# DEVELOPMENT OF PROCESSING PLATFORMS FOR THE CONTROLLED, SCALABLE AND COST-EFFECTIVE MANUFACTURE OF ALLOGENEIC THERAPIES DERIVED FROM iPSCs:

## *PROCESS DEVELOPMENT STRATEGIES FOR AGGREGATE-BASED CULTURE.*

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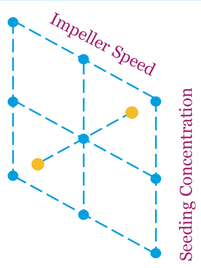
**Background and Aims:** Cell plasticity technologies herald a disruptive clinical potential for allogeneic cell based therapies. However, Cost of Goods and process complexity are key challenges for the commercialisation and competitiveness of iPSC-derived allogeneic therapy. Here, we presented The Cell and Gene Therapy Catapult (CGTC) strategy to develop a process for expansion of iPSCs as high-density, aggregate based culture in stirred tank reactors (STR) at 100-mL baseline scale of development. We investigate the effect of operational parameters on critical quality attributes of iPSCs expanded in STR and assessed scaling factors to support process translation from optimisation small-scale (Ambr®15) to baseline scale of development (100-mL).

**iPS cell line:** CGT-RCiB10 is an iPSC line established from episomal reprograming of $CD34^+$ cells from PBMNCs. This exemplar cell line was adapted to 2D culture in defined reagents and characterized to industry standards to support process development in different culture systems in this space.

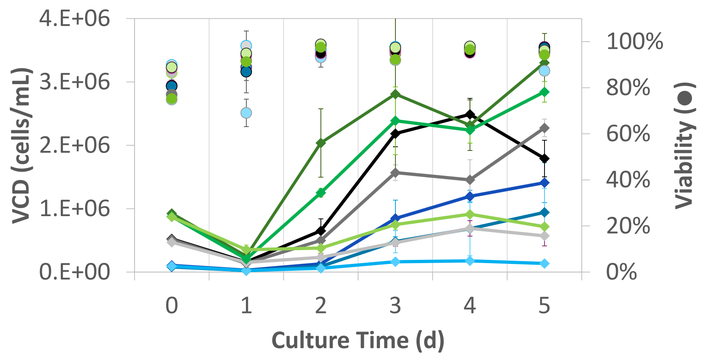
## Operational Design (Ambr®15)

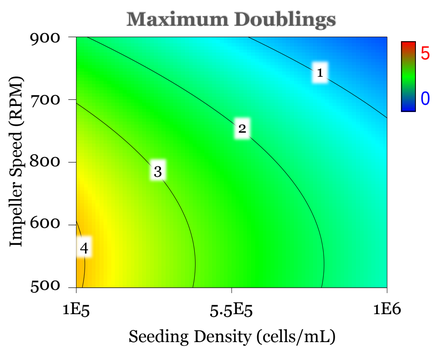
**Critical Parameters (CPP)**
- Seeding density
- Agitation rate
- Medium exchange



