T-cell therapy bioprocessing: application of Stratophase Ranger System and Automated Closed Loop Feeding Control

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Background

Translating clinically successful immunotherapy bioprocesses into scalable manufacturing strategies is a pan-industry challenge. Intelligent manufacturing solutions offer significant potential to reduce batch failure rates, maximise throughput and reduce CoGs for autologous cell therapies, by enabling adaptation to inherent starting material variability in real time.

Stratophase's Ranger system employs patented immersed sensor and control technology to detect minute changes in relative refractive index of cell culture micro-environments relative to the process (Process Trend Index – PTI). As PTI profiles reflect compositional changes in the medium, they typically correlate well to metabolite/protein concentrations and can be used to calculate the metabolic rate index (MRI) of cultures. Integration of decision logic based on MRI profile changes allows implementation of Automated Closed Loop Feeding Control (AFC) strategies.

This study, to the authors knowledge, is the first attempted application of Ranger technology towards the development of an adaptive, automated, feeding strategy for the bioprocessing of primary human T-cells expanded in a stirred tank bioreactor system.

PTI and MRI Characterisation of the SFB Process

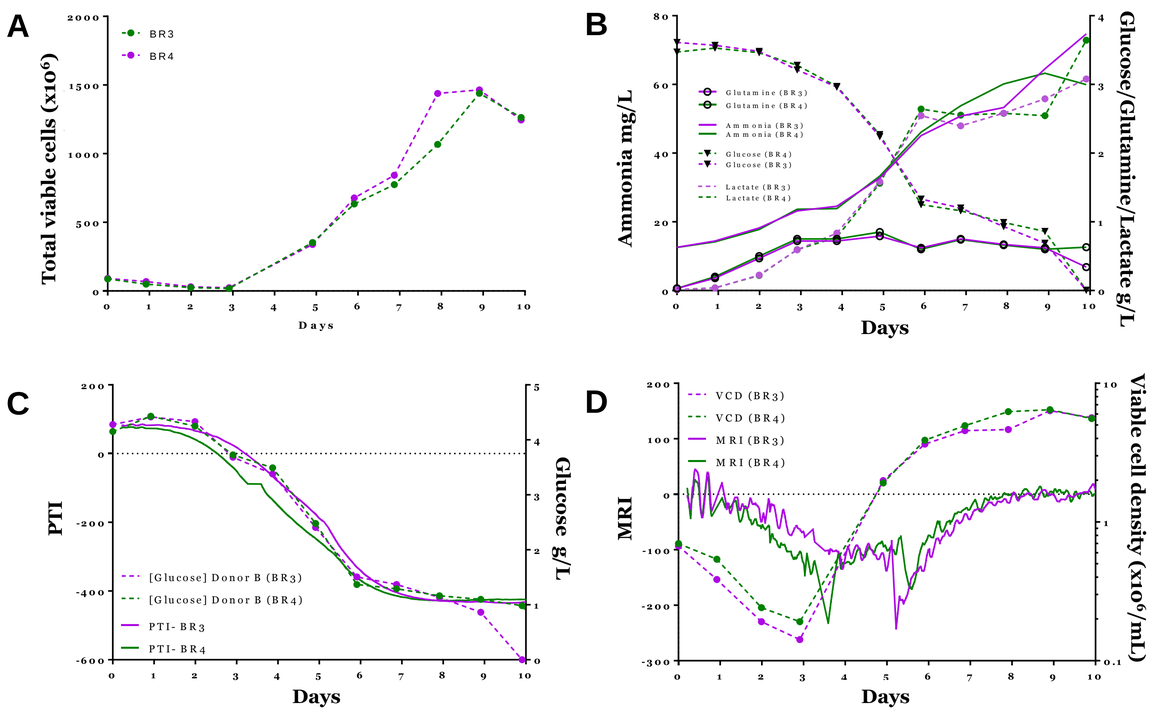

Figure 1. Characterisation of intra-donor reproducibility (n=2) with respect to viable cell number (**A**) and off-line metabolites (**B**). PTI exhibited a strong correlation to glucose consumption (**C**). Three distinct phases were identified in the MRI profiles corresponding to rates of change in viable cell density (**D**).

