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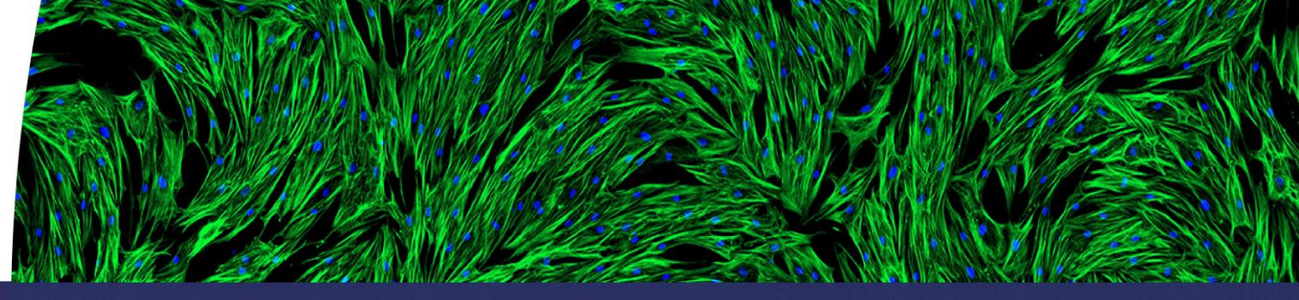
Engineering Bone Marrow for Discovery



Bioengineered models to study leukemic and pharmacological bone marrow remodelling

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INTRODUCTION AND BACKGROUND

The **haematopoietic niche**, located inside the bone marrow (BM), is a complex environment that **modulates the preservation or proliferation of haematopoietic stem cells (HSCs)** to maintain a steady pool of progenitor cells and a constant supply of mature blood cells.

This equilibrium is tightly regulated by a complex network of interactions between HSCs, other BM cells like mesenchymal stem cells (MSCs), and their biochemical and physical setting (*Pinho & Frenette, 2019*), which create distinct microenvironments within the BM (**Fig. 1**).

Abnormalities or dysregulation of the haematopoietic process may lead to **haematopoietic malignancies**. These include leukemic cancers, the 13th most common cancer group in the UK, which are associated with high reoccurrence rates after treatment resulting in a 10-year survival rate of 49% (*Cancer Research UK*).

The complexity of the BM plays a role in shielding leukemic stem cells (LSCs) from immune and pharmacological action, increasing LSC survival and proliferation, and decreasing HSC viability even after treatment. The interactions between LSCs and other niche components, especially MSCs, are being increasingly recognised as key drivers of cancer pathology and treatment success (*Brenner et al, 2017*).

Research into haematopoietic and leukemic dynamics is hindered by a lack of models that replicate leukaemia-driven changes to the BM in a manner physiologically relevant to human health. Therefore, there is an urgent need for humanised models that enable pathological research and pharmacological innovation (*Donnelly et al, 2024*).

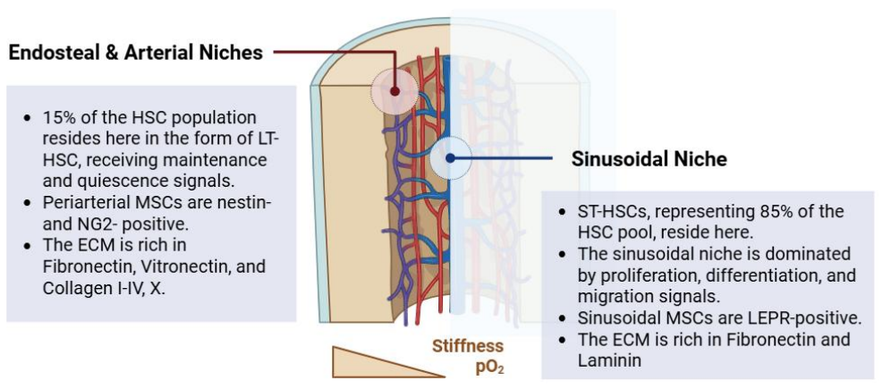

Figure 1. The distinct microenvironments in the haematopoietic niche are essential to drive and balance different haematopoietic behaviours.

