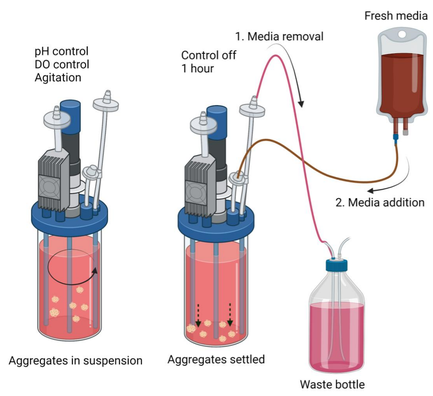
DEVELOPMENT OF AN END-TO-END CLOSED iPSC EXPANSION PROCESS USING A CELL SPECIFIC PERFUSION REGIME FOR PROCESS INTENSIFICATION

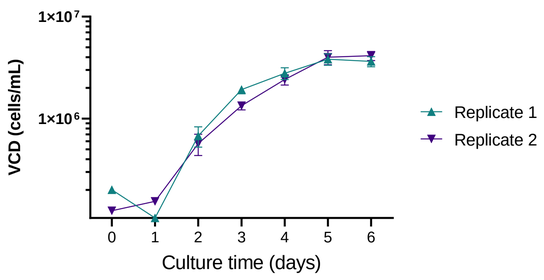
A R Podovei M Tregidgo A Karachaliou P Statham N Agabalyan M Mata J Guenat J Hasan

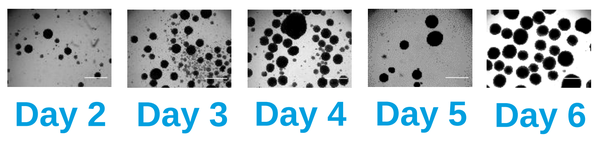
Contact: Alexandru-Robert.podovei@ct.catapult.org.uk

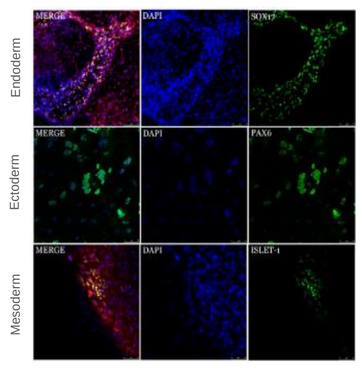
Introduction: Pluripotent stem cell (PSC)-derived therapies target large patient populations and require high cell doses making them unsuited to traditional 2D expansion. Here we present the development of a closed, scalable and automated process for the expansion of PSCs in high-density, aggregate cultures in stirred tank reactors (STR). The generation of PSCs in sufficient quantities to seed a bioreactor at scale can be closed and automated effectively using a hollow fibre bioreactor system. Resulting cells were single cell-seeded into DASbox® STR, using periodic settling to allow for automated medium exchange. However, periodic settling reduces ability to control aggregate size, a critical parameter which can impact expansion and differentiation. Therefore, an acoustic perfusion system was utilised to investigate the potential for process intensification and improved control of aggregate size, whilst maintaining pluripotency. Finally, integrated technologies were investigated for PSC concentration and wash to allow for automated passages.