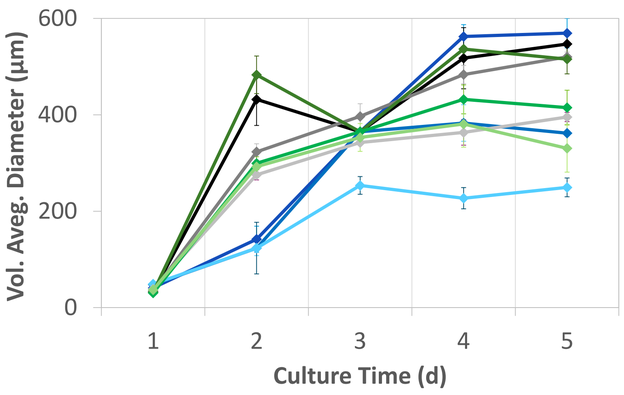

**Quality Attributes**
- Growth rate
- Viability
- Aggregate size
- Phenotype (flow cytometry)

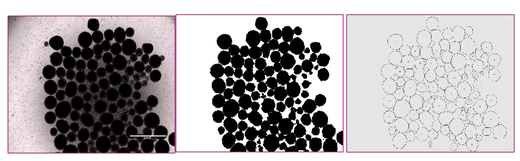
### Cell Growth



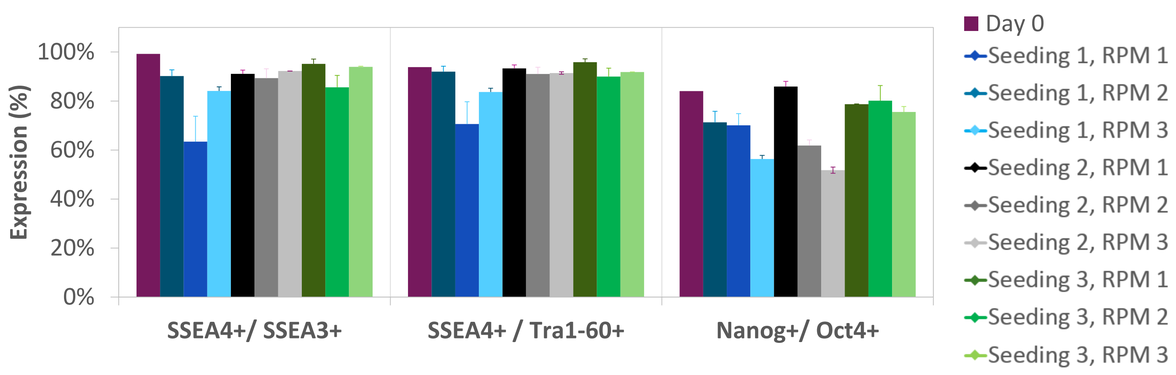

### Aggregate Size



A script was written to automate aggregate size distribution analysis. Phase-contrast captions of aggregates from culture samples were analysed using ImageJ software (image above with aggregates sampled at day 4 of culture in Ambr®15. Scale bar 1000 µm).

### Phenotype




Cell counts and viability were measured after dissociation of aggregates. Higher impeller speeds shows a positive effect on day 1 cell survival but a negative effect on total population doublings and co-expression of NANOG/OCT3/4. Aggregate size of approximately 400 mm is maintained after day 3 at all agitation rates of cultures seeded a Seeding 3. A 75% (v/v) daily medium exchange with two exchanges on day 4 was the medium exchange regime used herein as result from previous investigations.

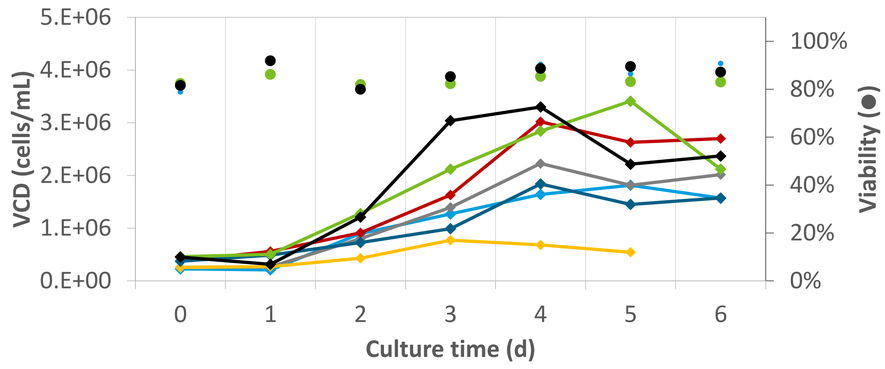
## Scale-up - Baseline Process 0.1-L

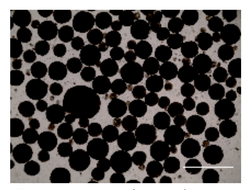
Ambr®15 → DASbox®

| DASBox® | RPM 1 | RPM 2 |
|---|---|---|
| $U_t$ (m.s$^{-1}$) | 0.43 | 0.52 |
| $\Lambda_{k,min}$ (µm) | 32 | 28 |
| P/V (W.m$^{-3}$) | 54 | 93 |
| $\tau_{max}$ (Pa) | 1.0 | 1.4 |

| Ambr®15 | RPM 1 | RPM 2 | RPM 3 |
|---|---|---|---|
| $U_t$ (m.s$^{-1}$) | 0.3 | 0.42 | 0.54 |
| $\Lambda_{k,min}$ (µm) | 35 | 27 | 22 |
| P/V (W.m$^{-3}$) | 18 | 50 | 107 |
| $\tau_{max}$ (Pa) | 0.60 | 1.0 | 1.5 |