Objective: This project aims to build a humanised model of the haematopoietic niche, incorporating the main physical and biological elements of the BM. As a first step, we are looking to use bioengineered substrates to replicate the stromal layer in vitro, to culture MSCs with an HSC-supportive phenotype

METHODS

Cells: Primary MSCs are extracted from human bone marrow aspirates precedent from clinical samples. Experimental MSCs are used P3-P5.

Culture substrate: Cell culture surfaces are enriched with laminin (LM), an extra-cellular matrix protein that promotes the expression of a quiescence phenotype through a decrease in mechanotransduction within the cell. To organise laminin into a higher bioavailability configuration, plates are coated with plasma-polymerised Poly(Ethyl Acrylate) (PEA).

Material validation: Laminin adsorption is confirmed through µBCA protein quantification. PEA coating is assessed via X-ray photoelectron spectroscopy (XPS) analysis..

Experimental design: Cells are cultured on the bioengineered substrates for up to 14 days and assessed for quiescence at the end of the culture period (**Fig. 2**). Immunofluorescence staining is performed for actin (cytoskeletal protein) and Ki67 (nuclear marker of active cell division). Results from cells grown on bioengineered substrates are compared against those grown on standard tissue culture plastic (TCP).

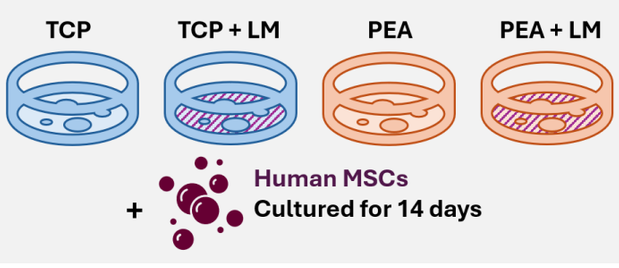

Figure 2. Experimental design. Human MSCs are grown in custom bioengineered culture surfaces or standard culture conditions for up to 14 days.

RESULTS

Substrate characterization

- XPS analysis of PEA-treated and untreated Poly(Styrene) (PS) plates reveals distinct carbon signatures, which correspond to the different molecular configurations of PS and PEA (***Fig 3A***).
- Cells grown on PEA-coated plates are less numerous, smaller, and morphologically distinct from cells grown on standard tissue culture-treated plastic (***Fig 3B***).

