Expansion of human pluripotent stem cells in stirred tank reactors; improved, scaled, intensified.

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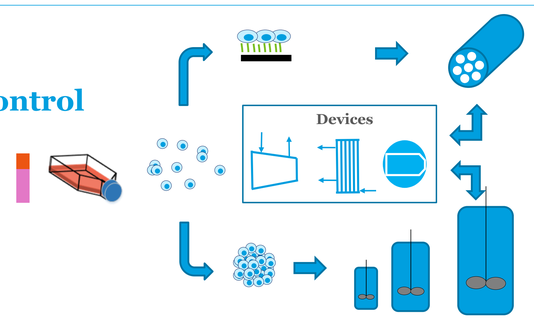
Motivation and Strategy

Despite more closely reflecting traditional biopharmaceutical manufacturing models, only one third of ongoing cell therapy clinical trials in the UK are allogeneic. Major barriers to the adoption of allogeneic approaches using pluripotent cells (PSCs) are the efficient and controlled expansion of cells to a relevant scale, the efficient and controlled differentiation and purification of target cell types, and the cost and GMP compatibility of current processing techniques. However, if these challenges are overcome, allogeneic therapies will have reduced variability in staring material and allow larger scale manufacture could more efficiently address indications with higher prevalence.

Key Challenges

- Automation and Control
- Scale-up
- Cost of Goods
- GMP manufacture



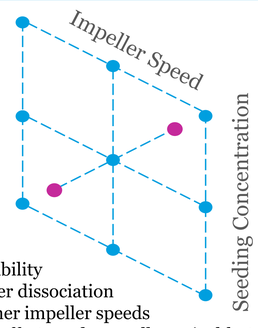
The Cell and Gene Therapy Catapult are working to overcome these barriers and develop a platform for large scale Pluripotent Stem Cell (PSC) manufacture. Using Shef6.1 and RCiB10 cell lines as exemplars of human embryonic and induced pluripotent stem cells respectively, we are; exploring design spaces to increase purity and yield, transitioning to automated 2D and 3D culture systems, developing novel monitoring systems, and investigating strategic bioprocessing approaches to scale-up PSC expansion to commercially and relevant scales.

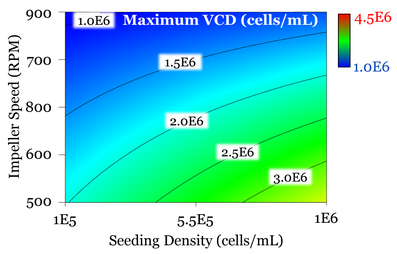
Development Targets

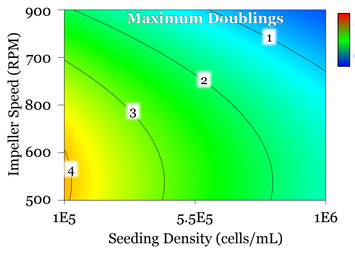
- Design Space 2D/3D Culture
- Automation of PSC Expansion
- Scale-up of PSC Expansion
- Monitoring and Control

Seeding, Stirring, and Feeding

We started investigating the design space for the 3D culture of PSC as aggregates by exploring a range of impeller speeds and seeding concentrations on the Ambr®15 micro-bioreactor system (Sartorius Stedim) using a face centred central composite design. An initial screen of feeding regimes, at the mid-point values, was also conducted. In addition to daily 75% volume medium exchange, we screened a twice daily 75% volume exchange and twice daily exchange with a volume dependent on cell concentration in accordance with the equation %Volume = 20+35 × [cell count, 10^6 cells/mL] (limited to a maximum of 80%). All Ambr®15 vessels were seeded with single-cell suspension and utilised a rho-kinase inhibitor for the first 24 hours. Cells were harvested after 5 days of culture. All conditions were screened in duplicate (n=2) except the centre point conditions (n=4).

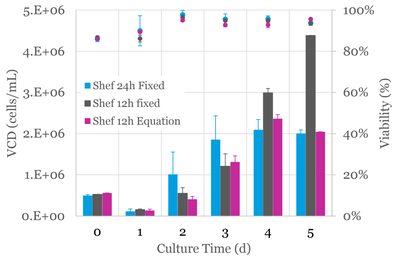


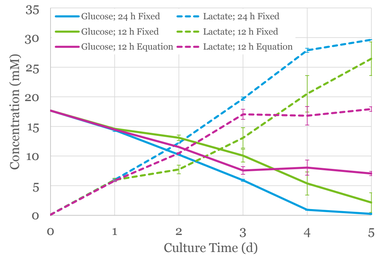




Cell counts and viability were measured after dissociation of aggregates. Higher impeller speeds showed a positive effect on day 1 cell survival but a negative effect on maximum viable cell density (VCD) and total population doublings achieved. Cell growth appeared to be inhibited at higher VCDs resulting in a positive effect of increasing seeding concentration on maximum VCD but a negative effect on total population doublings.

