CASE STUDY

Process Development for Scaled-up hESC/hiPSC Manufacturing

An optimized 14-day manufacturing workflow which produces > 10^{10}, cells was developed in < 12 months

CHALLENGE

To scale up, close and optimize a suspension-based manufacturing workflow for pluripotent stem cells (PSCs).

PROCESS DEVELOPED

A best-in-class, scalable manufacturing platform with closed passaging and in-line analytics.

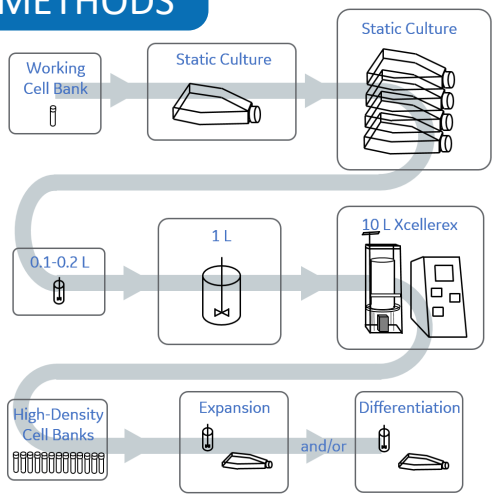
OUTCOMES

- **Scalable process capable of > 10^{10} cells per batch**
- **50% process time reduction**
- **Development of sample-free and closed workflows**

INTRODUCTION

Both human embryonic and induced pluripotent stem cells (hESCs and hiPSCs, together PSCs) have the ability to indefinitely self-renew and differentiate into any cell type. Stirred-tank reactors (STRs) for the large-scale expansion of PSCs have been reported by several groups and have the potential to generate clinically relevant amounts of cells. Here we report on a scale-up expansion seed train, able to produce 3.6 x 10^{10} PSCs in an 8 L volume of a Xcellerex disposable bioreactor.

METHODS



RESULTS

Scale-up of suspension-based, microcarrier-free PSC expansion was performed across a three-stage seed train (Figure 1). High-density seed banks enabled removal of the initial adherent culture and 200 mL STR stage, normalizing manufacturing input and reducing process time from four to two weeks. Utilization of a Quality by Design approach has ultimately improved manufacturability by developing closed passaging workflows and predictive biomass algorithms, both of which reduce process contamination risk and cell loss.

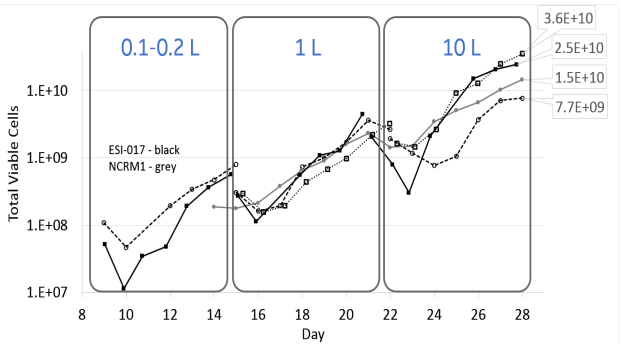


Figure 1: *Viable cell density.* *Three expansion runs are presented for ESI-017 (embryonic stem cell line, black series). Process transfer across cell lines was demonstrated with NCRM1 (iPSC cell line, grey series).*

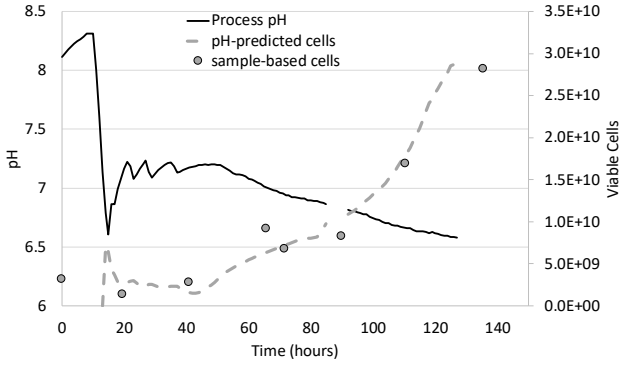


Figure 2: ***Process pH predicted viable cell counts.*** *Model fits and correlations between pH and cell growth were used to develop an equation to predict cell mass from process pH (dashed series).*

