DEVELOPMENT OF PROCESSING PLATFORMS FOR THE INDUSTRIAL MANUFACTURE OF PSC-DERIVED CELL THERAPY PRODUCTS:

CELL EXPANSION IS THE STARTING POINT

Ricardo P. Baptista, Rhys Macown, Daria Popova, Evangelia Rologi, Mark Bell, Isabel Uwagdboe, Celine Martin, Jahid Hasan, Ilaria Schena, Garikai Kushinga, Marcia Mata, Iris Valero, Nicole Nicoles, Shai Senderovich, Davide Grandolfo, Moira Francois, Marc-Olivier Baradez, Beata Surmacz-Cordle, Sarah Callens, Damian Marshall, Julie Kerby, Stephen Ward Contact: ricardo.baptista@ct.catapult.org.uk

Key Challenges

Product variations

Process translation to GMP

Process control

Cost-prohibition

CGT-RCiB10 iPSC

-0.79

-0.5<=x<=0.5 Comparable

x<-1.5

P39 (day4)

Priority Areas

- Materials
- **Automation/Scale-up/-out**
- **Process monitor/control**
- **Risk/cost/quality**

Strategy

- Design space 2D/3D culture
- Automation (closed/continuous)

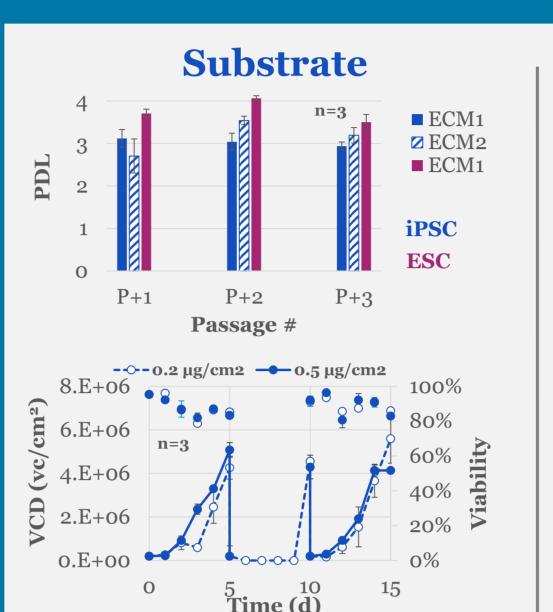
 0.66 ± 0.23

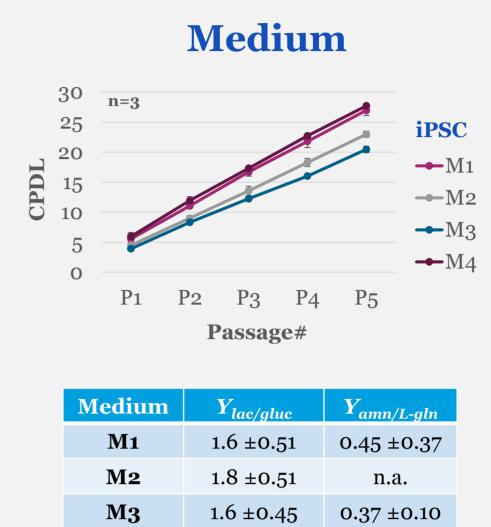
- Emerging technologies (fast processing)
- Control and quality (analytic development)
- Next Gen. CoGs reduction

Materials and CQAs

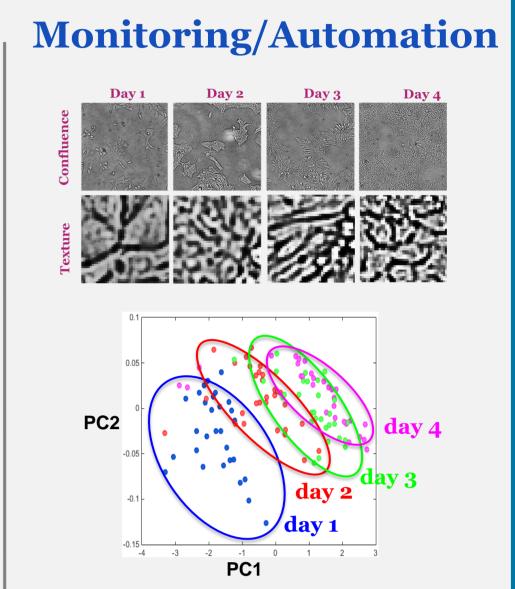








Seed Train



TRA1-60

CGT-RCiB10 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 (UKSCB). These exemplar cell lines were adapted to 2D culture in defined reagents and characterized to industry standards to support process development in different culture systems in this space. Potency was assessed by random and 6-day directed differentiation assays.

