APPLICATION OF QUALITY BY DESIGN TOOLS TO UPSTREAM PROCESSING OF PLATELET PRECURSOR CELLS TO ENABLE IN VITRO MANUFACTURE OF BLOOD PRODUCTS

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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.1 devised a novel process for producing megakaryocytes (MKs, the platelet precursor cell) in vitro. A transcription-factor driven, forward-programming (FOP) 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 which could lead to the production of a consistent, reliable supply of platelets which overcomes the logistical, financial and biosafety challenges for health organisations worldwide.

Here we follow a Quality by Design (QbD) approach to enable improvements to the upstream processing of FOPMKs. Firstly, we created a process flow diagram for production of in vitro platelets for transfusion, which segregated processes into individual unit operations for control and optimisation. Next, we developed a Quality Target Product Profile (QTPP) and identified Critical Quality Attributes (CQAs) for each stage.

We conducted a range of experiments utilising Design of Experiments (DOE) and mechanistic modelling2 tools to link Critical Process Parameters (CPPs) to CQAs. For adherent culture, we identified a productivity limit related to surface area available for growth and a cell loss phase which was dependent on cell seeding density, RhoK inhibitor usage and seed density. Using suspension cultures of FOPMK. We noted that TPO and Doxycycline concentration were CPPs as these impacted cell net growth rate and phenotype trajectory. Furthermore, we noted that medium exhaustion led to a 30% loss of viable cells over 8 hours. Proof of concept studies also showed that FOPMKs can be cultured in scaled-down suspension systems (ambr-15 and spinner flask culture) whilst retaining CQAs.

1. Moreau, T. et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. Nat. Commun. 7, 1–15 (2016).
2. Stacey, A. J., Cheeseman, E. A., Glen, K. E., Moore, R. L. L. & Thomas, R. J. Experimentally integrated dynamic modelling for intuitive optimisation of cell-based processes and manufacture. Biochem. Eng. J. 132, 130–138 (2018).