Previously, samples were withdrawn to determine cell concentration and to measure metabolite levels. In our initial, sample-based, manufacturing workflow perfusion rates were increased in response to increasing cell concentration. As the manufacturing approach became more defined, we were able to draw correlations between continuous process data (e.g. pH and dissolved oxygen) and viable cell production (Figure 2). Subsequent production runs have relied on these correlations to enable sample-free expansion with predicted cell density, perfusion feed rate changes and vessel harvest timing. This development not only reduces labour and contamination risks associated with sampling, but also allows for automated metabolic control of perfusion feed rates. As PSCs grow, they consume glucose and dissolved oxygen and produce lactic acid, driving down pH. Together, these traditional sensor outputs provide a metabolic proxy for cell activity and allow for a cell metabolism-specific feeding regime.

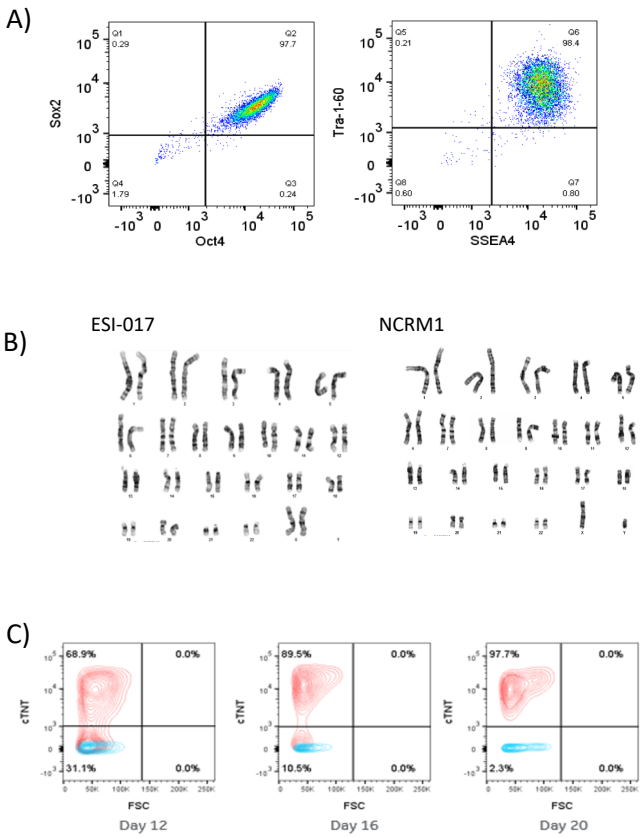


Figure 3: ***Pluripotency, Karyotype and Differentiation of STR-derived PSCs.*** *(A) Expression of Sox2, Oct4, Tra-1-60 and SSEA4. (B) Karyotypic stability (ESI-017; 46,XY and NCRM1; 46,XX). (C) Differentiation to cardiomyocytes with > 97% cTNT positive cells on Day 20.*

DISCUSSION

The manufacturing workflow developed offers a path to the production of clinically-relevant amounts of PSCs. For clinical use, these cells must maintain their critical quality attributes (CQAs) and differentiation ability. For PSCs, CQAs include expression of pluripotency markers and maintenance of karyotypic stability (Figure 3A & 3B) and, in our case, differentiation to cardiac cells was monitored by cTNT expression (Figure 3C).

SUMMARY

This work has established a clinically-relevant, Good Manufacturing Practices (GMP)-ready manufacturing platform for PSCs. The original three-stage seed train has been improved to two-stage, reducing manufacturing time by 50%. In-line process data have been correlated to cell density, removing the need for sample withdrawal. Finally, PSCs from this process demonstrate maintenance of CQAs and the ability to differentiate to a clinically relevant cell lineage.

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