Type of substrate (ECM) and media (M) impact cell growth, metabolism, differentiation efficiency, and manufacturing costs. We tested recombinant human LN-521 and Vitronectin as substrates, and different medium formulations for 2D culture of PSCs in GMP. Expansion profiles of RCiB10 cells were found similar in two substrate concentrations, and media M1 and M2 yield similar cell numbers as shown by the cumulative doublings (CPDL) after five passages. We have developed imaging analysis algorithms to automate and control confluence assessment and remove operator variability from the process.

Aggregate Culture in STR

hESC

DASbox RMP1

Culture Time (d)

(48 genes)

Operational Design



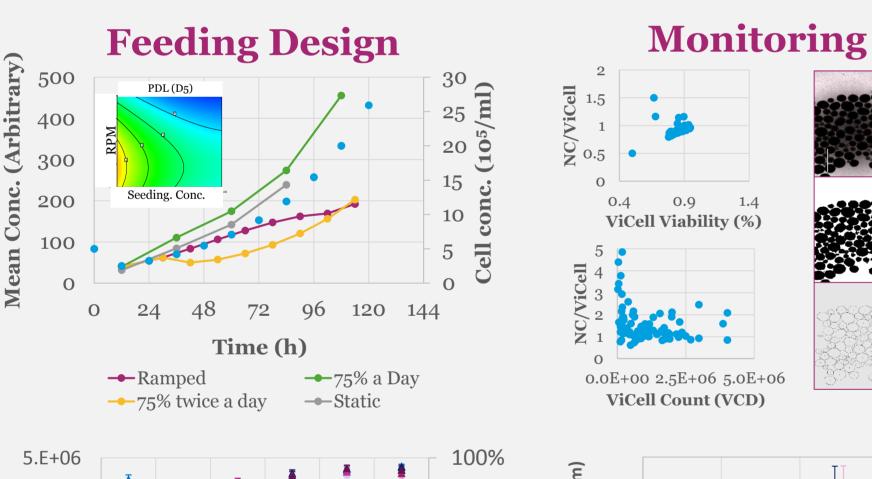
TRA1-60 + 93 %

NANOG + 90 %

Seed Master Working

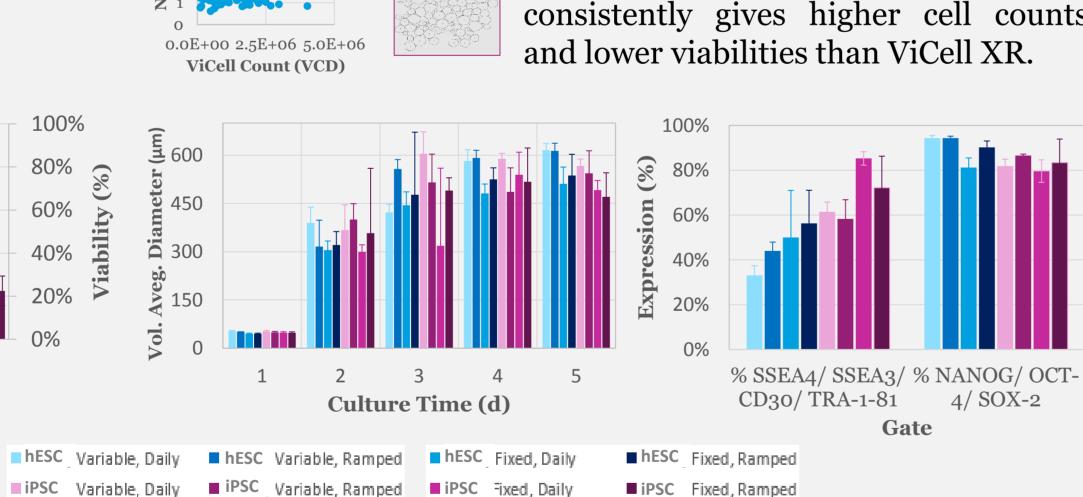


Risks
Baseline process
Quality
Productivity/ Scalability
Cost



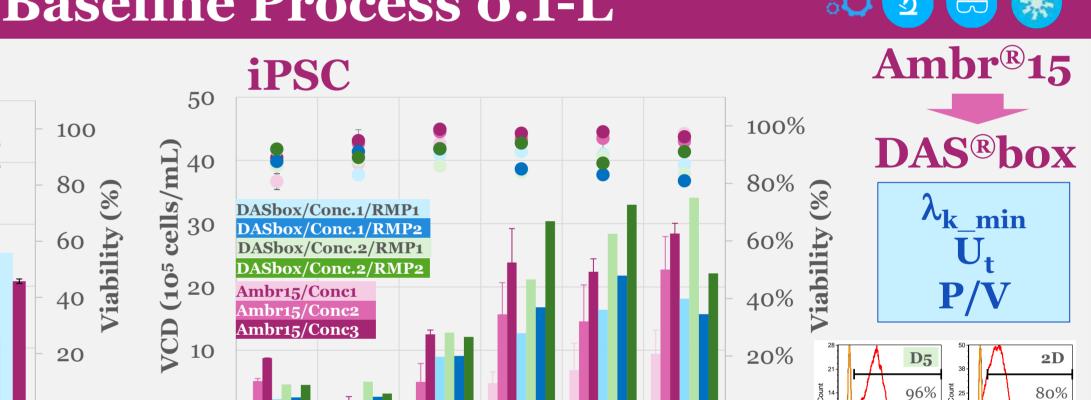
Culture Time (d)

Higher impeller speeds show a positive effect on day 1 cell survival but a negative effect on total population doublings (feeding design was needed). Lactate accumulation can inhibit cell growth at higher seeding concentrations. Nucleocounter NC-200 consistently gives higher cell counts and lower viabilities than ViCell XR.



Day 1 cell survival increased for higher impeller speeds (variable). However, a reduction in impeller speed from 24 hours did not cause the higher cell numbers to persist to day 2. From day 3, a clear increase was observed in the rate of expansion of hESC with increasing medium exchange frequency (ramped), relative to daily exchange. Increased medium exchange frequency did not increase the rate of expansion of iPSC. Fixed impeller speeds resulted in higher expression with both lines.

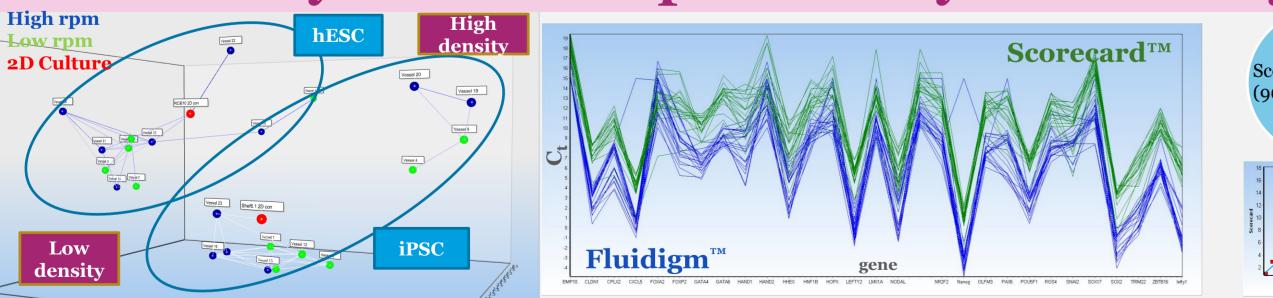
Baseline Process 0.1-L



Culture Time (d)