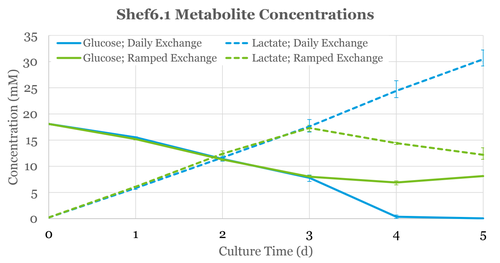
Relative to daily, 75% volume medium exchange, twice-daily, 75% volume medium exchange achieved significantly higher VCDs of Shef6.1 cells during the later days of culture. Twice-daily medium exchange with a volume based on cell count did not have a similar effect, despite similar exchange volumes over the final day of culture. RCiB10 cells showed the highest expansion with daily, fixed-volume medium exchange. Twice daily exchange of 75% of the culture medium offsets metabolite limitations by approximately 24 hours in Shef6.1 cultures and this is likely responsible for the higher VCD achieved. Equation based medium exchange may have resulted in insufficient addition of growth factors early in cell culture.

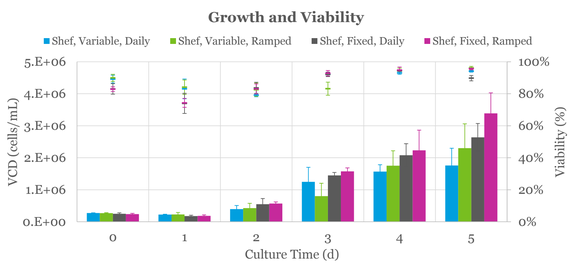


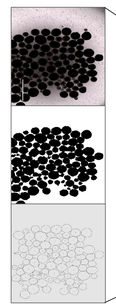


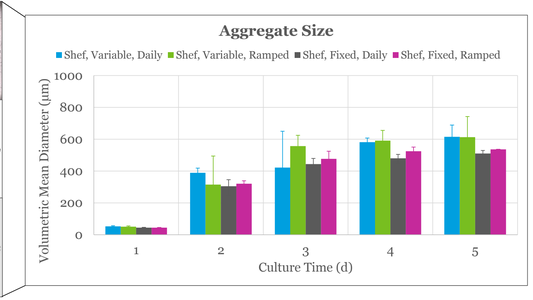
Stirring and Feeding Profiles

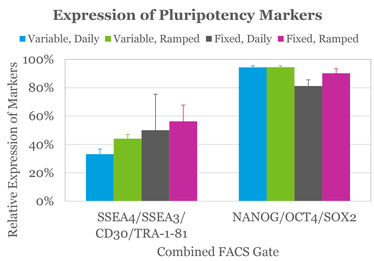
A second experiment using the Ambr®15 micro-bioreactor system built on the Shef6.1 findings of the first. In contrast to a fixed impeller speed of 700 RPM, a variable regime was investigated using, for the first day, the 900 RPM impeller speed that achieved the highest day one survival, before reducing to 500 RPM until day 3.5 to promote growth, and then increasing to 700 RPM aiming to limit aggregate size. In contrast to daily medium exchange, a ramped exchange frequency was investigated with additional exchanges at 3.5, 4.3 and 4.7 days (n=3). While the variable impeller speed approach had a detrimental effect on cell yields, the ramped medium exchange frequency controlled metabolite concentrations as planned and increased cell yields.

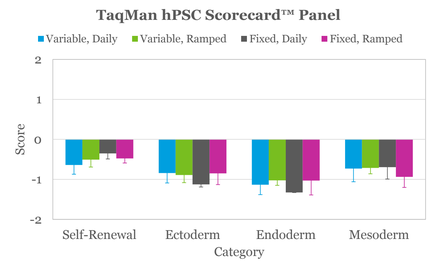












Microscope images of aggregates were analysed using an ImageJ macro designed in-house. Masks were generated and particle analysis was used to determine the cross sectional area of each aggregate. Form these areas a volumetric mean size was calculated by assuming the particles to be spherical. A lower impeller speed from day 1 to day 3.5 resulted in slightly larger aggregate diameters and these were not reduced when the impeller speed increased to 700 RPM. FACS and qPCR analysis indicate retention of a pluripotent phenotype by all cultures.

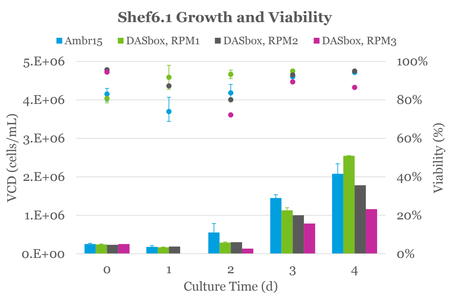
Scale-up

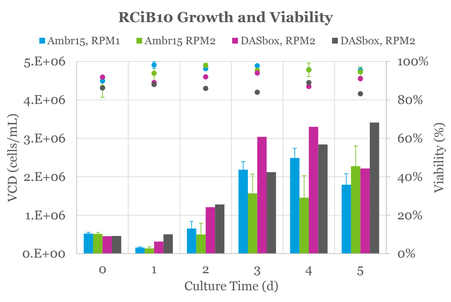
Potential scaling factors for impeller speed include tip speed, volumetric power input, and minimum Kolmogorov scale (eddy size). To determine which factors are the best predictors of scale-up a range of impeller speeds were investigated in the DASbox® mini-bioreactor system (Eppendorf AG) .

