Engineering a bone marrow microenvironment for red blood cell manufacturing

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Introduction

Blood transfusion is an essential medical intervention that saves millions of lives every year. However, it relies entirely on donor availability, which creates significant global inequalities in access. Approximately 120 million blood donations are collected annually, but nearly 80% of the world's population has access to only 20% of the supply [1].

Ex vivo production of red blood cells (RBCs) has emerged as a promising strategy to address this imbalance. Nevertheless, current approaches based on liquid suspension cultures remain prohibitively expensive due to the large volumes of costly culture media required to sustain cell growth at low densities [2].

To overcome this challenge, we aim to mimic the body's efficient RBC production in the bone marrow by engineering high-cell-density, low-media cultures. Here, we established two 3D cultures of peripheral blood (PB) cells within a microenvironment combining 3D-printed scaffolds and fibrin hydrogels, replicating bone marrow-like RBC production from erythroblastic islands.

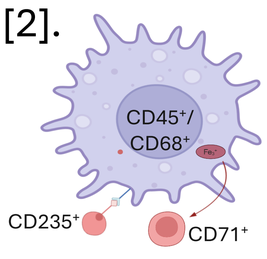


Figure 1. BM Erythroblastic island

Methods

We engineered 3D-culture systems by combining melt electrowritten (MEW) polycaprolactone (PCL) scaffolds embedded within fibrin hydrogels.

Two culture strategies were developed.

a) Peripheral blood mononuclear cells (PBMNCs) from healthy donors were seeded onto MEW PCL scaffolds. Cells were cultured for eight days in erythroid differentiation medium to evaluate their ability to adhere, proliferate, and initiate erythroid differentiation.

b) PBMNCs were fractionated into $CD3^-$ T-cell-depleted cells and $CD34^+$ hematopoietic stem and progenitor cells (HSPCs). The $CD34^+$ cells were first expanded in 2D erythroid cultures. In parallel, $CD3^-$ cells were seeded onto MEW scaffolds to promote macrophage formation and establish a supportive niche. After six days, the expanded HSPCs were suspended in a fibrin hydrogel and incorporated into the macrophage-coated scaffolds, creating a hybrid hydrogel–scaffold co-culture system.

