Evaluation of a novel bench-top stirred-tank bioreactor for process development of autologous T-cell therapies

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Background and Aim

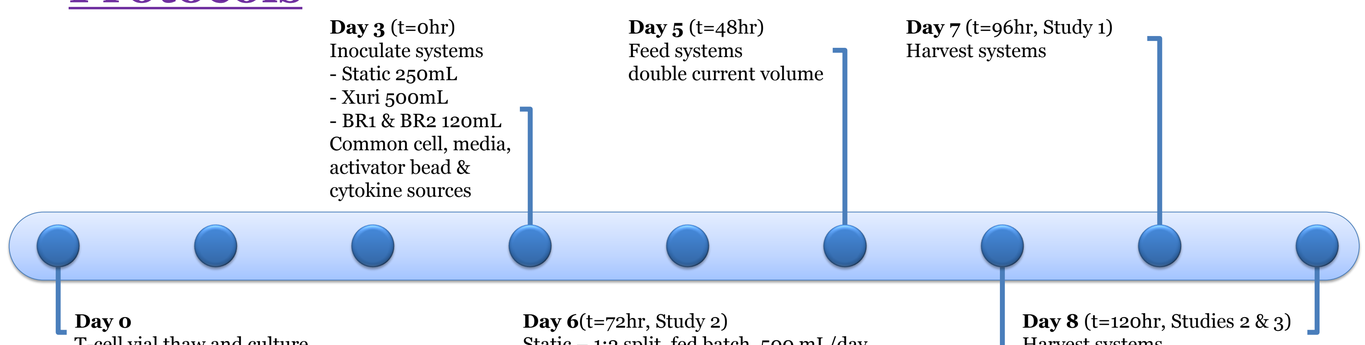
- Current manufacturing solutions for autologous cell therapies consist of stationary vessels (flasks or bags), requiring a high level of manipulation and costly scale-out throughout the product expansion phase. Agitated vessels, such as stirred-tank reactors (STR) and lateral movement culture systems which are more amenable to scalability, have enhanced control of the culture environment, and provide automation of culture maintenance and analysis.
- Sartorius Stedim Biotech (formerly TAP Biosystems) has been developing a prototype small scale single-use STR from process development activities, and in partnership with the Cell and Gene Therapy Catapult, wished to evaluate performance of the prototype when used in an autologous suspension cell therapy scale-up and scale-out application. The prototype system contained two separate bioreactor stations (denoted, BR1 and BR2) under individual control by the system software, and single-use bioreactor vessels with integrated reagent reservoirs.
- A cell bank of lymphocytes was generated for use within system testing (see Protocols below) and used to evaluate the prototype bioreactor in supporting T-cell expansion and how it compared with both static bags and lateral movement alternatives.

Parameter	Static Bag	Lateral Motion GE Xuri 2L	Prototype Bioreactor
Temp	37°C (AC)	37°C (AC)	37°C (AC)
CO_2	5% (AC)	5% (AC)	5% (AC)
pH	-	7.0 - 7.6 (NAC)	7.0 - 7.6 (AC)
DO	-	NAC	>50% utilising stirring cascade
Headspace gas (air) flow rate	-	300 mL/min (AC)	75 mL/min (AC)
Agitation	-	500 mL: 15 rpm @ 6° (AC) 1000 mL: 15 rpm @ 8° (AC)	125 mL: 150 rpm (AC) 250 mL: ≤250 rpm (AC)
Mixing Time (assessed by dye injection)	-	500 mL: 42s	125ml: 2s

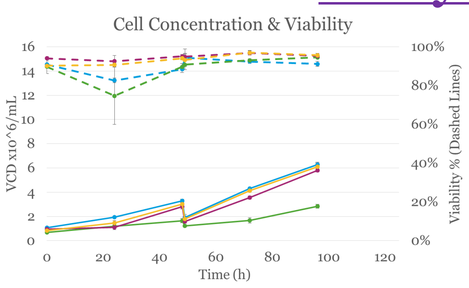
(AC = Active Control, NAC = Non-Active Control)

- **Cell count and viability assessed with Vi-Cell XR, data shown is mean ± 1 S.D., n=3**
- **Supernatant [Glucose] and [Lactate] quantified via Cubian HT-270, data shown is the mean of duplicate measurements**
- **Off-line pH measurements were conducted using a Mettler Toledo SevenEasy™ pH meter, data shown is n=1**
- **The volume of daily samples taken was 2.5% of the vessel working volume**
- **Expression analysis was conducted using the MACSQuant® Analyser 10 Flow Cytometer , data shown is mean ± 1 S.D., n=3**

