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Experimentally integrated dynamic modelling for intuitive optimisation of cell based processes and manufacture

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ABSTRACT

Dynamic mechanistic modelling of cell culture is a key tool in bioprocess development to support optimisation and risk assessment. However, the approach is underutilised in the bioprocess industry due to challenges including lack of accessible tools to support a structured approach, the difficulty of realising computationally tractable (low parameter) yet realistic models, and the specialised skill sets required. We have proposed that these issues could be partly addressed by developing a parsimonious framework comprising a set of model building blocks, based on the ordinary differential equation modelling paradigm, representing common cell culture dynamics and modulation thereof. The framework is designed to avoid obvious pathological behaviours. Further, specific model instances within the framework can be simply visualised as a directed graph with vertices representing system species, dynamics and modulations, and arcs representing the interactions between them. The directed graph can be extended to describe the timing and nature of experimental interventions. A visual and intuitive route to describing models with an associated mathematical framework enables realisation in a software interface and integration with standard mathematical tools such as those for sensitivity analysis and parameter estimation. Such a framework is sufficient to represent some of the simple mechanisms underpinning bioprocesses that nonetheless lead to highly non-linear and counterintuitive outcomes. It also has a relatively low learning burden for users without formal mathematical training. The concept could be extended to include, for example, partial differential equation-based approaches to stochastic or spatially complex systems built up from a small number of parametrically parsimonious and well-behaved building blocks.

1. Introduction

Cell culture processes are a major element of manufacture in many biologic and emerging cell-based therapies. The dynamics of these processes, such as cell growth, consumption and production rates, have long been recognised as important determinants of process outcomes. For instance, in protein producing cell line cultures, reduction of major nutrients and accumulation of metabolic by-products such as ammonia and lactate can result in growth inhibition, reduced culture viability, and altered product titre [1]. Evidence is accumulating that newer cell-based products proposed in the regenerative medicine field add further complexity. Cell-released factors (identified or unidentified), metabolic substrate availability, and metabolic by-products can have tissue specific feedback relationships with lineage trajectory and growth rate, and as such are likely to be highly product specific [2–4]. Each candidate process and product will require appropriate understanding, description, and control of these dynamic relationships to achieve product optimisation and robust process control [5,6].

Best practice of process development and optimisation, as articulated in structured approaches such as Quality by Design or Six Sigma, has quantitative modelling at its heart [7]. In cell culture, current models fall roughly into two camps, namely those that empirically map between process parameters and target outcomes and those that consider the dynamics and mechanisms by which process parameters affect process outcomes. Empirical mapping treats the biological and experimental system as a "black box" and thus provides limited process insight for risk assessment, extrapo-

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lation or hypothesis-based iterative development [8]. Further, due to the complex and dynamic nature of the cell culture environment, experimental design or manufacturing conditions optimised for single time points or across time intervals often fail when considered over longer relevant time-courses. Conversely, a mechanistic approach entails the formulation and evaluation of hypotheses concerning the dynamics of the culture in terms of their consequences for culture trajectory. Mathematical and mechanistic formalisation of hypotheses concerning these dynamics represents the starting point effectively to link short time-scale dynamics with their consequences over a longer time-scale. There is increasing awareness, particularly within cell therapy, that such process insight is critical in diminishing risk in transfer to manufacture and in delivering consistent product quality [9,10].

Low parameter-count approaches of the empirical paradigm (exemplified by Design of Experiments) have been used extensively to improve cell based manufacture processes, including for cell therapies, facilitated by low-barrier and generic software tools for experimental design and analysis [11–13]. The mechanistic approach is employed by specialist groups within academia to elucidate biological systems and process knowledge, but application within commercial process development remains sporadic [14–16]. Consequently the hypothesis-testing power, and precision of hypothesis expression that mechanistic modelling enables, is significantly under-utilised, particularly early in product and process development when teams or companies are small and resources relatively limited. Reasons for restricted application include lack of low barrier turnkey tools and standardised workflows necessitating a diverse skill-set covering both biological hypothesis development and specialist modelling techniques. The tendency therefore is for the majority of biological experimentation and hypothesis testing, or of mathematical modelling of biological systems, to proceed in isolation, or in a poorly integrated manner. The requirement to address such deficiencies has been recognised in several technology road-mapping exercises such as that conducted by the National Cell Manufacturing Consortium [17].

We aim to facilitate wider application of dynamic mechanistic modelling by addressing the challenges that prevent the development of a broadly accessible turnkey software package for mechanistic cell process modelling. Such a package must (i) minimise mathematical knowledge required for model development (ii) enable precise articulation and interdisciplinary communication of population-dynamic hypotheses (iii) support development of relevant and robust mathematical models and (iv) allow linking of models to data from complex time-course experimentation for verification. To achieve this we aimed to develop a conceptual framework and mathematical formulation that could facilitate the expression of a broad range of biological phenomena in a consistent form and that could be conveniently expressed within a visual (software) interface to provide an intuitive bridge between biological description of a dynamic system and precise mathematical expression thereof.

2. Methods

2.1. Model framework development

Many complex time-courses can be efficiently described within an ordinary differential equation (ODE) modelling paradigm, in which the evolution of the system is described in terms of the dependency of the rates of change of the system variables on other system variables or additional temporal factors. Within this paradigm, mathematical expression of biological dynamics conventionally uses established functions, such as logistic and Monod (for macroscopic kinetics) and flux equations (microscopic kinetics) [16,18]. However, such formulations often conflate multiple mechanisms, for example, in terms of growth and saturation, which prevents straightforward reconfiguration to express a full range of dynamic hypotheses. To address this we developed a modelling approach in which - by restricting the repertoire of mathematical forms to a set of carefully chosen building blocks - the constituents of a system, and the relationships between them can be expressed intuitively, the former in terms of natural language, and the latter in terms of directed graphs (digraphs). A digraph constitutes a system of asymmetric relationships in which directional arrows (arcs) define the relationships between points (vertices) that represent the elements in an organisational structure. A secondary benefit of a limited ODE approach is that it can be designed to ensure that ill-formed or badly behaved model formulations are naturally avoided (for example, those that would, under certain conditions, predict a negative quantity of a necessarily positive system constituent), in contrast to a naïve deployment of, for instance, flux equations.

From the perspective of a population or sub-population of cells the bulk of these dynamics can be characterised as:

- population growth due to cell division
- population decline due to cell death, and
- concomitant decline of one population and growth of a second due to interconversion of cells from one to the other, for example, due to a phenotype change.

On an instantaneous time-frame, these dynamics can be approximated as being essentially additive. In terms of the primary modulators of population dynamics e.g. non-cell species within the media or direct cell feedback, the number of general forms is similarly small:

- production of a species by cells (such as a by-product of metabolic activity or a signalling molecule such as a cytokine)
- consumption (or destruction) of a species by the cells
- species decay, and
- conversion from one species to another, for example due to cell activity.

In the absence of further data during model formulation, each of these dynamics can be assumed to take the simplest possible form that avoids pathologies in the model, namely:

- cell growth:

$$\frac{dX}{dt} = rX$$

where X is the cell density and r the (positive) specific growth rate

- decline of cell population or species:

$$\frac{dX}{dt} = -rX$$

where X is cell density or species concentration and r the (positive) specific decay rate

- interconversion of population or species:

$$\frac{dX}{dt} = -rX$$

And

$$\frac{dY}{dt} = rX$$