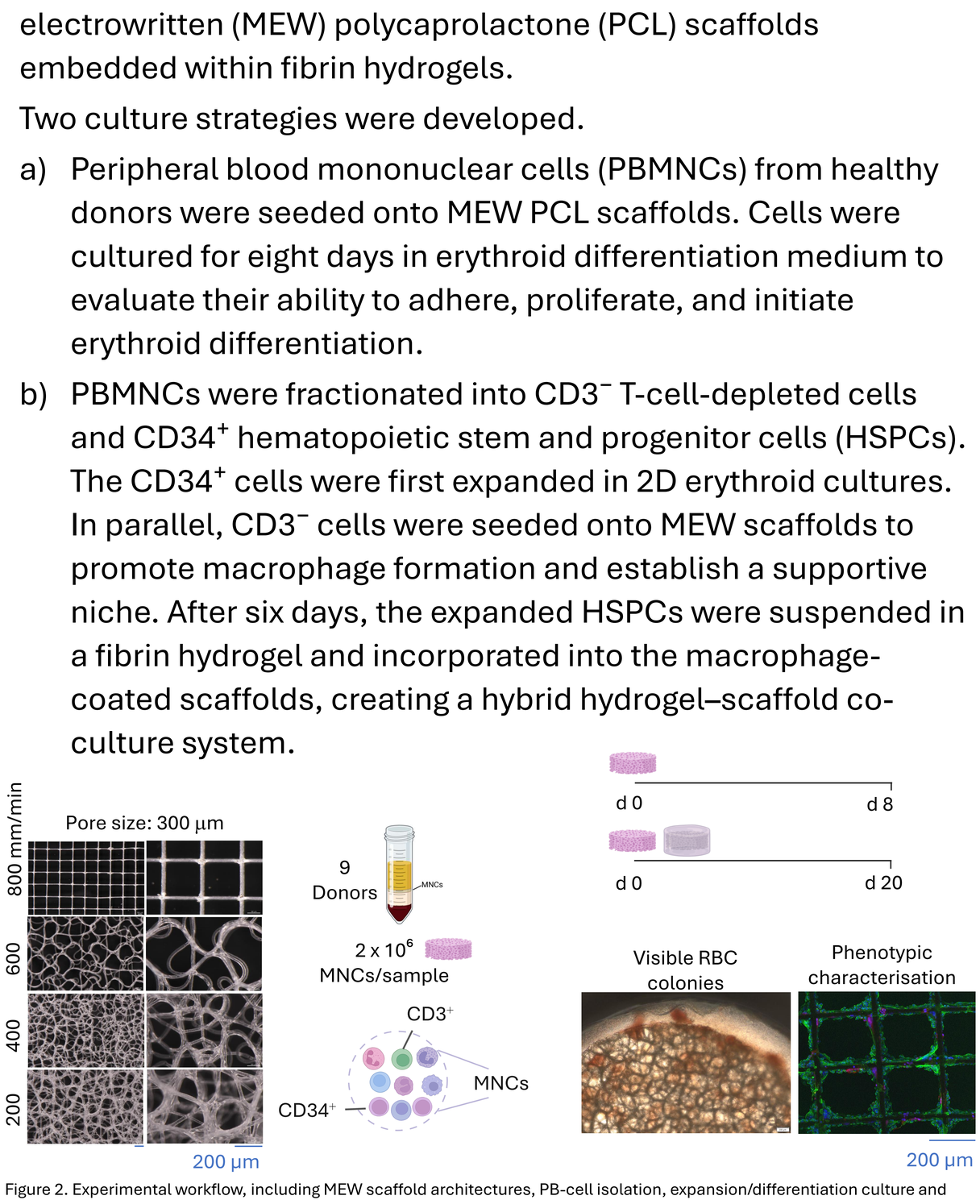


Figure 2. Experimental workflow, including MEW scaffold architectures, PB-cell isolation, expansion/differentiation culture and characterisation

Results

Early erythroid differentiation in MEW scaffolds

PBMNCs readily adhered to the MEW scaffolds and initiated erythro/myelopoiesis.

Cell expansion was significantly improved compared to suspension controls, with a 1.9-fold increase observed after eight days. Microscopic analysis revealed the formation of macrophage-coated fibres surrounded by clusters of $CD71^+$ and $CD235a^+$ erythroid cells. This organization closely resembled native erythroblastic islands found in bone marrow.