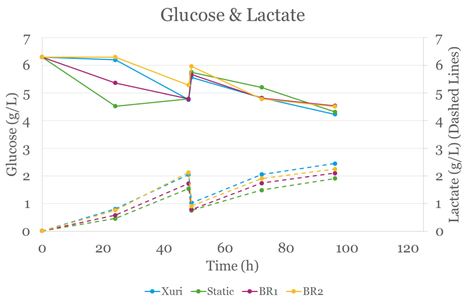
Protocols



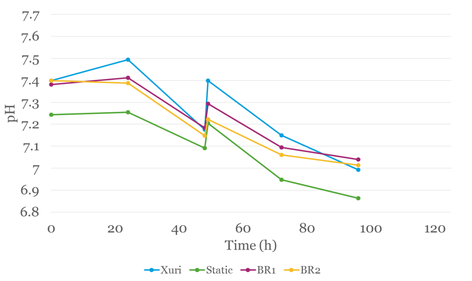
Study 1



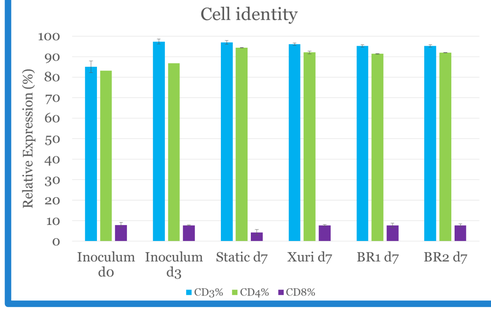
- Xuri, BR1 and BR2 (~6x10⁶ cells/mL) outperforming static bag culture (~2.5x10⁶ cells/mL) during the 7 day in batch culture
- Viability >80% in all systems (dashed lines)



- A decrease in VCD and lactate coupled with an increase in pH and glucose at t = 49 h on all data plots indicative of culture feed (doubling of working volume)
- Varied glucose uptake during first 24 h may be indicative of adaptation to agitated systems – highest uptake observed in static culture uptake (a continuation of first 3 day culture technique)
- Trend in lactate (dashed lines) accumulation comparable between all systems, mirroring cell concentration trends observed

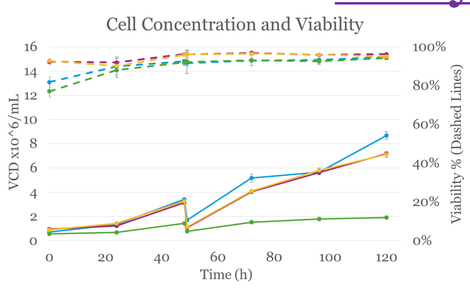


- pH range from 7.5 to 7.0 for agitated vessels during expansion period, with similar daily readings across three systems (n=1)
- pH within static culture consistently lower throughout, dropping to ~ 6.9 by Day 7

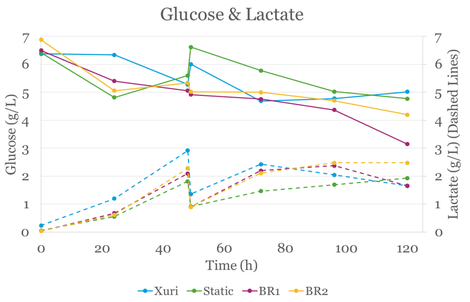


- Phenotype of harvested material is comparable between all culture systems (>90% expression of both CD3 and CD4)
- Expression of CD8 is maintained during culture in agitated systems. A decrease in CD8 expression is observed on day 7 in static culture.

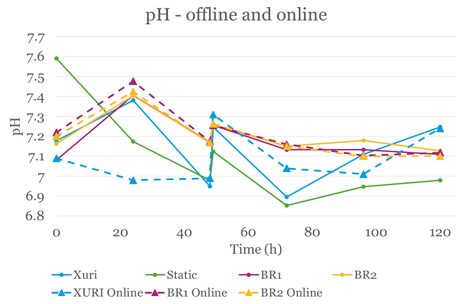
Study 2



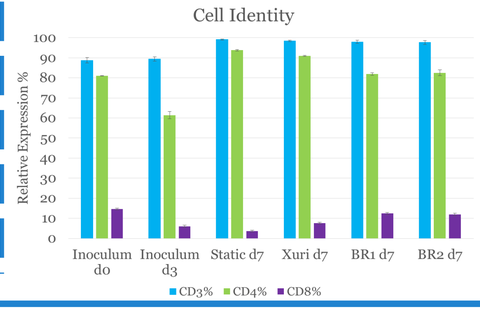
- Good consistency demonstrated by fed-batch BR1 and BR2 cultures (~7x10⁶ cells/mL), outperforming the fed-batch static bag culture (~2x10⁶ cells/mL)
- Fed-batch productivity almost matching that of perfusion culture, despite its predicted elevated performance



