ENABLING HUMAN PLURIPOTENT STEM CELL DERIVED MEGAKARYOCYTE MANUFACTURE

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Annually 4.5 million platelet units are transfused in Europe and the United States. These are obtained solely from allogeneic donations and have a shelf life of 5-7 days. To address the corresponding supply challenge, Moreau et al. have devised a novel process for producing megakaryocytes (MKs, the platelet precursor cell) in vitro. A transcription-factor driven, forward-programming approach converts human pluripotent stem cells into MKs. This strategy has the unique advantage of generating high yields of pure MKs in chemically defined medium through the establishment of 2-3 month long-term cultures. This could lead to the production of a consistent, reliable supply of platelets which overcomes the logistical, financial and biosafety challenges for health organisations worldwide. However to enable commercialisation of platelet manufacture, process optimisation and scale-up are essential.

Medium can contribute a significant proportion of the cost of a cell based product. We have used tissue culture flasks to represent static culture and compared this to a scaled-down automated bioreactor system (ambr15, Sartorius) to evaluate feasibility and optimisation factors for the growth of forward programmed (FoP) MKs in scalable stirred-suspension culture. The medium supply and exchange strategy were analysed using high temporal resolution growth curves for three medium exchange regimes. We assessed the productivity of the medium, showing that approximately 1.3 million cells are produced per millilitre of medium. Common metabolites lactate and ammonium were unlikely to be limiting proliferation and only 20% of glucose was depleted.

Using novel deterministic modelling software developed by our group, we have constructed a model of forward-programmed MKs growth. Based on inhibitor production, the model demonstrates the most efficient expansion strategy using the exchange strategies and observed growth characteristics of proliferating populations. Cell populations were identified using flow cytometry and phenotype analysis. This type of mechanistic modelling can be used to inform and optimise manufacturing strategy for scaled production of FoPMKs for platelet production and more generally for the manufacturing of cell based therapies.