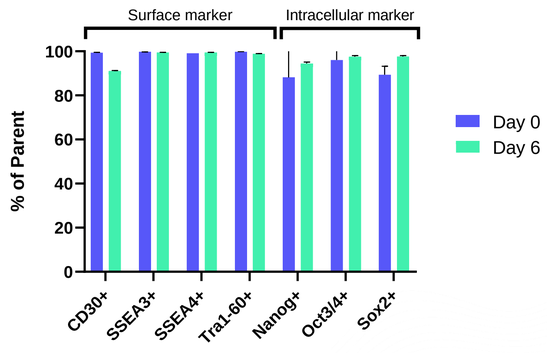
3D EXPANSION REPEATED BATCH PLATFORM





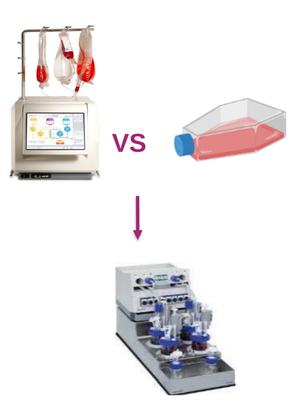


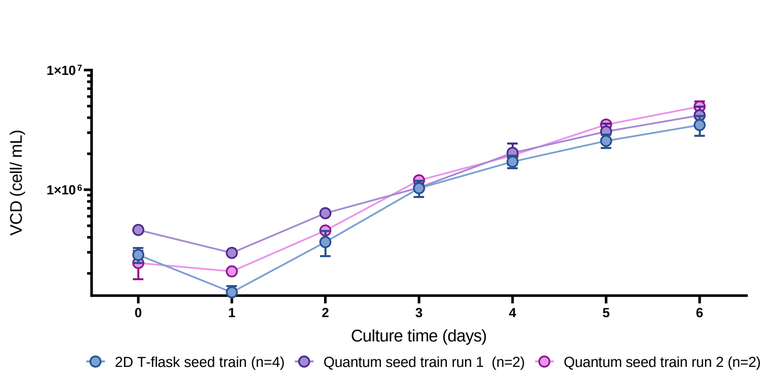




- Easily **translatable platform** for aggregate formation and iPSC expansion in 3D.
- Automated medium exchange, and DO/pH control.
- **Larger aggregates** due to settling step.
- Feeding regime supports VCD up to **5E6 cells/mL**.
- Maintenance of pluripotency and **tri-lineage** differentiation capability.

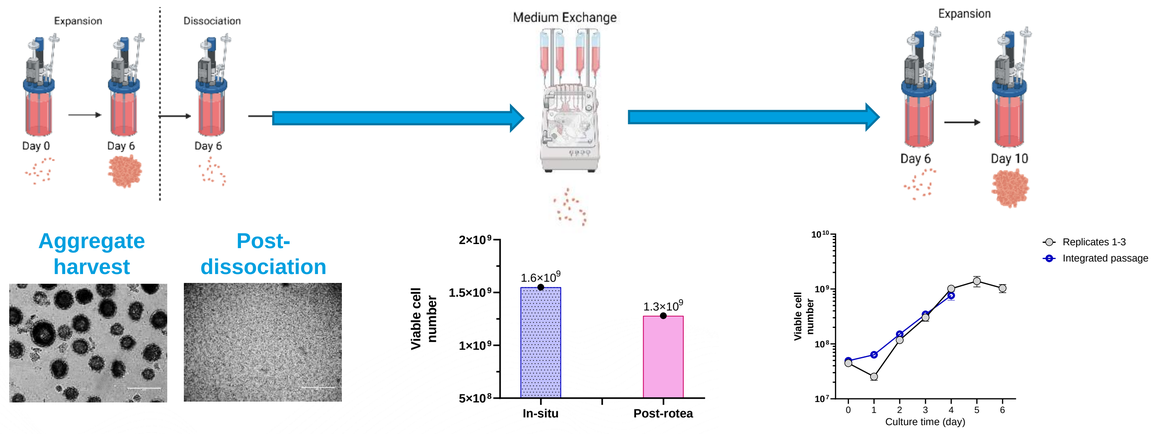
CLOSED SEED TRAIN





Reproducible process achieving **10-fold** expansion **in 6 days** with retention of pluripotency markers. **Comparable expansion** observed in 3D in DasBox using repeated batch process from traditional **2D seed train vs. Quantum** seed train.

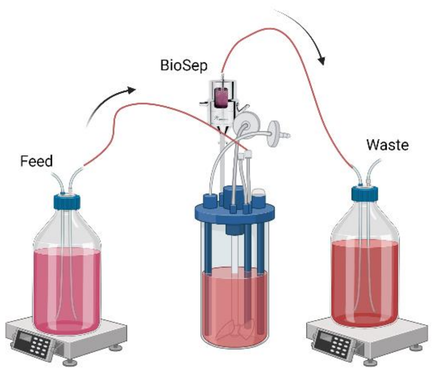
INTEGRATIVE PASSAGE CAPABILITY



- In-house developed process for ***in situ* aggregate dissociation**.
- Rotea™ for **automated medium exchange** with good viable cell recovery **(85%)**.
- Following integrated passage an **equivalent growth curve** was observed as in initial expansion.
- Demonstrates capability for process **intensification**.

