Bioengineering Surfaces to Preserve Mesenchymal Stromal Cells' Growth *in vitro*

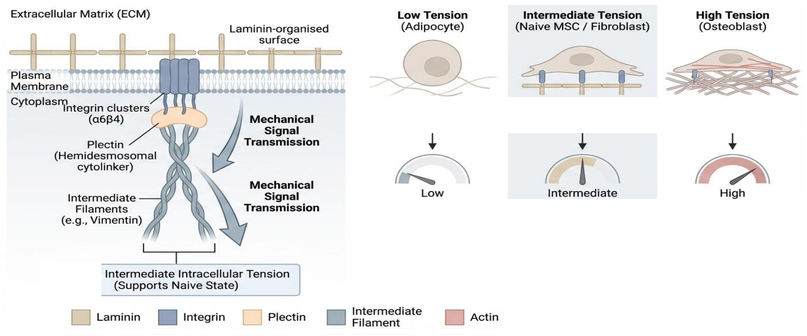
Yusuf Ayten[1], Monica P Tsimbouri[1], Vineetha Jayawarna[2], Richard, O.C. Oreffo[3], Manuel Salmeron-Sanchez[2], Catherine C Berry[1], Matthew J Dalby[1]

[1]Centre for the Cellular Microenvironment, School of Molecular Biosciences, University of Glasgow
[2]Centre of Cellular Microenvironment, James Watt School of Engineering, University of Glasgow
[3]Bone and Joint Research Group, Centre for Human Development, Institute of Developmental Sciences, University of Southampton

1. Introduction

Mesenchymal stromal cells (MSCs) are promising candidates for cell-based therapy; however, during in vitro expansion, the stressful properties of the tissue culture environment cause a dramatic loss of their proliferative capacity and phenotypic stability. We hypothesise that laminin-presenting PEA substrates provide a biomimetic microenvironment which is imtermediate intracellular tension level that preserves MSC function (Figure 1). We further investigate whether α6 integrin mediates this effect.

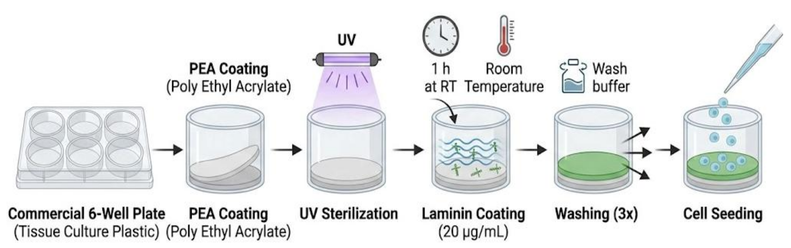
Figure 1.



2. Approach

- PEA-coated surfaces functionalised with laminin (20 µg/mL)(Figure 2).
- Senescence- associated secretomes (SASPs) and proliferation assessed by ICW (p16, p21, p53, Ki67, TERT, AKT)
- Cell cycle analysed by flow cytometry
- Mechanism probed via α6 integrin blocking antibody (10 µg/mL).
- YAP1 localisation and MSC phenotype evaluated

Figure 2



3. Results

3.1 The effect of the material on MSC's phenotype and senescence

Figure 3. PEA+LM delays MSC senescence and preserves phenotype.