### Cell Growth and Aggregate Size

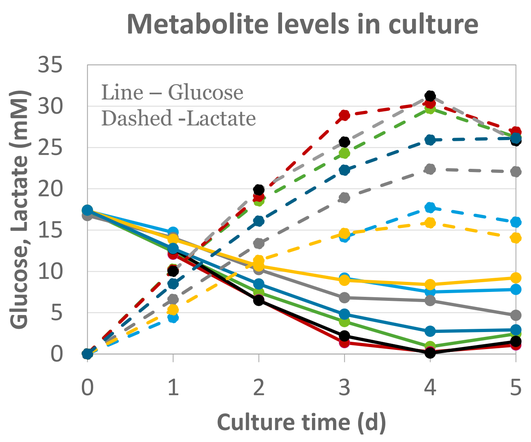


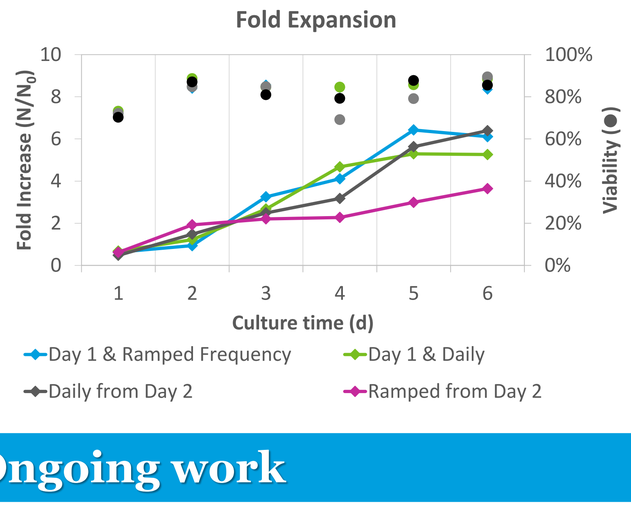
Representative image from DASBox® at day 4 (scale bar 1000 µm).

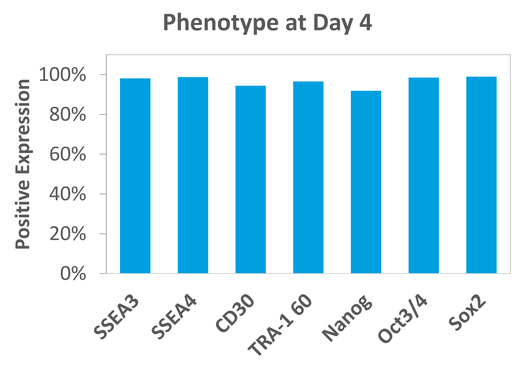
### Medium Exchange

Different agitation rates were tested in 100-mL STR to explore scaling parameters impeller speed, Kolmogorov scale, dissipated power, and shear stress based on Ambr ®15 data. Vessel geometry impacts fluid patterns and culture environment at different scales. Seeding concentrations and agitation rates are being tested to compare quality attributes between the two scales (DASBox:n=1). Preliminary screening of feeding strategies were performed in order to increase final yield. A twice daily 75% volume exchange and ramped 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%).




Cell growth arrest may be caused by a combination of several factors: aggregate size or due to metabolite depletion/accumulation. A 6.4-fold increase in cell numbers ($N/N_0$) corresponding to viable cell concentration of $3x10^6$ cells/mL were achieved after 5 days of expansion in 100-mL STR. Flow-cytometry data showed maintenance of pluripotent phenotype (>90% positive expression of pluripotency related proteins).

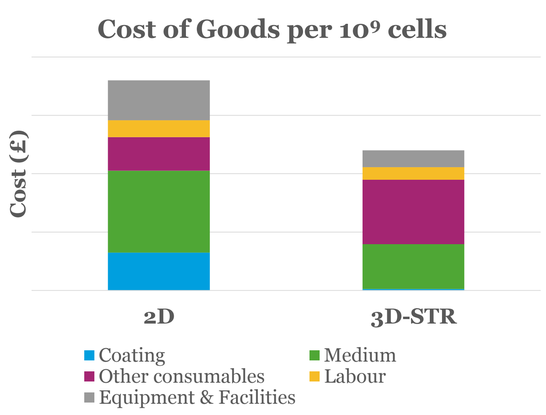



## Relevance and Ongoing work

**Towards a clinical grade automated closed system**

- ✓ **Cell line with Industry Standard QC**
- ✓ **Aggregate culture in 100 mL STR**
- ✓ **Adherent culture in Quantum®**
- ? **Scale-up STR culture**
- ? **Feeding Regime**
- ? **Process Intensification**
- ? **Downstream Processing**




- ➢ Operational ranges of agitation rate, seeding concentration, to assist translation from Ambr15 and establishment of process at baseline scale of development and scale-up of PSC expansion in STR.
- ➢ Intensification of aggregate culture (e.g. perfusion) and potential of continuous and closed culture for several expansion cycles is being developed.
- ➢ Impact of automation in process cost/risk is being evaluated, as well as the potential for the use of medium key component mimetics.

We work with Innovate UK

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