital properties they possess.

The main aim is to **improve MSC phenotype** using:

- Tissue engineering approaches with the help of **biomaterials.**
- Synthetic **material – extracellular matrix (ECM) protein** interfaces,

Problem:
Conventionally used tissue culture plastic (TCP) surfaces promote **senescence associated phenotypes** and inhibit large-scale MSC expansion.

Proposed Solution:
ECM proteins like Fibronectin (FN) and Laminin (LM) provide a more **native** environment for MSCs to grow on.
Biomaterial Poly (ethyl acrylate) (PEA) needed to unravel globular structure.

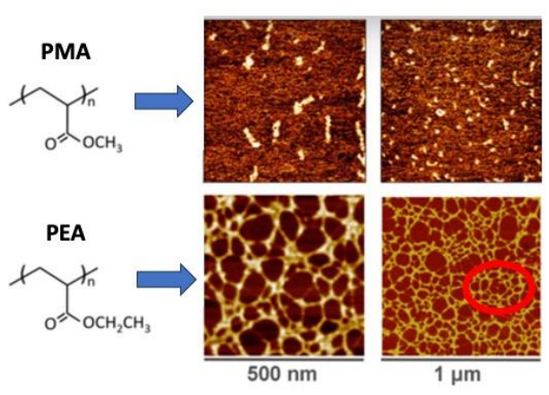
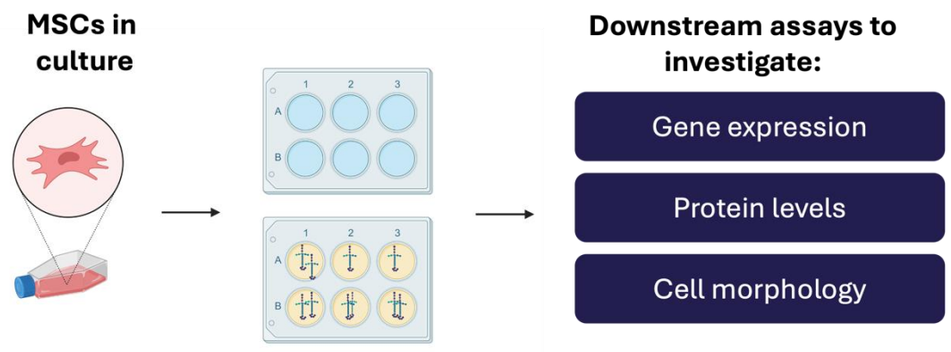


Figure 2: FN adsorption onto PEA can form a mesh-like nanonetwork, whereas on the PMA polymer, a globular FN conformation is observed[1].

Methodology



PEA+LM Supports an Improved Cellular Homeostasis

Ascertaining Protein Levels Using an In-Cell Western Assay

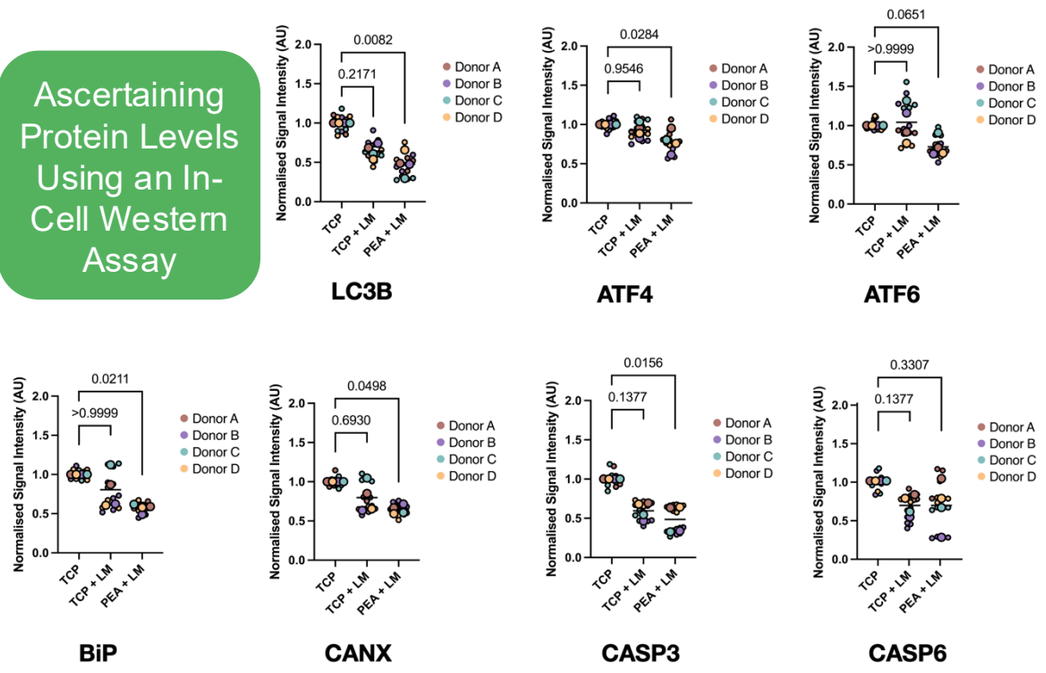


Figure 4: MSCs grown for 7 days exhibit decreased protein expression on PEA+LM in markers pertaining to autophagy, the unfolded protein response, protein folding and stress induced apoptosis. *Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons correction.*