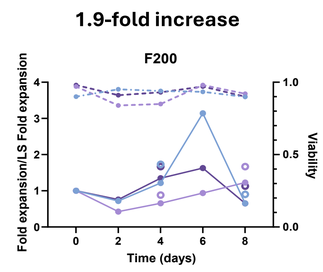


Figure 3. Liquid suspension expansion

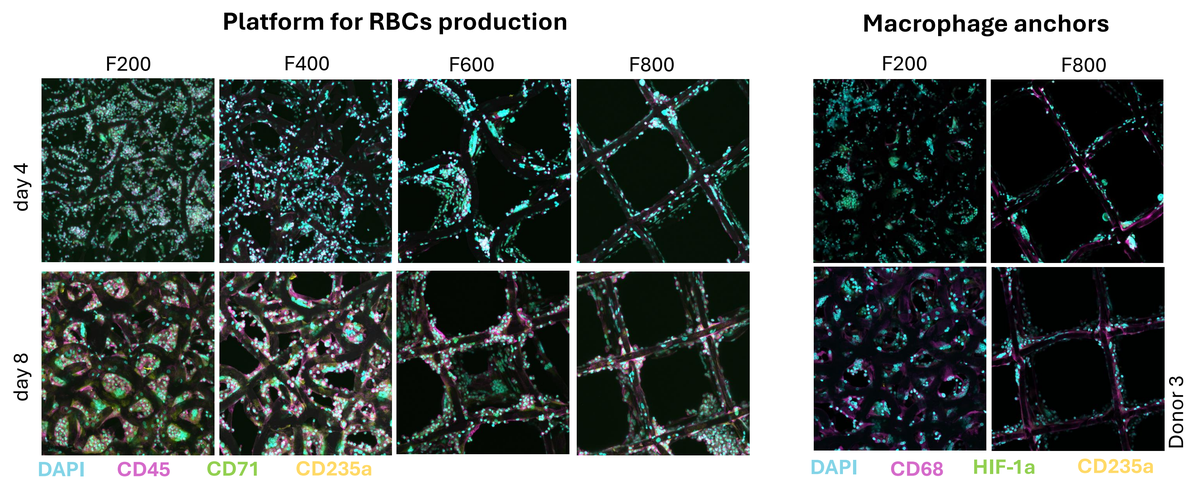


Figure 4. Phenotypic characterisation of PBMNC-derived RBCs and macrophage attachment to MEW scaffolds

Full erythroid differentiation in a fibrin hydrogel/MEW scaffold 3D-system

The hydrogel–scaffold co-cultures achieved cell densities of up to 2.6×10^7 cells/mL. This represented a substantial increase compared to current liquid suspension methods (10^6 cells/mL [3]).

Erythroblast expansion reached up to 347-fold, demonstrating the strong proliferative capacity of the system.

Importantly, these results were achieved while consuming 15.5 times less culture medium than standard suspension cultures, highlighting a major improvement in resource efficiency.

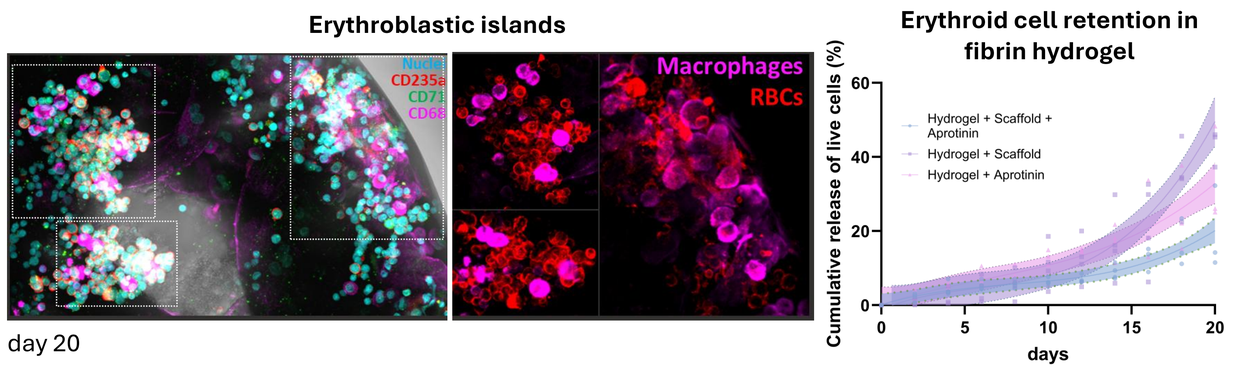
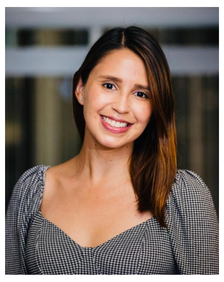


Figure 5. Full erythroid differentiation from CD34+ and CD3-depleted PBMNCs in a fibrin hydrogel/MEW scaffold 3D-model.

Conclusions

The use of MNCs introduces key accessory cells, enabling us to establish a model of erythroblastic islands.

Further investigation into the role of macrophage activation is essential to fully understand and enhance the overall performance of the model.



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