Development of a cost efficient platform for the industrial manufacturing of pluripotent stem cell derived products for cell therapy: cell expansion is the starting point

Jahid Hasan, Rhys Macown, Daria Popova, Celine Martin, Evangelia Rologi, Isabel Uwagboe, Mark Bell, Ilaria Schena, Nicole Nicholas, Shai Senderovich, Garikai Kushinga, Marc-Olivier Baradez, Beata Surmacz-Cordle, Julie Kerby, Damian Marshall, Sarah Callens, Ricardo Baptista, Stephen Ward. Contact: jahid.hasan@ct.catapult.org.uk

Strategy The Cell and Gene Therapy Catapult aims to develop cost-effective processes for the industrial manufacture of therapies derived from pluripotent stem cells (PSC) in both 2D and 3D culture systems. Data is shown for the development of industrialisable processes and control systems using an iPSC cell line generated from GMP-grade material.

Analytical framework / IPC

Generation of cell banks and characterisation assays → **2D and 3D operational design space (small scale)** → **Intensification and scale-up** → **Investigation of automated wash/ concentration technologies**

Systematic process assessment – Risk/Cost/Quality

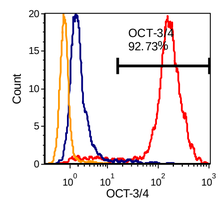
Industry reference materials

Cell banks and CQAs

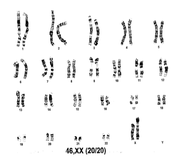
Donor selection → **Tissue Acquisition (leukapheresis)** → **Reprogramming (CD34+/Oct4/Sox2/Klf4/ L-Myc/Lin28)** → **Pre-seed Lot** → **Seed Lot** → **Master Cell Bank** → **Working Cell Bank**

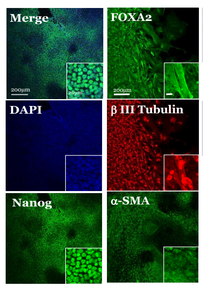
GMP — Research-grade

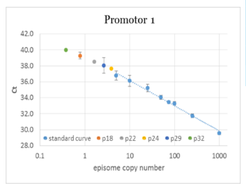
CGTRCiB10 is a research-grade iPSC line established from a cGMP seed lot, and Shef6.1 a ESC line kindly gifted to CGT by University of Sheffield. These exemplar cell lines have been adapted to 2D culture in defined reagents and characterized to industry standards in order to support process development in different culture systems in this space.



SSEA-1 +	3.1 %
SSEA-4 +	98.2 %
SSEA-3 +	91.8 %
TRA1-60 +	92.6 %
OCT4 +	92.7 %
NANOG +	90.1 %
SOX2 +	95.3 %





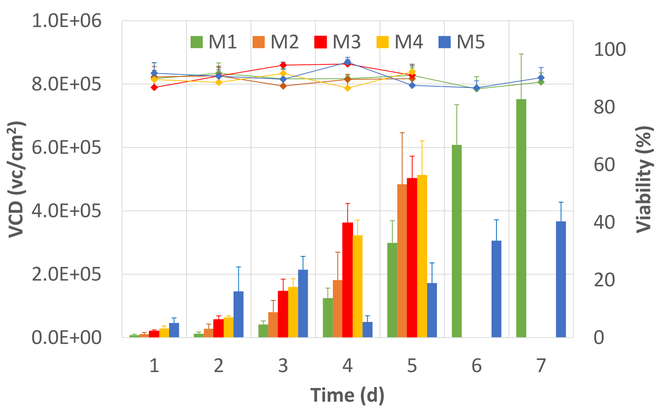


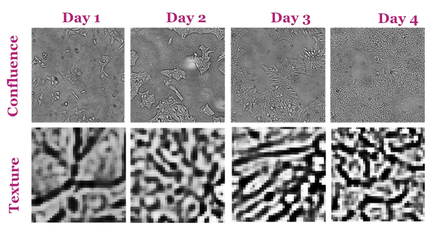
Self-renewal	Ectoderm	Mesoderm	Endoderm
-0.65	-1.20	-1.02	-1.91
-0.77	-0.70	-0.75	-1.33
-0.75	-0.82	-0.84	-1.34
-0.61	-1.15	-1.00	-1.78
-1.23	-0.79	-1.32	-1.55

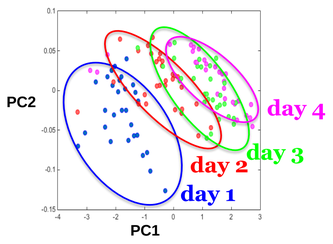
-0.5 to 0.5	Comparable
-1.0 to -0.5	
-1.5 to -1.0	
<-1.5	Downregulated

2D design space: label-free quantitative imaging

Several media formulations and protocols commercially available were screened to support the growth and maintenance of iPSC cultures. We applied an image-analysis algorithm to measure and monitor at-line specific features of cell morphology during the expansion process.

