A semi-automated cost-efficient process for the closed expansion and harvest of pluripotent stem cells using a hollow fibre bioreactor and continuous centrifugation

Jahid Hasan, Mark Bell, Garikai Kushinga, Nicole Nicholas, Julie Kerby, Ricardo P. Baptista, Stephen Ward

Jahid.hasan@ct.catapult.org.uk

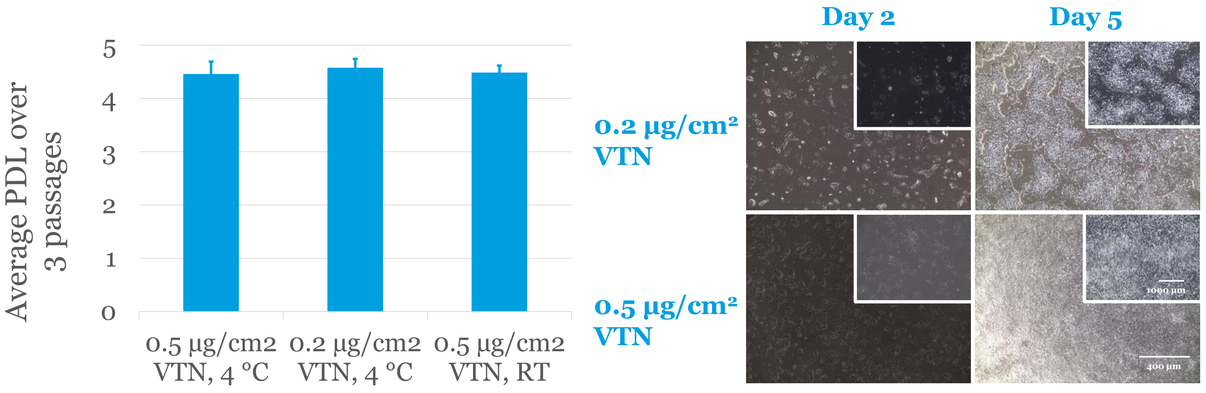
Aim and Introduction

Pluripotent stem cell (PSC) culture at commercial scale requires a shift from the current manual processing methods and costly cell culture reagents to deliver affordable high quality product in a repeatable and robust manner. Here we propose a strategy for using a hollow fibre bioreactor to generate large numbers of PSCs in a semi-automated expansion process, with concentration and wash steps utilising fluidised-bed centrifugation technology in a single, closed unit operation.

Determination of Quantum operating parameters

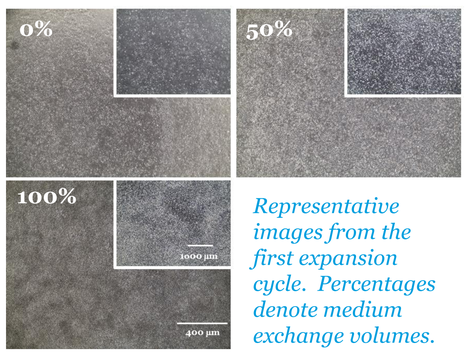
In order to determine the suitability of iPSC culture to the Quantum® hollow fibre reactor, several small scale studies were undertaken to either reduce the final Cost of Goods or provide insight on the operational design space in the Quantum®. An in-house iPSC line (CGTRCiB10) generated from a GMP-grade seed lot and adapted to defined culture conditions (Essential™ 8 medium, Vitronectin (VTN) substrate and Accutase™ passaging) was thawed and cultured for three passages prior to use in these studies.

The first study aimed to reduce the substrate concentration by 60% from that typically used in 2D culture whilst also looking at the stability of culture medium at two temperatures. This would allow the medium to be prepared in bulk and stored above the Quantum® further reducing the labour requirement.