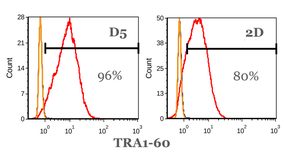
Ambr®15 → DAS®box

Scaling Factors

λ_{k_min}
U_t
P/V

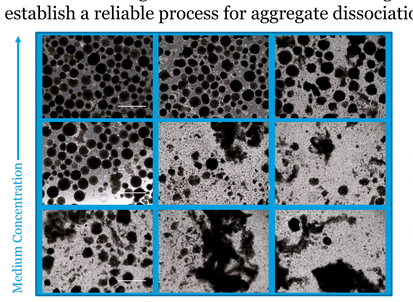


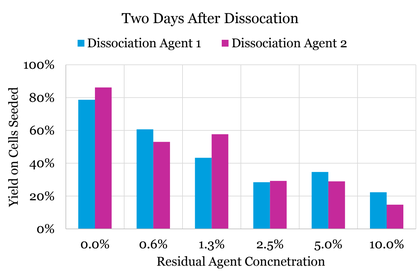


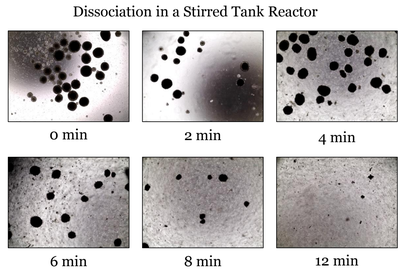


Dissociation

Automation of the dissociation of aggregates in stirred tank reactors (STRs) presents unique challenges; cell retention and vessel geometry limit volume reduction prior to addition of dissociation agent, closely packed aggregates limit contact with the agent to the outer layer requiring shear to break up aggregates, and the residual levels of dissociation agent after passage have significant effects on both cell survival and aggregate formation. We have investigated different dissociation agents, dissociation agent concentrations, residual medium concentrations and dissociation times to establish a reliable process for aggregate dissociation in STRs. We have also investigated the impact residual agent on subsequent cell expansion.

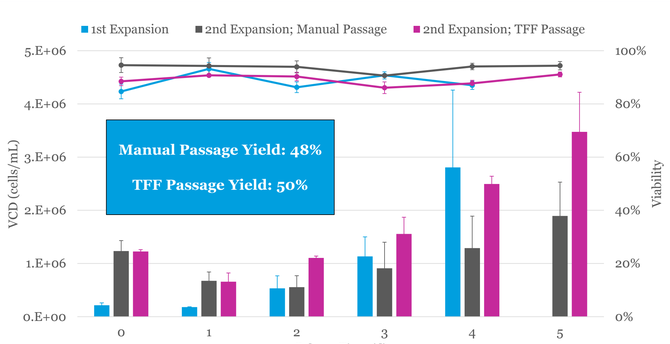






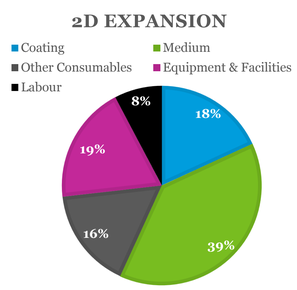
Intensification

Closed and automated passaging of aggregates to single cell inoculum is critical to scale-up and industrialisation. It may also facilitate the intensification of the PSC expansion process through multiple cycles of expansion in the same vessel at increasing seeding densities. The Cell and Gene Therapy Catapult are investigating a range of cell separation technologies for their potential to rapidly replace passaging agent with fresh medium while retaining single cells. Automated and closed cell retention systems have demonstrated comparable yields to manual processing and higher subsequent cell growth. Further work is required to minimise cell losses during aggregate dissociation and achieve higher cell densities in subsequent expansion cycles.

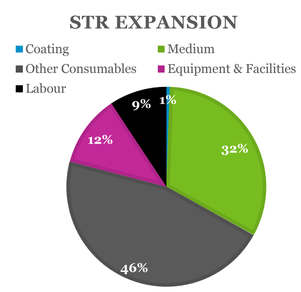


Cost of Goods

We have generated Cost of Goods models to assist our strategic platform development. Shown here are the costs to expand cells using entirely manual 2D culture vs. using a combination of 2D culture and STRs. Assumptions include an annual production of 20×10^{12} cells. Modelling suggests that in this example the shift to 3D culture would result in a 33% reduction in the cost of expanding PSCs. We continue to update our models with performance data established during bioprocess and analytical development of our manufacturing platform and can expand them to incorporate differentiation formulation and fill operations as required.



£7,200 per 10^9 cells



£4,800 per 10^9 cells

We work with Innovate UK

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