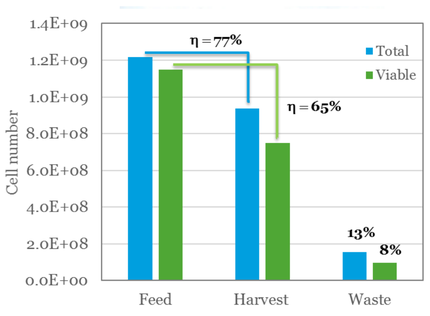






2D scale up and seamless DSP

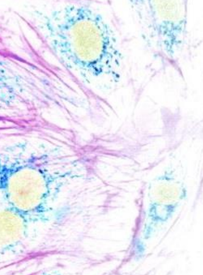
Development of closed and automated large-scale systems for integrated PSC culture, wash and concentration are imperative to improve process reproducibility and reduce process risks and cost. We are investigating the Quantum® system to expand iPSC which are then washed and concentrated using the kSep®. In preliminary experiments, we achieved a 10-fold expansion in the Quantum® and 8%6 recovery of iPSC undergoing a 10-fold concentration.

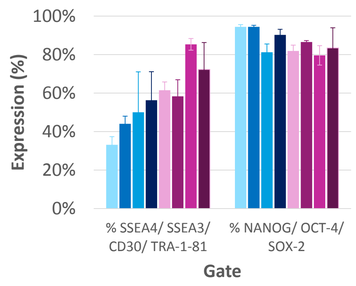


3D design space: aggregates

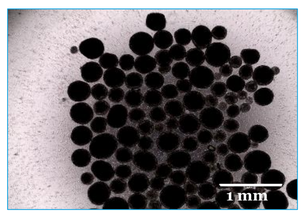
Stirred-tank reactors (micro-scale)

The AMBR15® micro-bioreactor system has been used to investigate the operational design space of stirred suspension culture of PSCs. Fixed and varied impeller speeds were investigated in combination with fixed and ramped medium exchange frequencies of two cell lines.





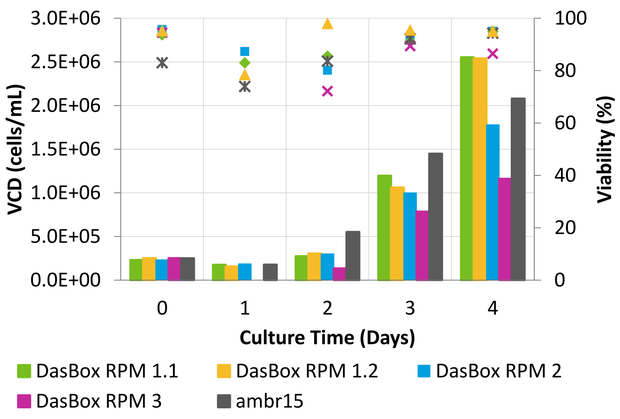
A fixed impeller speed resulted in higher cell expansion from day 2 onwards when compared to a variable impeller speed. Harvested cell yield was also higher for fixed impeller speeds across both cell lines.



Increasing the feeding frequency from day 3 onwards resulted in higher cell expansion of cell line 1. However the same effect was not seen for cell line 2. Fixed impeller speeds resulted in higher expression of pluripotency markers.

3D baseline scale and intensification

Process scale up and intensification are necessary if cell therapy bioprocesses are to benefit from economies of scale enjoyed by the biopharmaceutical industry. Our data suggest that the mixing parameters volumetric power input (P/V) and Kolmogorov size could support the scale-up of the vessel mixing properties and enable control of the size of PSC-aggregates in larger STR.



Dynamic stirred 3D suspension cultures employing various cell retention devices (CRD) are being investigated to automate and close medium exchange and cell passaging, in order to increase cell concentration in the bioreactor. Current progress shows comparability between manual manipulation and CRD integration.

Remarks and landscape

- An iPSC line has been generated from GMP-grade material and baseline processes established for the expansion of PSCs in 2D and 3D (aggregates) culture systems. PoC integration of USP-DSP operations to grow and recover large amounts of iPSCs has been demonstrated.
- An engineering strategy is proposed to support scale-up of intensified aggregate-based cultures of PSCs in STRs.
- Risk-cost based assessment is required to determine and model the impact of processing platforms on CQAs and the labour and facility requirements, prior to scale-up.
- Smart multi-parametric control systems and 3D culture systems for cell expansion and differentiation are under investigation.

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