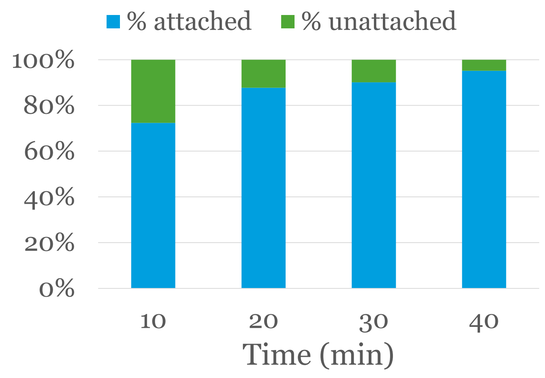


Average population doublings over 3 passages were comparable between all culture systems. Cell morphology was also comparable with cells cultured on 0.2 µg/cm² VTN displaying similar morphology to those cultured on 0.5 µg/cm² VTN.

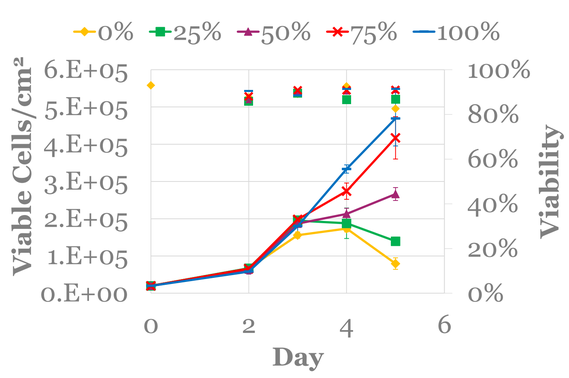
The second study focused on operating parameters during expansion in the Quantum looking at the attachment kinetics of CGTRCiB10 cells and the medium demand over the culture cycle. This was to then help inform the seeding and feeding strategy in the Quantum®. iPSC were cultured for three cycles and then seeded into multi-well plates. Medium was exchanged at a percentage of the standard volume over the course of three expansion cycles.



Representative images from the first expansion cycle. Percentages denote medium exchange volumes.



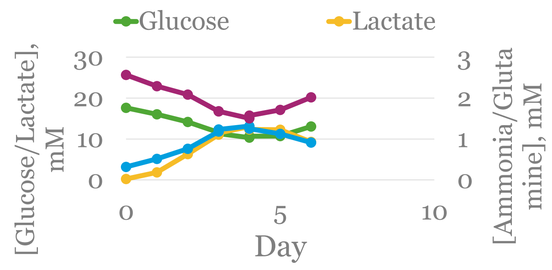
Cell attachment kinetics for CGTRCiB10. Cells were seeded onto multi-well plates and the supernatant removed and counted every 10 minutes. >95% of cells were attached 40 minutes after seeding. Remaining cells displayed low viability suggesting no further attachment of cells beyond this time.

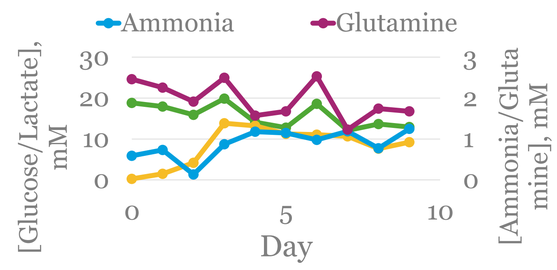


Average count and viability data of CGTRCiB10 cells over three passages where different proportions of culture medium was exchanged. The medium requirement on each day can be seen allowing the determination of a cell number based feeding strategy.

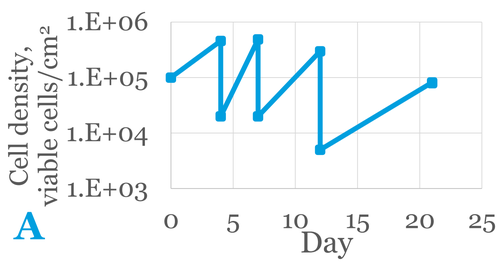
Large scale, automated expansion of iPSC

Development of closed and automated large-scale systems for integrated PSC culture, wash and concentration are imperative to improve process reproducibility and reduce process risks and cost. We are investigating the Quantum® system to expand iPSC. In preliminary experiments, we have achieved a 20-fold expansion in the Quantum® with cells retaining high viability and markers for pluripotency.