Different agitation rates were tested to explore scaling parameters based on Ambr15 data. Vessel geometry impacts fluid patterns and culture environment at different scales. Process parameters have been identified for Shef6.1 aggregate culture in the DASbox system, and seeding concentrations and agitation rates have been tested to compare cell growth at different scales to establish baseline process for aggregate culture of CGT-RCiB10 in 100 mL STR with maintenance of cell pluripotency (Ambr15:n=5, DASbox:n=1).

Analytical Development – dynamic array



iPSC gene expression clustering is affected by impeller speed and seeding concentration. Both dynamic arrays and ScorecardsTM show high level of similarity in gene expression. Dynamic arrays show overall lower C_t values compared to ScorecardsTM due to differences in the methods however the trend between different genes remains similar. ScorecardsTM were run on Quantstudio 7 (Thermo-Fischer) and 48.48 dynamic arrays were run on Biomark[®] HD (FluidigmTM). Statistical analysis was performed using Genex[®] 6 (MultiD).

Intensification STR+Separation Devices Manual Automated Automated STR+Separation Devices STR+Sep

Integration of cell retention devices to close and automate cell passage step in STR is being explored. The effects of type, concentration and incubation time of the dissociation agent in cell viability were assessed, and the residual amount of agent supporting the subsequent expansion cycle was determined. The concentration of Shef6.1 cells increased after two cycles of expansion in aggregate culture in STR coupled with a tangential flow filtration device. Process parameters for the dissociation step should be further explored to reduce cell death during the dissociation step.

Relevance and Ongoing work

- > The results provide operational ranges of impeller speed, seeding concentration, and feeding regimes to assist establishment of baseline process and further scale-up of PSC expansion in STR.
- > Data is promising for intensification of aggregate culture and potential continuous closed expansion and differentiation should be considered.
- > Impact of automation in process cost/risk is being evaluated, as well as the potential for the use of medium key component mimetics.