Figure 3.A

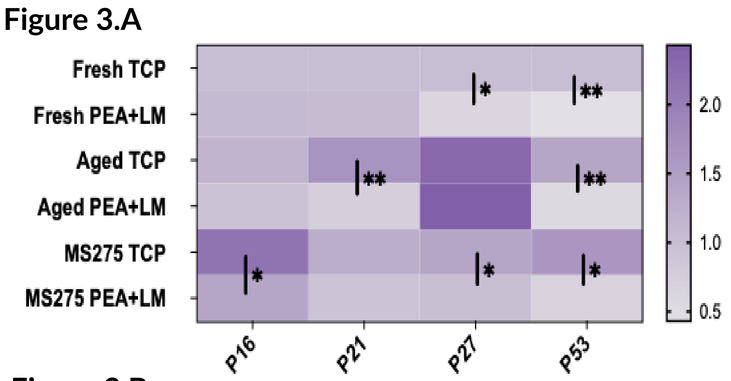


Figure 3.B

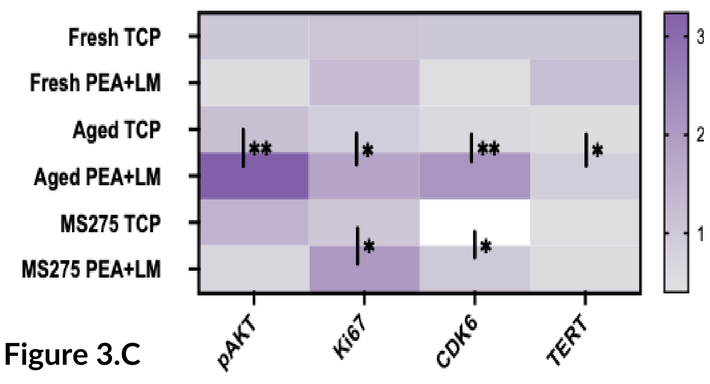
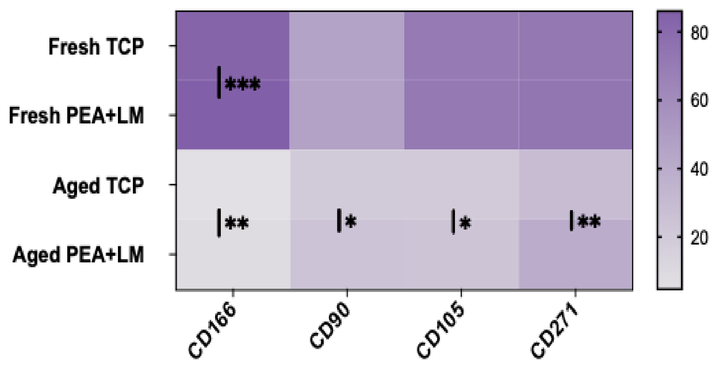


Figure 3.C



A–B: Heatmaps showing senescence and proliferation markers. Standard culture conditions increase senescence (↑ SASP) and reduce proliferation and survival markers (↓ pAKT, Ki67, CDK6, TERT). PEA+LM mitigates these effects, maintaining a more proliferative state. **C:** Heatmap of MSC surface markers (CD166, CD90, CD105, CD271). Marker expression declines under standard conditions but is preserved with PEA+LM. Data represent mean (n = 3); *p < 0.05, **p < 0.01, ***p < 0.001.

3.2 MSC's self-renewal: ERK1/2 signalling and cell cycle regulation

Figure 4. PEA+LM restores cell cycle progression and signalling activity.