Glucose, lactate, ammonia and glutamine measurements taken throughout the expansion in the Quantum®. Both expansions display comparable rates of consumption/production over the culture period. Metabolites plateau over the extended culture (right) owing to the control of medium feed rate.



A

B

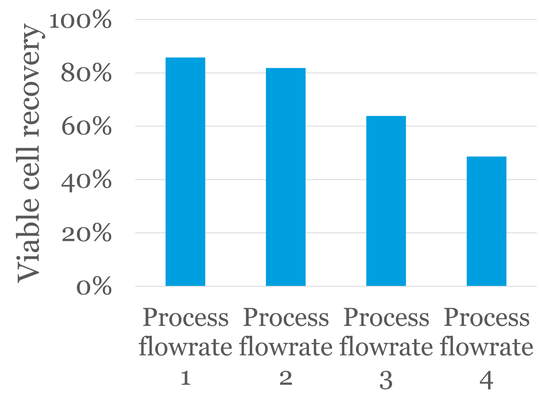
	SSEA-3	SSEA-4	TRA-1-60	SOX-2
Run 1 Inoculum	92%	99%	91%	99%
Run 1 Harvest	65%	99%	95%	99%
Run 2 Inoculum	98%	99%	95%	91%
Run 2 harvest	97%	99%	70%	99%
Post-kSep	99%	99%	94%	100%

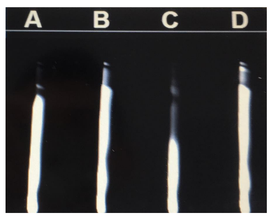
A) Representative seeding and harvest densities for the seed train and Quantum expansion. A markedly lower harvest density is observed for the Quantum expansion compared to flask-based seed culture. B) Surface marker analysis for inoculum and harvest.

A six-day expansion in the Quantum® yielded 7.4 x10^8 viable cells (9.3-fold expansion) whereas a nine-day culture yielded 1.4 x10^9 cells (23.5-fold expansion) with high cell viability (>90%).

Automated wash and concentration of harvested iPSC

Dissociated cell expansion typically uses open, batch centrifugation to remove dissociation agent and buffer exchange into the desired medium for subsequent processing. Here we have used the kSEP® and determined operating parameters for washing and concentrating cell harvest from the Quantum®.





Reactor conc.	Viability	Harvest conc.	Viability	Total viable cells recovered
0.96 x10^6	92%	9.6 x10^6	93%	**84%**

Determination of wash and concentrate processing parameters revealed a direct relationship between establish bed flow rate, processing flowrate and cell recovery. The image (far right) shows formation of the bed in the processing chamber.

Cost of Goods analysis

To determine the economic benefit of moving towards an automated process, we developed a Cost of Goods model comparing a typical manual manufacturing process expanding PSC's in flasks and concentrating and washing using centrifugation to the process described in this poster expanding cells in a Quantum® hollow fibre reactor followed by concentration and wash processes using a kSEP® continuous centrifugation system.

This work demonstrates the feasibility of an automated upstream and downstream platform to grow and recover large amounts of viable high quality PSCs. Automated platforms will provide a step change in PSC therapeutic use, and the potential to integrate additional expansion and differentiation unit operations are being investigated for the development of cost-effective manufacturing of PSC-derived products for cell therapy applications.

We work with Innovate UK

Cell and Gene Therapy Catapult
12th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
+44 (0) 203 728 9500 | info@ct.catapult.org.uk | ct.catapult.org.uk

CATAPULT Cell and Gene Therapy