- Elevated glucose presence and reduced lactate accumulation indicative of slow proliferation in the static system
- Perfusion within Xuri resulting in plateau of both glucose and lactate from 72 h until harvest

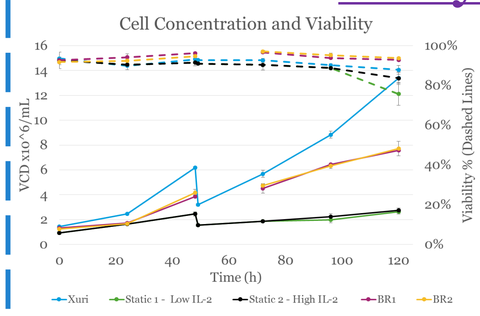


- Largest pH range again observed within static culture, ranging from 7.6 - 6.85, with a return to 7 following feeds
- Good consistency between online and offline pH data for BR1 and BR2, remaining within 7.1 – 7.5 throughout culture

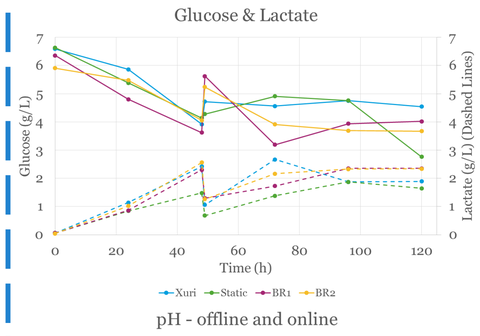


- Flow cytometry analysis demonstrating all harvested material >95% expression of CD3
- BR1 and BR2 culture vessels demonstrate highest proportion of CD8 presence following Day 8 harvest, with lower CD4 expression reflecting this

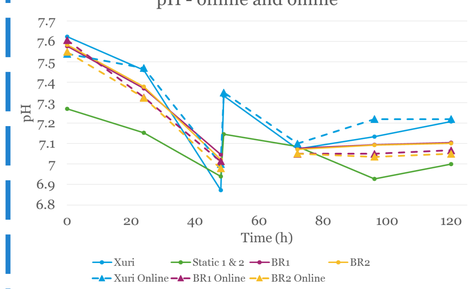
Study 3



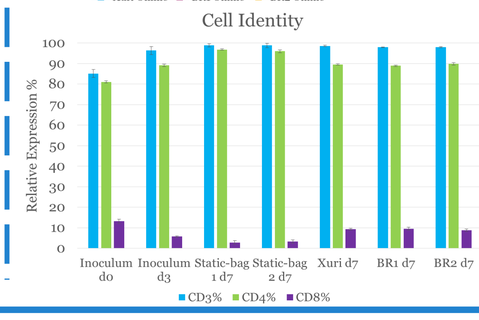
- An increase in IL-2 concentration within the inlet perfused media appeared to have a positive impact upon proliferation within the Xuri (~13x10⁶ cells/mL)
- Consistency demonstrated by fed-batch BR1 and BR2 cultures (both ~8x10⁶ cells/mL), despite difference in IL-2 concentration within feed (note: no data recovered at t = 49 hr)
- Agitated vessels again outperforming the fed-batch static bag cultures (~2.5x10⁶ cells/mL) during the 8 day expansion



- Glucose consumption and lactate production consistent across both BR1 and BR2 throughout expansion
- Perfusion/feeds at 72 hr lead to plateau of glucose and lactate concentrations within agitated vessels



- High level of consistency between online and offline pH data within agitated vessels
- Static Culture pH consistently lower than agitated vessels



- Higher ratio of CD8:CD4 within CD3 population in agitated vessels compared with static culture

Conclusions

- The prototype bioreactor supports T-cell expansion and merits further investigation for optimisation of T-cell expansion processes
- Industry standard, static bag culture, is consistently outperformed by agitated alternatives
- Distribution of nutrients, waste product and pH gradient is improved by agitation, and is achieved more rapidly in the prototype bioreactor
- Efficient mass transfer pivotal to successful proliferation of cell populations
- Exhaustion markers such as KLRG1 and CD57 only detected at low level <5% for all harvested material (data not shown)

Acknowledgements

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