Figure 4.A

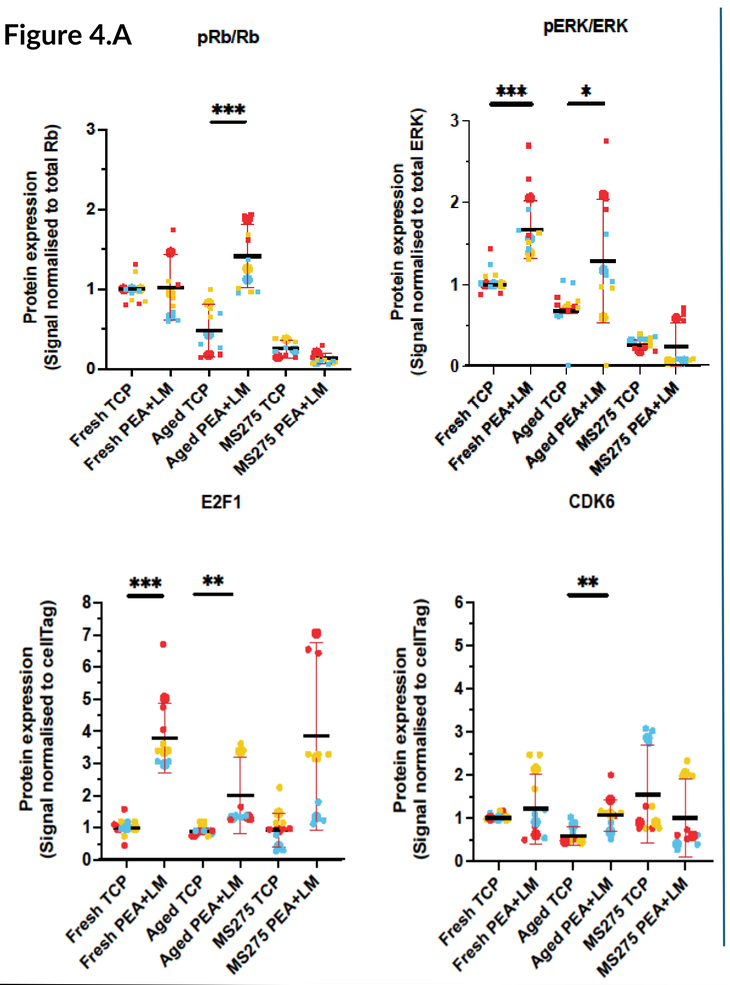
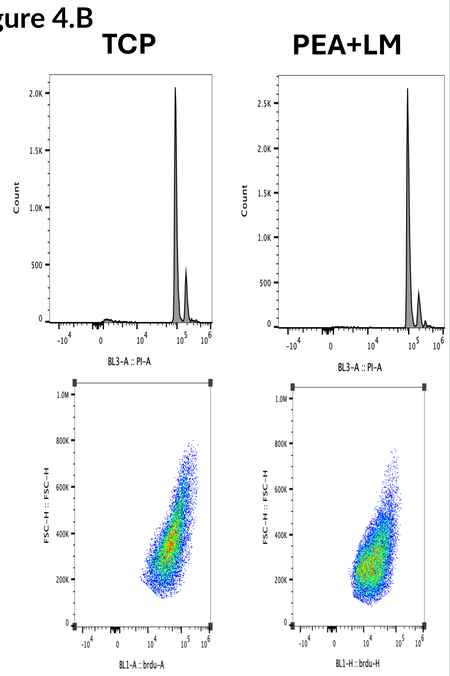


Figure 4.B



	G1	S	G2
Fresh TCP	68.7	1.97	15.53
Fresh PEA+LM	64.33	1.437	15.50
Aged TCP	64.97	3.44	18.03
Aged PEA+LM	61.67	2.24	17.37

A: Relative expression of key regulators (pRb/Rb, pERK/ERK, E2F1, CDK6). PEA+LM enhances proliferation-associated signalling compared to standard conditions. **B:** Cell cycle arrest analysed by flow cytometry. PEA+LM promotes more cell cycle progression. Cells were synchronised by serum starvation prior to analysis. Data represent mean ± SD (n = 3); *p < 0.05, **p < 0.01, ***p < 0.001.

3.3 Controlling the mechanotrunsduction: blocking α6 integrin

Figure 5. α6 integrin regulates YAP1 translocation and MSC function.

