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Relationship between preparation of cells for therapy and cell quality using artificial neural network analysis

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ABSTRACT

Objective: The successful preparation of cells for therapy depends on the characterization of causal factors affecting cell quality. Ultra scale-down methods are used to characterize cells in terms of their response to process engineering causal factors of hydrodynamic shear stress and time. This response is in turn characterized in terms of causal factors relating to variations as may naturally occur during cell preparation, i.e., passage number, generation number, time of the final passage stage and hold time in formulation medium.
Methods: To investigate the influence of all of these causal factors we have adopted a non-linear, multivariate predictive artificial neural network (ANN) based modeling approach to help create clearer insights into their effect on cell membrane integrity and surface marker content. A prostate cancer cell line candidate for cancer therapy (P4E6) was used and cell surface markers CD9, CD147 and HLA A-C were investigated.
Results: All causal factors studied were found to be significant in establishing an ANN model for the prediction of cell quality parameters with the extent of exposure to shear stress being the most significant and then passage number (range 57–66) and generation number (range 10–19) determining most strongly the cells' resistance to shear stress. Both the operation of the final cell passage and the hold time of the cells in a formulation buffer also determine the cells' resistance to shear stress. The processing parameters related to cell handling after preparation, i.e., shear stress and time of exposure were found to be the most influential affecting cell quality.
Conclusion: CD9 surface marker loss was the most sensitive indicator of the effects of shear stress followed by loss of membrane integrity and then HLA A-C, while CD147 remained unaffected by shear stress or even prone to increase. Also greater stability of cell surface marker presence was noted for cells generated at greater passage numbers or generation numbers or for reduction in hold time in formulation buffer.

1. Introduction

The biopharmaceutical industry has taken the first steps into production of 'therapeutic cells' where cells are introduced to the patient that are capable of restoring function and helping to combat disease. This type of therapy is particularly complex and the industry faces the challenge to manufacture cells at different scales in a reproducible and cost effective manner. All phases from initial cell sourcing to cell expansion and processing to formulation, storage and administration require a great deal of research and development to achieve the required cell quality [1].

Allogeneic cell therapies offer the opportunity to manufacture at scales of up to10s and even 100s L of culture. At such scales processing considerations become even more of a key issue than for bench scale, e.g., for cell transfer, hold stages, etc. The example studied in this paper is a cell candidate for a therapeutic whole cell vaccine [2] with cells selected to promote a potent immune stimulus [3]. For example, several prostate cell lines representing different stages of prostate tumor progression may be incorporated

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into an allogeneic whole cell tumor vaccine [2,4] to target multiple tumor specific antigens [5]. An overriding important factor is to ensure the required cell quality is maintained throughout the culture and manufacturing processes. Methods of cell characterization range from cell membrane integrity analysis which is of importance for the responsiveness of the immune system [6], surface marker analysis [7–9] the cells' cytokine release profile [10] and their potency in creating an appropriate immune response [11]. Similar processing and characterization challenges are faced in the production of cells for regenerative medicine therapies [12–16] as well as their ability to act as an inoculum for continued growth [17].

A record of cell history is important when producing a therapeutic whole cell product [18,19]. The most common record is the passage number assigned to the culture which increases by one each time sub-culturing occurs. Other information such as cell generation number, the extent of cell expansion especially at the final stage, time between passages, time of exposure to a dissociative enzyme (at passage) and confluency at passage all provide the user with further quantitative information about the history of the cells prior to bioprocessing and subsequent administration to the patient.

Cell preparation for patient administration involves a sequence of operations during which the cells are exposed to a wide range of hydrodynamic forces. Such forces can often lead to membrane rupture and death [20–22]. Physical conditions that must be considered include the extent and duration of shear stress (or intensity of power dissipation), e.g., during mixing and vialling and the use of holding stages, e.g., during preparation for centrifugation or for formulation. Ultra scale-down devices of rotating disk [8,9] and capillary [7,23] configurations have been developed to help inform of the effects on cells of the process environments which may exist during large-scale preparation.

The nature of the interaction of biological systems with physical bioprocess conditions is inherently nonlinear, making their effects difficult to interpret. This is compounded when the effects of multiple parameters are considered. Such nonlinearity and co-correlation rule out the use of linear based statistical methods such as multivariate regression. Nonlinear predictive approaches such as artificial neural networks (ANNs) can overcome these problems; for example for the identification of "biologically relevant" molecules, in pyrolysis mass spectrometry [24]; genomic microarraying of tumor tissue [25]; discrimination between multiple classes for blind data for MALDI mass spectrometry [26,27]; or SELDI-MS-derived data containing a high level of background noise [28]. Generalized models may be produced without relying on predetermined relationships (e.g., in medical diagnostics [29], prostate cancer [30]) and can be subjected to further development through continued interpretation to improve the accuracy of outputs [31].

