SCALABLE AND CONTROLLED PRESENTATION OF SURFACE IMMOBILISED FACTORS FROM THE BONE MARROW NICHE FOR HEMATOPOIETIC CELL EXPANSION

Rebecca Moore, Loughborough University
R.L.L.Moore@Lboro.ac.uk
Katie Glen, Loughborough University
Matthew Worrallo, Loughborough University
Robert Thomas, Loughborough University

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Umbilical cord blood provides a source of hematopoietic stem cells for transplantation with immunological and availability advantages over conventional bone marrow sources. Limited cell numbers and slower engraftment from umbilical cord blood units has led to the clinical development of immobilised Notch ligand Delta-Like 1 to promote ex vivo expansion of a rapidly engrafting cell population. Further, bone marrow stromal cells also promote ex vivo proliferation and differentiation of hematopoietic stem cells, supporting the concept that bone marrow niche cues may influence their fate. Surface receptors for a number of proteins released from the bone marrow niche have been identified on hematopoietic stem cells. The immobilisation of these niche proteins in a controllable manner presents an exciting opportunity for the improved expansion of cells with high clinical potential.

The aim the work was to demonstrate the biological function, and integration with a scalable system, of a highly controllable immobilised protein approach. A micro-scale stirred tank bioreactor platform (ambr®*;* Sartorius Stedim, UK.) was used to determine the scalability of the immobilisation method. Initial studies were carried out using Delta-Like 1. Delta-Like 1 was immobilised onto streptavidin coated magnetic particles via a heterobifunctionalised polyethylene glycol linker molecule. $CD34^+$ cord blood cells were treated with Delta-Like 1 immobilised particles, and phenotype markers measured to monitor population distributions using spanning tree progression of density normalised events software. Surface concentration of Delta-Like 1 was simply controlled via reagent concentration and beads could be efficiently removed from static or stirred experimental systems. Immobilised Delta-Like 1 is functional and stimulates qualitatively similar lineage skewing toward high expressing CD34 CD133 CD33 myeloid progenitors in both static and stirred culture platforms. We have further demonstrated the controlled immobilisation of seven further niche proteins, sonic hedgehog, delta-like 4, jagged 1, jagged 2, bone morphogenetic protein 2, bone morphogenetic protein 4 and wingless MMTV integration site 3A, alongside 4 hematopoietic relevant cytokines.

Immobilised niche proteins have the potential to improve the manufacturing efficiency and control of final *ex vivo* expanded cell products through compatibility with highly controlled and characterised suspension culture systems and scalable surface volume ratios.