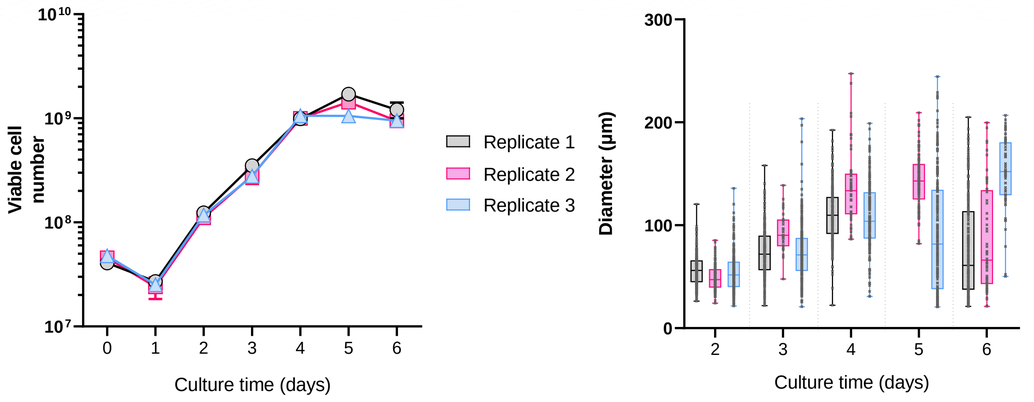
INTENSIFIED PERFUSION PROCESS ENABLES HIGH DENSITY iPSC EXPANSION

ADAPTABLE PLATFORM

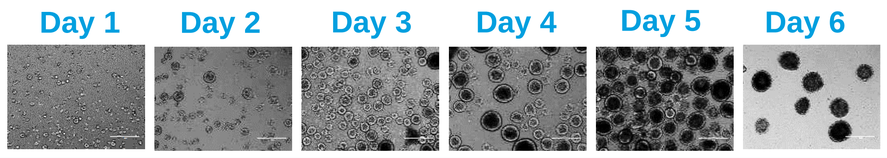


- Seeded with single cell, with **aggregate formation** by Day 2 .
- **Perfusion** system using BioSep® cell retention device.
- Medium addition/removal is **automated**.
- Fed at **cell specific perfusion** rate.
- Control over DO, pH, agitation.

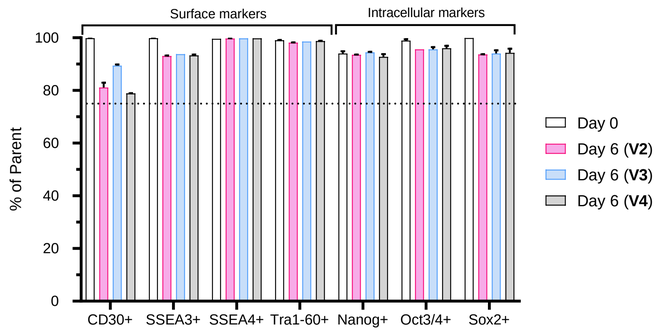
HIGH YIELDS



- Perfusion enables high density cultures with reproducible yields of **>10E6 cells/ml**, as well as control over aggregate diameter between **100-200 µM**.



PLURIPOTENCY



- In-house pluripotency panel demonstrates maintenance of **pluripotency** following 6-days expansion.

Conclusions: Following a closed seed-train, the repeated batch process demonstrates a means of repeatable iPSC expansion, whilst maintaining pluripotency. Importantly, this process is easily translatable, in principle, to groups wishing to work with iPSCs. The BioSep® acoustic filtration system uses a more complex set-up to permit perfusion, allowing for further optimisation and intensification of the process, through overcoming feeding limitations and allowing for greater control over aggregate size. Results of this intensification are high yields of ~1E9 viable pluripotent iPSCs with an aggregate size between 50-200 µM. A comparable growth profile was seen post-integrated passage, which is valuable for further intensification within this platform. Future work will focus on incorporating iPSC differentiation into the process, after encouraging proof of concept data.

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