PEA+LM Reorganises Cytoskeletal Proteins and Alters MSC Stiffness

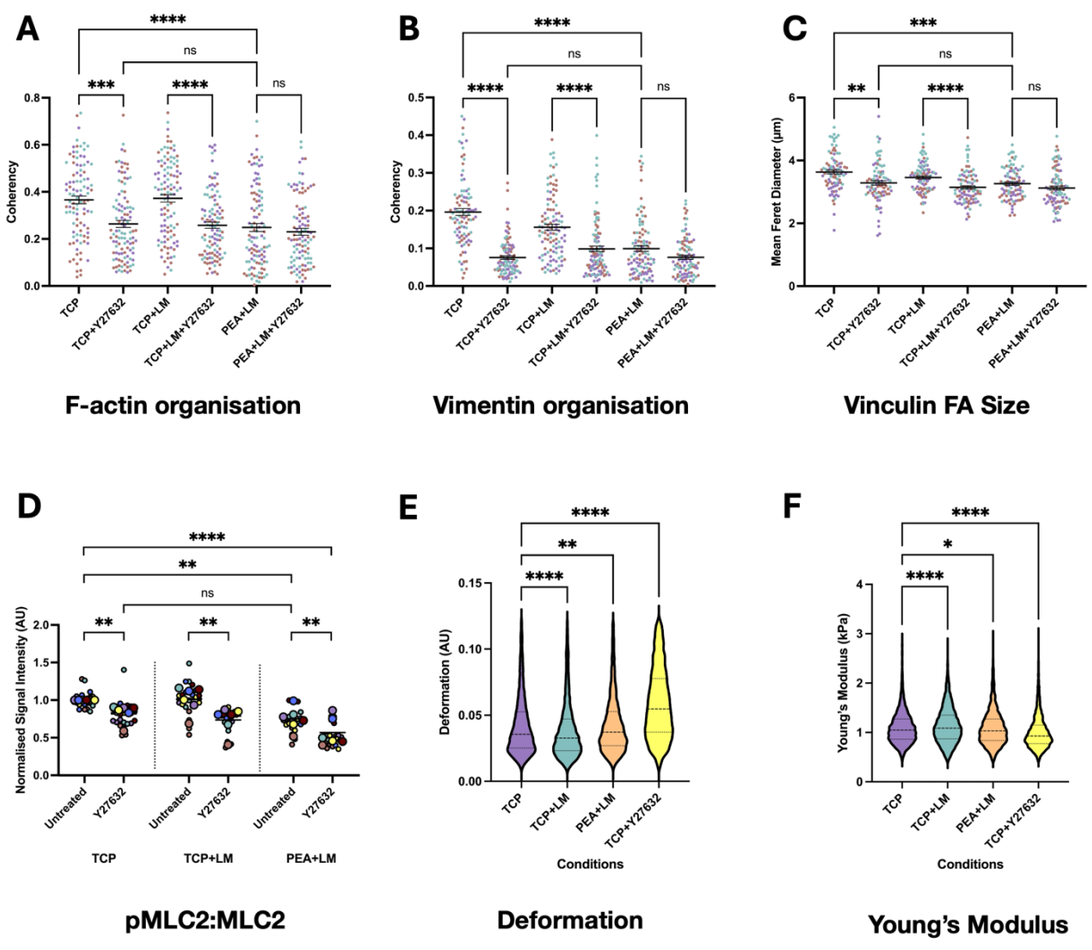


Figure 3: ROCK inhibition using Y-27632 rearranges cytoskeletal organisation and decreases cytoskeletal tension. Organisation of **A)** F-actin and **B)** vimentin filaments in MSCs cultured on different material substrates over 7 days. **C)** Mean feret diameter of focal adhesions formed on different material substrates. **D)** Normalised signal intensity ratio of pMLC2 to MLC2. **E)** Deformation and **F)** Young's modulus of detached MSCs in 3D on different material substrates. ***A-D)*** *Two-way ANOVA followed by Šidák's test for multiple comparisons.* ***E-F)*** *Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons correction.*

Conclusions

The PEA+LM surface is **superior** in comparison to TCP and TCP+LM.

↑ Proliferation	↓ Stress-induced Apoptosis	↓ Mechanotransduction
↓ Senescence	↓ Autophagy	↓ Cell Stiffness
↓ Chaperones	↓ ER Stress	↑ Deformability

Future Work

1. Build on the mechanobiology data – Investigate the nuclear localisation of YAP.
2. Further understand the mechanism behind the improvement in cellular homeostasis.
3. Investigate any relationships between the mechanophenotype of cells and the improvement in cellular homeostasis.

1. **Llopis-Hernández, V., *et al*., Science advances, 2016. 2(8): p. e1600188.**
Images created using BioRender and analysis was performed using GraphPad Prism.

Acknowledgements: We thank EPSRC and SFI for funding the lifETIME Centre for Doctoral Training (EP/S02347X/1).