Biochemical Engineering Journal 133 (2018) 28–38

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

A mechanistic model of erythroblast growth inhibition providing a framework for optimisation of cell therapy manufacturing

Katie E. Glen [a], Elizabeth A. Cheeseman [a], Adrian J. Stacey [a,b], Robert J. Thomas [a,*]

[a] Centre for Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering, Loughborough University, Loughborough, Leicestershire, LE11 3GR, UK
[b] Advanced Bioprocess Design Ltd, 90 Main Street, East Leake, Loughborough, LE12 6 PG, UK

ARTICLE INFO

Article history:
Received 27 July 2017
Received in revised form 15 January 2018
Accepted 27 January 2018
Available online 31 January 2018

Keywords:
Erythroblast
Cell therapy
Manufacture
Blood
Model
Bioprocess

ABSTRACT

Manufacture of Red Blood Cell based products *in vitro* requires highly efficient erythroblast culture for economic viability. It has previously been shown that efficiency of erythroblast culture in scalable bioreactors is not primarily limited by mass transfer, availability of medium components, or commonly recognised inhibitory metabolites or cytokines. We have developed a dynamic mechanistic model that describes an autocrine feedback loop in which a cell-derived factor accumulates in culture medium resulting in reversible erythroblast growth inhibition. Cells exhibited two phases of growth: a relatively uninhibited followed by an inhibited phase. Cell cycle analysis during inhibition identified slight accumulation of cells in S phase, distinct from the G1 accumulation anticipated in growth factor or nutrient deprivation. Substantial donor to donor growth rate variability (mean 0.047 h^{-1}, standard deviation 0.008 h^{-1}) required the growth rate parameter to be refitted for different donors. The model could then be used to predict growth behaviour with full medium exchange, but showed some reduced predictive ability after partial medium exchange. The model could predict the growth inflexion point over a range of phenotypic maturities from early to late maturity erythroblasts; however the secondary phase of growth differed substantially with less inhibition observed in more mature cells. The model provided a framework to optimise culture economics based on cost of production time and input consumables. It also provided a framework to evaluate the benefits of biological process engineering in medium design or cell modification *vs.* operational optimisation depending on the specific cost scenario of a process developer.

1. Introduction

A significant number of cell based therapeutic products (CBTs) are in development, and are projected to represent an increasing proportion of the total therapeutics market over the coming decade [1]. The manufacturing approach for CBTs builds on the extensive science and engineering base of the biologics industry and more than forty years of optimisation of cell line processing for protein production. However, CBTs pose certain unique challenges. In conventional biologics production the focus is on protein productivity and quality, allowing adaptation of other cell characteristics for manufacturing benefit. This opportunity is limited for CBTs due to the requirement for minimal alteration of many cell functions for safe and effective function *in vivo* [2]. Furthermore, CBT cell populations are often relatively heterogeneous and can produce paracrine signalling factors that alter cell function creating acute sensitivity to manufacturing operations such as medium supply regimens [3,4]. These factors combine to challenge the manufacturing goals of either maintaining fidelity with the source cell material or controlling phenotypic trajectory to a suitable endpoint.

CBTs require an appropriate level of bioprocess optimisation for quality, economics and risk for commercial success and regulatory approval. A validated mathematical model defining the manner in which bioprocess operation determines process outcomes could provide a powerful tool for such optimisation. Efficient empirical approaches, such as 'Design of Experiments', have been widely applied in bioprocess design to cost-effectively model control parameter effects on outcomes [5]. However, such techniques require substantial elaboration for complex non-linear relationships such as those that commonly occur over time series in biological systems. This decreases their efficiency for application to the dynamic processes underpinning cell culture [6]. Although high parameter approaches such as neural networks produce 'black-box' models that can achieve prediction and control goals, they are challenging to develop if data are scarce or expensive, a com-

* Corresponding author.
E-mail addresses: k.e.glen@lboro.ac.uk (K.E. Glen), e.a.cheeseman@lboro.ac.uk (E.A. Cheeseman), adrian@conceptiverse.com (A.J. Stacey), R.J.Thomas@lboro.ac.uk (R.J. Thomas).

https://doi.org/10.1016/j.bej.2018.01.033

Table 1
Erythroblasts were cultured in the ambr™ bioreactor under a series of different experimental process conditions, A1-D3, for development and validation of the model of growth and inhibition. The table details the starting density of each culture, the time at which any process operation was undertaken, and the nature (cell density adjustment or media exchange) and magnitude of the operation.

Process Condition	Seeding Density (cells/mL)	Medium and/or Cell Density Adjustment Time (hrs)	Proportion of medium exchanged with fresh	Adjusted Cell Density (cells/mL)
A1	3.1×10^6	–	–	–
A2	4.2×10^6	–	–	–
A3	4.2×10^6	5	0.3	–
B1	2.5×10^6	16	–	4×10^6
B2	2.5×10^6	16	–	2×10^6
B3	2.5×10^6	16	–	0.65×10^6
C1	2.5×10^6	18.5	1	3.1×10^6
C2	3.1×10^6	18.5	1	0.5×10^6
D1	2.8×10^6	18.5	0.4	3.5×10^6
D2	2.5×10^6	18.5	0.3	0.5×10^6
D3	2.4×10^6	18.5	–	0.5×10^6

mon scenario during CBT product and process development [7,8]. Further, regulatory initiatives such as Quality by Design emphasise a degree of mechanistic understanding of process which such approaches alone rarely provide [9]. An alternative approach is to employ mechanistic models that describe known or hypothesised phenomena. This approach enables the description of non-linear dynamics in a relatively parsimonious form through incorporation of prior system knowledge (such as cell transitions or influences).