Figure 5.A

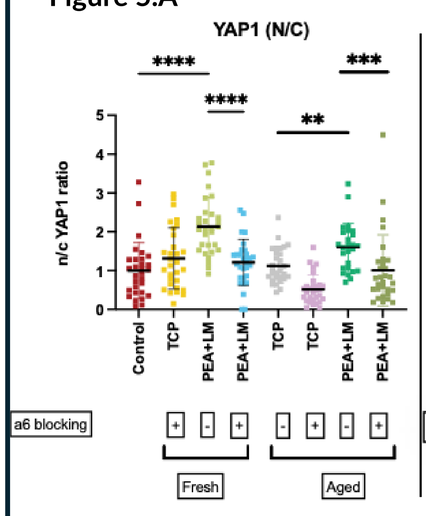


Figure 5.B

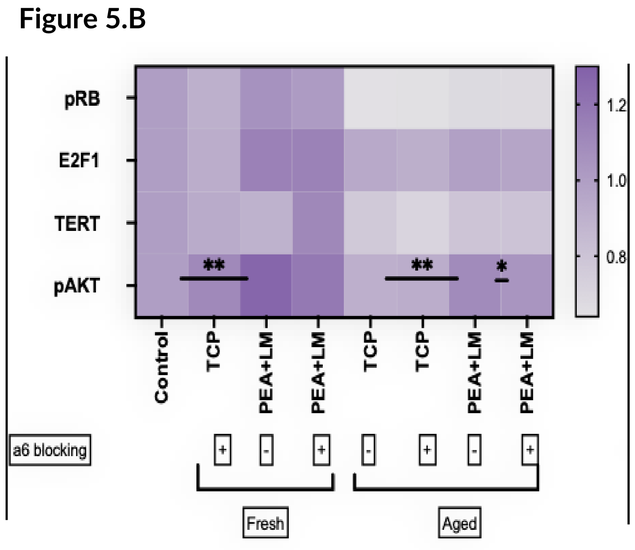
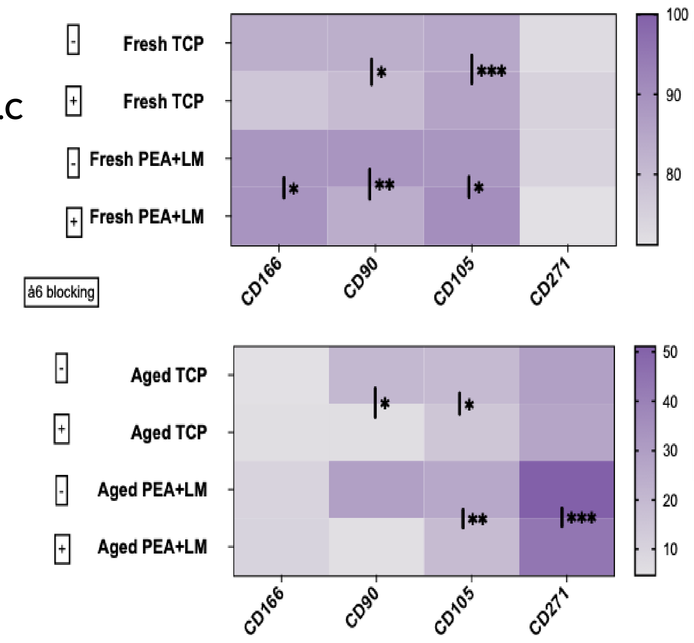


Figure 5.C



5. A: α6 integrin inhibition disrupts YAP1 nuclear localisation in day 3.
5. B: Reduced expression of survival and proliferation markers under α6 blockade.
5. C: Loss of MSC surface marker expression following α6 inhibition.
Overall, α6 integrin blockade impairs mechanotransduction, leading to reduced proliferation and loss of phenotypic stability.
Data represent mean ± SD (n = 3); *p < 0.05, **p < 0.01, ***p < 0.001.

4. Conclusion

Conventional tissue culture conditions drive MSCs toward a senescent, functionally compromised state marked by loss of proliferative capacity and phenotypic identity. In this study, we demonstrate that bioengineered PEA+LM substrates actively maintain the fitness of MSCs by sustaining proliferation-related signalling, stabilising surface marker expression, and suppressing senescence-related pathways. Inhibition of α6 integrin with an α6 integrin-blocking antibody abrogates these protective effects and controls YAP1 localisation and downstream signalling networks, identifying it as a critical regulator of MSC mechanobiology. Collectively, our findings reveal laminin-presenting biological interfaces as a powerful strategy to counteract senescence-induced functional decline and enhance the stability and therapeutic potential of MSCs.

5. Acknowledgement

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References

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