



Technical Note:

Scaling Up Production of Human Induced Pluripotent Stem Cells (hiPSC) using Spinner Flasks.

Introduction

One of the shortcomings of stem cell therapy is that it requires a significant number of cells to produce an effective therapeutic outcome for the patient. For example, one to two billion cardiomyocytes are required to treat damaged heart tissue after myocardial infarction, and about 1.3 billion insulin producing β -cells are needed to realize insulin independence for insulin patients. It is not practical to generate the required numbers of cells using static culture flasks, so efficiently transitioning the culture from a static process to a continuously agitated microcarrier based suspended culture platform is the optimal way to create the large numbers of cells required in a cost-efficient manner.

Process Summary

Step 1.

Evaluation of whether O-negative blood derived hiPSCs lines expanded under agitation on a microcarrier platform during the pluripotent expansion and mesoderm stages could be further differentiated through the hematopoietic and erythroid induction stages under continuous agitation in suspension culture, on microcarriers, in 6-well ULA plates using an O-neg hiPSC cell line D5. Control was using static conditions using normal tissue culture plates using a monolayer static protocol. Pluripotent expansion (7 days) and mesoderm differentiation (3 days) were established.

All cell lines were cultured on Laminin-5231 (LN-521) coated Cytodex 1 microcarriers.

Step 2.

After 3 days of hematopoietic mesoderm differentiation individual cells (derived following trypsinization from the microcarriers) from the following cell lines, were further differentiated into 50mL shaker flasks which were incubated for 27 days

- O-negative erythroid progenitors (D9, D12, X13 and BR7),
- Bone marrow derived hiPSCs (BM1)
- Dermal fibroblast derived hiPSCs (FR202 and IMR90)

Step 3.

Following the demonstration that multiple hiPSCs lines can be differentiated under constant agitation in 50mL shaker flasks, the next step is to demonstrate scale up of differentiation and reproducibility in 125mL and 500ml spinner flasks.

- 2 cell lines, X13 hiPSCs and FR202 hiPSCs which demonstrated the best differentiation outcome in shaker flasks were used.
- 3 spinner flasks for each cell line were used. Media was exchanged every 3 days but increased to daily in the erythroid expansion phase.
- In 125mL spinner flasks, X13 achieved approximately a 10-fold expansion during the pluripotency stage while maintaining pluripotency levels based on the data from analysis of the following markers, Oct-4, Tra1-60, SSEA-4, T-bra, and FDR+.
- By day 27 of the X13 culture all 3 spinner flasks yielded hemoglobinized erythroid cells with an average cumulative fold expansion of 58.6 +/-8.1 fold.
- In 125mL spinner flasks FR202 hiPSCs also produced the following markers T-bra, KDR+, CD34+, and CD43+.

- Erythroid cells differentiated from FR202 hiPSCs were able to achieve a cumulative fold expansion between 206-fold and 805-fold, and a total yield of $3.3 - 5.9 \times 10^8$ erythroid cells in a final media volume of 60mL
- The erythroid differentiation process was expanded into 500ml spinner flasks (with 200ml of media) and generated approximately 1.6×10^9 erythroid cells at densities up to 8.2×10^5 cells/mL

Step 4.

Functional evaluation of the erythroblasts differentiated from hiPSCs in spinner flasks was performed to determine equivalency to adult RBCs from peripheral blood.

- hiPSCs derived erythroblasts expressed alpha, beta and gamma globins. Blood derived RCB's expressed on alpha and beta globins.
- The P_{50} values for oxygen equilibration curves were significantly lower in erythroblasts derived from hiPSCs, (12.1 – 12.7) than from adult RBC's (17.9).
- Evaluation by qRT-PCR of the expression levels of genes involved in hemoglobin switching, HbF to HbA showed these genes to be expressed at higher levels in hiPSC-derived erythroid cells than adult RBCs.

Summary.

1. A suspension agitation culture differentiation process was developed for differentiating hiPSCs toward erythroblasts
2. The process can be volumetrically scaled up
3. The hiPSC-derived erythroid cells were highly similar to adult derived erythroid cells at the molecular level.

References.

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