Expansion of human pluripotential 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 starting material and allow larger scale manufacture could more efficiently address indications with higher prevalence.

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 seed densities. Using 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 range of volume and daily exchange volume dependent on cell concentration according to the equation Volume = initial volume (10^5 cells/mL) (limited to a maximum of 80%). All Ambr®15 vessels were seeded with single-cell suspension and utilised a rho-kitinhin 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 seed density 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 Shef.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. RCB10 cells showed the highest expansion with daily, fixed-volume medium exchange. Twice daily exchange of 75% of the culture medium offsets metabolic limitations by approximately 24 hours in Shef.1 cultures and this was more consistent for the higher VCD achieved. Equation based medium exchange may have resulted in insufficient addition of growth factors early in cell culture.

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).

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 shearing 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.

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 × 10^11 cells. Modelling suggests that in this example the shift to 3D culture would result in a 33% reduction in the cost of producing 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.

We work with Innovate UK

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