Systems biology researchers have developed highly parameterised dynamic mechanistic models, including considerations of parameter stochasticity, that can potentially describe complex biological systems such as intracellular signalling events [10]. Alternative approaches to describe non-intuitive outcomes based on mechanistic behaviour include agent based modelling, potentially in combination with more conventional approaches such as flux-balance models [11]. However, a CBT process developer has specific requirements of a modelling approach. It needs to be cost effective to develop and amenable to validation. Accessibility to the bioprocess team will ensure model development benefits from wide input. These drive towards the simplest model that will describe the system complexity sufficiently to deliver the predictive power required for optimisation and control goals. The complexity of goals, whether prediction of simple cell yield or complex definition of sub-populations, will dictate the model complexity required.

Manufactured red blood cells are a therapy of interest for transfusion medicine or as engineered delivery agents for other therapeutics [12,13]. We have previously shown that erythroblasts, the lineage committed and rapidly proliferating cell population underpinning red blood cell production, proliferate *in vitro* followed by relatively rapid growth inhibition [14]. We further identified that mass transfer, common metabolic limitations, or previously reported paracrine signals were not responsible for this inhibition creating a potential economic barrier to *in vitro* production of red blood cells. Here, we propose that a series of hypotheses regarding the mechanism of inhibition could be tested via the development of incrementally more complex deterministic mechanistic models based on the dominant phenomena of cell culture (such as growth and inhibition). We further aim to show that a sufficiently predictive model could be achieved in a relatively parsimonious form. Finally, we aim to show that this approach supports understanding of current manufacturing limits as well as hypothesis refinement for mechanism(s) of inhibition.

2. Materials and methods

2.1. CD34+ cell culture

Fresh umbilical cord-derived mononuclear cells (MNC) were supplied by the Anthony Nolan Cell Therapy Centre (http://www.anthonynolan.org/clinicians-and-researchers/cord-blood-services) with informed consent and NREC ethical approval. CD34+ cells were isolated via positive selection using CD34 antibody-labelled microbeads as per the manufacturer's instructions (Miltenyi Biotec). Mixed donor CD34+ cells (>90% purity) were cryopreserved prior to cell culture. On thaw, CD34+ cells seeded at a density of $1–2 \times 10^5$/mL were cultured in IMDM (Merck Millipore) supplemented with 3% (v/v) AB Serum (Sigma), 2 mg/mL Human Serum Albumin (Irvine Scientific), 10 μg/mL Insulin (Sigma), 3 U/mL heparin (Sigma), 500 μg/mL iron saturated Transferrin (R&D Systems), 10 ng/mL SCF (R&D Systems), 1 ng/mL IL-3 (R&D Systems) and 3 U/mL EPO (Peprotech). Cells were cultured in tissue culture flasks at 37 °C, 5% CO_2 prior to bioreactor culture; they were fed by addition of medium (including previously listed cytokines) on day 3 of culture; thereafter they were fed daily to maintain density between 2 and 3×10^5/mL. On day 5, 7, 8 or 9 cells were seeded according to Table 1 in the ambr™ bioreactor system (Sartorius Stedim); non-sparged vessels were pre-conditioned as described previously [15], and temperature, impeller speed, pH and DO were set to 37 °C, 450 RPM, pH 7.5 and 52% respectively.

2.2. Culture analysis

2.2.1. Cell count and viability

Online cell counting and viability was measured using a Vi-Cell XR (Beckman Coulter) set to the following parameters: Minimum diameter (μm) = 6, Max diameter (μm) = 20, Cell brightness (%) = 85, Cell Sharpness = 100%, Viable cell spot brightness (%) = 65, Viable spot area (%) = 10, Minimum circularity = 0.8. A coefficient of variation of 4% was calculated for counts from parallel bioreactor technical replicates.

2.2.2. Assessment of cell morphology

1×10^5 cells were sampled, re-suspended in 200 μL of medium, and centrifuged onto poly-lysine coated microscope slides (Sigma 3–16 PK centrifuge with a cytology rotor) at $60xg_{av}$ for 5 min at room temperature. Slides were left to air dry overnight, stained using Leishman's stain (VWR International) and mounted. Slides were examined by bright field microscopy using a Nikon Eclipse Ti (Nikon) at 40× magnification.

2.2.3. Flow cytometry of erythroid lineage markers

Cells were sampled (1×10^5/tube) into flow buffer (PBS containing 1% BSA) and centrifuged at $300g_{av}$ for 5 min. Cell pellets were re-suspended and mixed with the appropriate volume of antibody in three separate panels to a final volume of 100 μL in flow buffer, incubated for 30 min at room temperature, washed once and analysed using a BD FACSCanto™ II flow cytometer (BD Biosciences) and gated against specific isotype and fluorescence-minus-one