Glucose augmentation fails to improve T-cell expansion

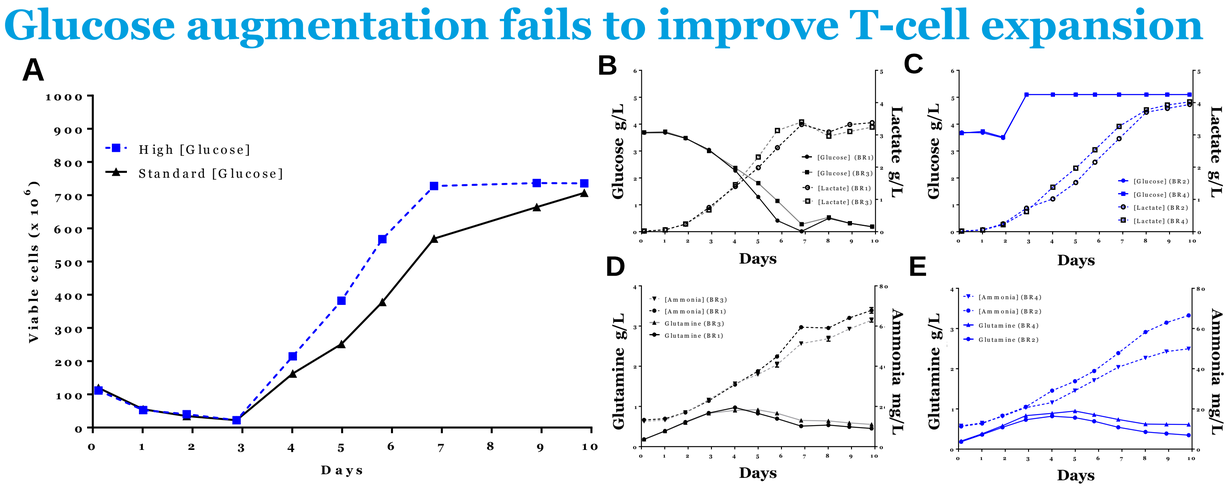

Figure 2. Total viable cell yield as a function of glucose concentration for a single donor (n=2 per condition) (**A**). Maintaining high glucose concentrations increased peak lactate levels (**B** and **C**), but didn't impact glutamine or ammonia concentrations (**D** and **E**). T-cell expansion is known to be sensitive to lactate concentrations > 2 g/L and ammonia concentrations >30 mg/L (Janas et al., 2015). This data suggested optimising adaptive strategies to control toxic metabolite accumulation rather than providing glucose availability would have greater benefit.

Methods

Cryopreserved banks (N=3 Donors) of purified CD4+/CD8+ cells were established (Viability ≥90% and a CD3 T-cell purity ≥90%). Post thaw, cells were activated and expanded in a DASbox Parallel Mini Bioreactor System equipped with Eppendorf BioBLU 300mL single-use vessels.

A series of 5 bioreactor runs were performed. The first characterized the PTI and MRI for our standard, scheduled fed-batch (SFB) expansion protocol. The next 3 runs explored the impact on culture performance (cell yield and phenotype profile) by adapting the feeding regime (timing & volumes) in response to notable changes in the MRI profile. A final verification run then implemented an optimal MRI trigger point identified from the previous studies and compared culture performance to the historical SFB process.

Evaluating an MRI initiated feeding regime

The three distinct phases (lag, exponential and stationary) of cell growth (Figure 1, D) were characterised in terms of MRI profile (Table 1). Levelling of the maximum metabolic activity (MRI plateau) appeared indicative of cultures entering exponential growth phase. Based on the previous metabolite analysis, we hypothesised, initiation of a feeding regime once $\frac{dMRI_{average}}{dt} = 0$ for at least 12h, whereby the feed rate applied closely matched the maximum growth rate, would facilitate a longer period of an optimal culture microenvironment for T-cell proliferation (Due to system volume limitations and lack of perfusion capability, the feeding regime was applied for 48h only).

MRI	growth phase
$MRI_{average} = 0 \pm 10$	Inoculation
$\frac{dMRI_{average}}{dt} < 0$	Lag phase
$\frac{dMRI_{average}}{dt} > 0$	Exponential phase and stationary
$\frac{dMRI_{average}}{dt} = 0$	Switching between phases

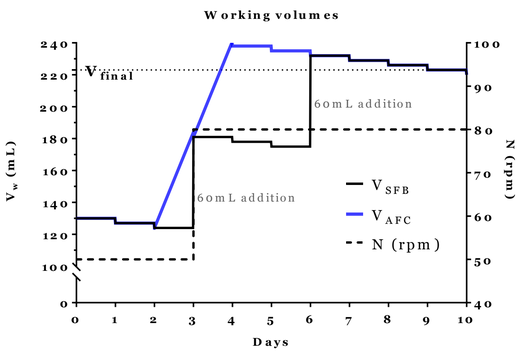

Table 1. Relationship between MRI and growth phases. **Figure 3.** Process feeding profiles: working volumes for SFB (black) and MRI initiated (blue) cultures.

