A MECHANISTIC MODEL OF ERYTHROBLAST GROWTH INHIBITION; OPTIMISING RED BLOOD CELL MANUFACTURE

Katie Glen, Loughborough University, United Kingdom
K.E.Glen@lboro.ac.uk
Adrian Stacey, Advanced Bioprocess Design Ltd, United Kingdom
Forhad Ahmed, Loughborough University, United Kingdom
Rachel Bayley, Loughborough University, United Kingdom
Robert Thomas, Loughborough University, United Kingdom

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Manufacture of red blood cells (RBCs) from progenitors has been proposed as a method to reduce reliance on donors. Such a process would need to be extremely efficient for economic viability given a relatively low value product and high 2E12 cell dose. To achieve efficient process optimisation and scale-up an integrated approach comprising both experimentation and modelling is required.

Using an automated stirred tank micro-bioreactor (ambr®, Sartorius Stedim, UK) we have shown that initially erythroblasts rapidly proliferate but then enter an inhibited growth phase. Experimentally we have confirmed that the conventional constraints on cell manufacturing efficiency, such as mass transfer, common metabolic limitations, or previously reported paracrine signals were not responsible for this inhibition.

To further understand the mechanisms underlying the growth inhibition, we have used our own novel software interface, designed for the description, testing and manipulation of hypothetical dynamic mechanistic models. CD34+ cells derived from cord blood were grown in culture under erythroid expansion conditions. Cells were transferred to fresh culture medium and subject to different operating conditions. High time resolution growth curves were generated for each condition to distinguish between alternative models of growth and inhibition. The software, along with the experimental data, enabled a series of hypotheses regarding the mechanism of inhibition to be tested via the development of incrementally more complex mechanistic models based on the dominant phenomena involved in cell culture (e.g. substrate-dependent growth, cell death). Further experiments were performed under different operational conditions to test the predictive capabilities of the model and allow optimisation. These iterations produced a relatively simple deterministic mechanistic model based on inhibitor production and decay that could predict erythroblast growth behaviour as a consequence of medium provision and cell density strategy.

We have described an experimentally efficient approach to model key cell growth behaviour and operational consequences for manufacturing, which has general relevance across therapeutic cell culture systems where feedback signals are prevalent. The approach supports a high degree of confidence in manufacturing control due to mechanistic underpinnings and is complimentary to a hypothesis driven approach to further understand influences of cell growth.