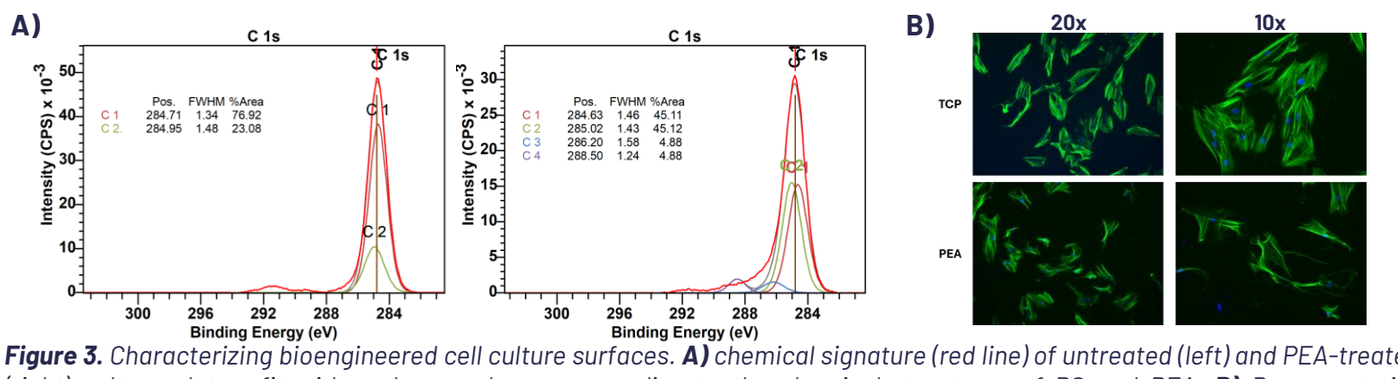

Figure 3. *Characterizing bioengineered cell culture surfaces.* ***A)*** *chemical signature (red line) of untreated (left) and PEA-treated (right) culture plates, fit with carbon peaks corresponding to the chemical structures of PS and PEA.* ***B)*** *Representative fluorescence images of MSCs grown on untreated (top) and PEA-treated (bottom) plates. Samples were stained for* ***DAPI****,* ***actin****.*

Growing cells for quiescence

FBS supplementation in culture media was optimised to maintain MSCs quiescence, avoid over-proliferation or differentiation, and preserve the HSC-supportive, stem phenotype (***Fig. 4***).

- Cells grown in 2% FBS were less confluent at the end of the 14-day culture period than cells grown at 10% FBS, in all experimental groups.
- The addition of laminin to PEA-treated culture surfaces restores normal cell numbers and morphology.
- Supplementation with 10% PromoExQ for the last 24 hours of the culture period restores proliferative activity in all experimental groups.

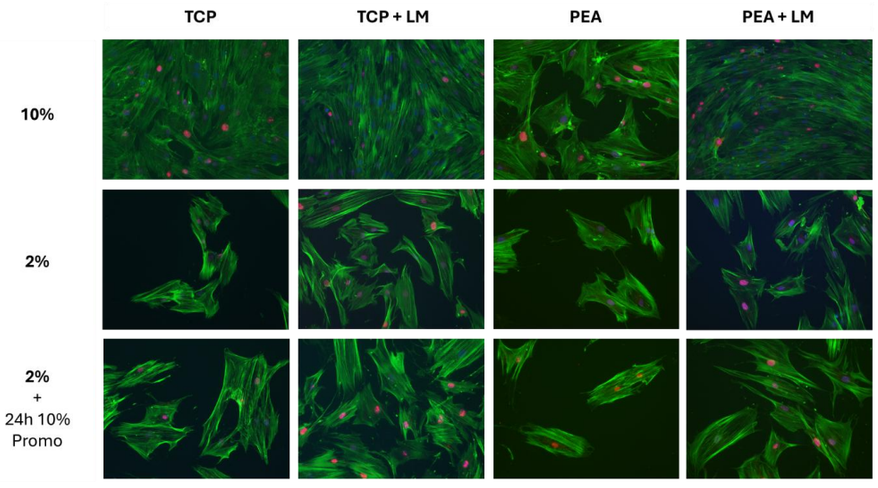

Figure 4. *Representative fluorescence images of primary MSCs cultured on standard TCP or on PEA-treated surfaces, with or without laminin enrichment. Cells were cultured for 14 days with standard 10% FBS supplementation, 2% FBS supplementation, or 2% FBS plus 10% PromoExQ for the final 24 hours. Samples were stained for* ***DAPI****,* ***actin****,* ***Ki67****.*

Quantification of proliferative activity

- There are no significant differences in the number of Ki67+ nuclei across groups. Supplementation with PromoExQ produces an increase in Ki67 expression, confirming that quiescence is maintained across groups.
- Laminin enrichment of the cell culture surfaces produces notable differences in the trends of nuclear Ki67 expression, especially in PEA-treated surfaces.