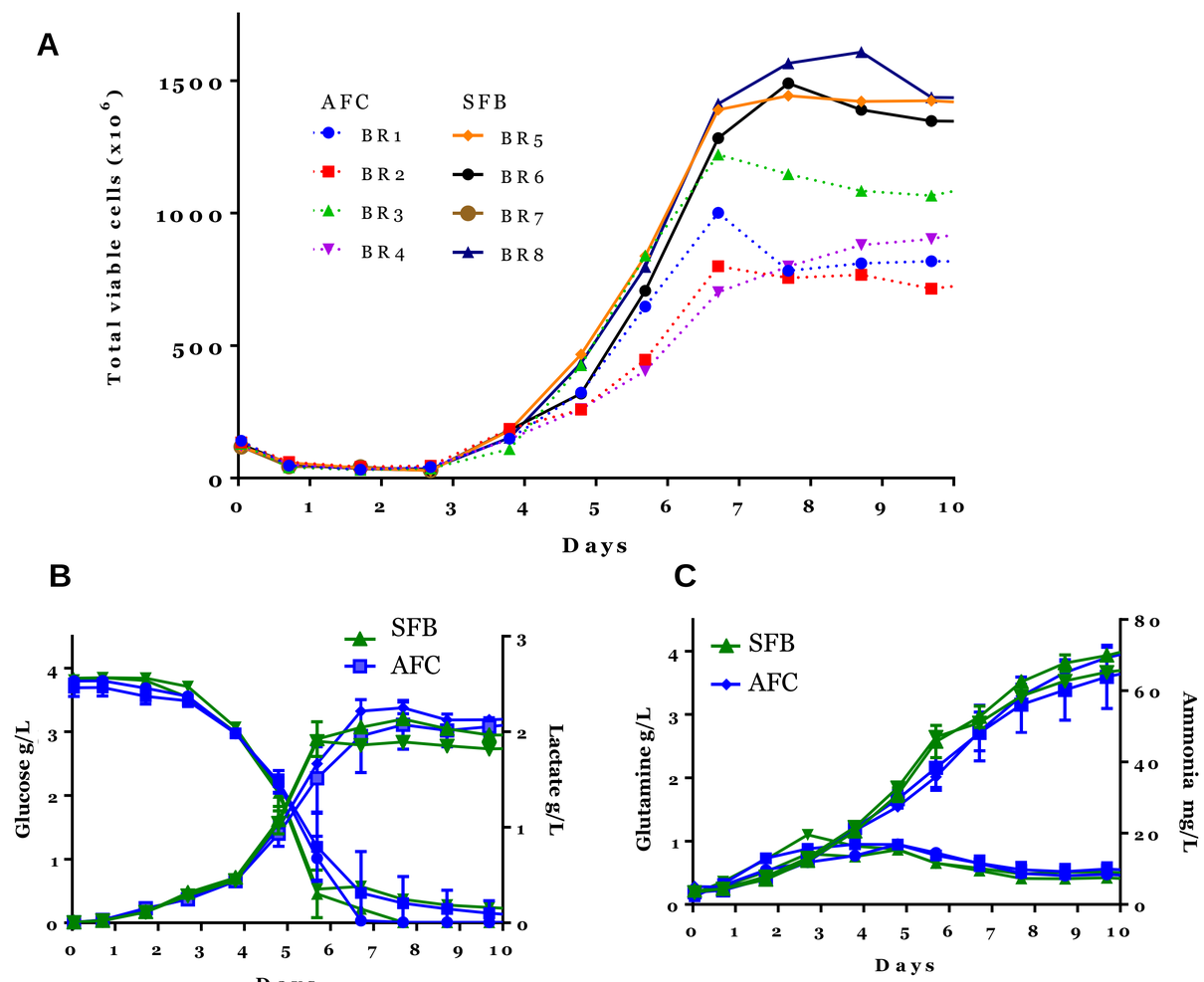

Figure 4. Total cell yield (A) and corresponding metabolite profiles (B and C) for SFB and MRI initiated cultures (n=2 per donor, n=2 donors). The SFB process outperformed the MRI initiated cultures across all donors and replicates, whilst no difference in metabolite profiles were observed.

Conclusion & Significance

This study was the first application, that the authors are aware of, in applying Ranger Technology to cell and gene therapy manufacture. It has highlighted numerous additional complexities in applying monitoring and control technologies in this field compared to the biopharma field. The data presented in this feasibility study demonstrates the potential of the Ranger technology to monitor the functional behaviour of cells in real-time during cell therapy bioprocessing. The manual control strategy informed by Ranger was not sufficiently responsive to the real-time requirements of the cells, however, further refinement of such techniques to realise closed-loop control are expected to enable manufacturing processes to adapt to donor-specific cell behaviour in the future.

We work with Innovate UK

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