The research presented here uses an ANN to mine sets of experimental data parameters comprising cell culture and cell bioprocessing operating variables and resulting cell properties, e.g., membrane integrity or surface marker content, and help identify relationships between the parameters studied.

2. Materials and methods

The input and output data analyzed in this paper are based on the study of bioprocessing of a prostate cancer cell line [8]. A brief description is given of the cell preparation and processing along with the nature of the data recorded during experimentation.

2.1. Cell preparation and processing

A prostate cancer cell line, P4E6 (provided by Onyvax Limited, London, UK) was grown at 37 °C in a humidified atmosphere containing 5% CO_2 using keratinocyte serum free medium (Invitrogen, Paisley, UK) supplemented with 2% (v/v) fetal calf serum (PAA laboratories, Linz, Austria) and 5 μg/L epidermal growth factor (Invitrogen, Paisley, UK) and sub-cultured when the cells reached approximately 70% confluency [8]. Cells were harvested by removal of spent medium, detachment in Accutase (Sigma–Aldrich, Poole, UK), quenching in complete growth medium, separation by centrifugation at 500 g for 3 min followed by washing twice in Hank's balanced salt solution (HBSS; Sigma–Aldrich, Poole, UK), and final resuspension in HBSS containing 8% (v/v) dimethyl sulfoxide (DMSO; Sigma, Poole, UK) to the required concentration range of 2–5 × 10^7 cells/mL [8]. In all cases the cell passage number and the number of cells at the start and end of each passage stage was recorded. The cells were held in static suspension at 21 °C for 1.5–3.5 h, and then gently resuspended and then exposed to a defined hydrodynamic stress environment in a 20 mL rotating disk shear device, disk diameter 40 mm operating at 4000 rpm (equivalent maximum shear rate 90 × 10^3 s^{-1} or maximum power dissipation, ε, 14 W/mL), 5000 rpm (130 × 10^3 s^{-1} or 31 W/mL) or 6000 rpm (175 × 10^3 s^{-1} or 52 W/mL) for up to 3 h [8,9]. Cells held in static suspension were used as a non-stressed control. Cells were sampled every 45 min in triplicate and each sample analyzed in triplicate for cell membrane integrity (trypan blue exclusion method using a haemocytometer) or for cell surface marker analysis, CD9, CD147 and HLA A-C using flow cytometry (FACScan; Becton Dickinson, New Jersey, USA with data analyzed using FCS Express software, De Novo Software, Ontario, Canada).

3. Data sets for analysis

The process sequence studied is described in Fig. 1 along with the classification of the various input factors and resulting

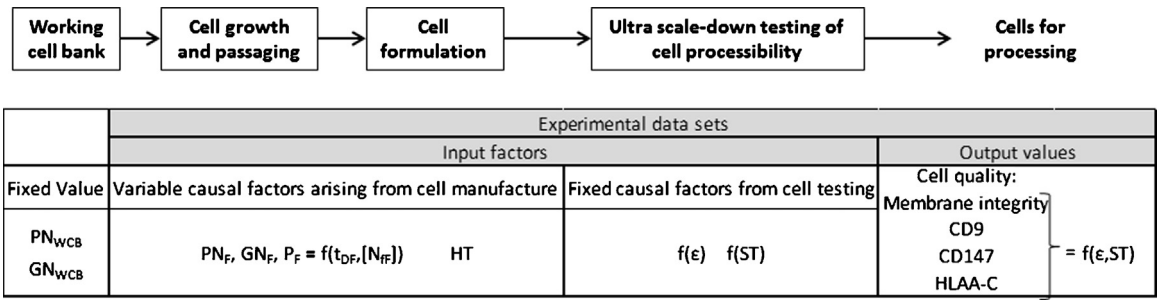

Fig. 1. Process sequence studied and experimental data collected for the development of an ANN model linking output values to input factors related to cell preparation and cell testing. The flow sequence is related to factors and experimental data sets as described directly below the individual operating stages. The key to the factors used: *Fixed values*: passage number (PN_{WCB}) and generation number (GN_{WCB}) for working cell bank (see Eq. (1)). *Input factors for cell manufacture:* passage number (PN_F) and generation number (GN_F) (Eq. (1)). Proportion of time spent in stationary phase, time (t_{DF}) and concentration [N_{fF}] for final phase (Eq. (2)) and holding time after formulation (HT). *Input factors and output values for cell testing:* maximum power dissipation (ε) and time of exposure to shear (ST).