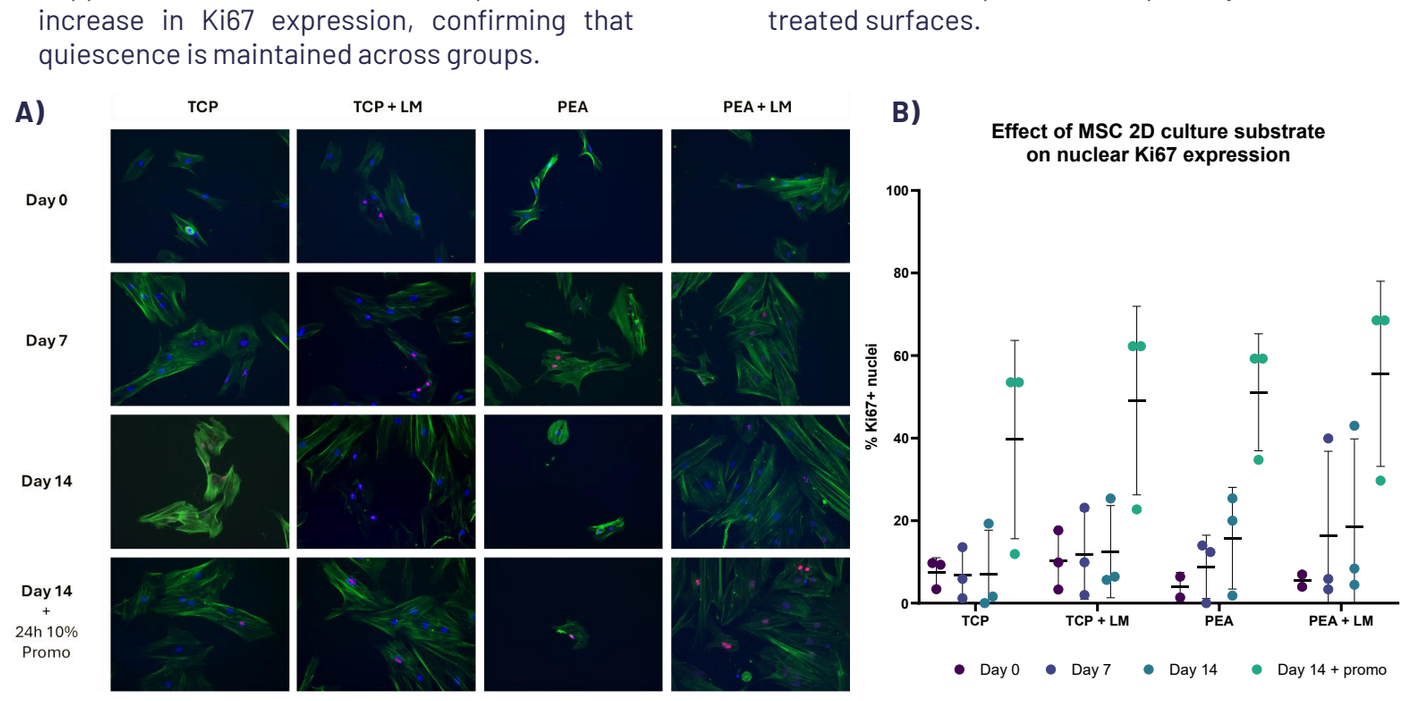

Figure 1. A) *Representative fluorescence images of primary MSCs cultured on standard TCP or on PEA-treated surfaces, with or without laminin enrichment at each of the experimental timepoints: day 0 (24 hours post-seeding), day 7, and day 14.* ***B)*** *Percentage of Ki67+ nuclei per group. Data is presented as mean, SD (n = 3). Samples were stained for* ***DAPI****,* ***actin****,* ***Ki67****.*

DISCUSSION

These preliminary results support the use of bioengineered surfaces to grow a population of HSC-supporting MSCs. More work is needed to characterise phenotype markers and secretome.

The development of human BM-derived stromal layers will enable the study of HSC interactions with the haematopoietic niche in health, disease, and to screen pharmacological interventions.

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