CASE STUDY

Manufacturing of pluripotent stem cell-derived cardiomyocytes for pre-clinical animal studies

Translation of a cell therapy protocol from bench-scale to bioreactor

CHALLENGE
Generating cryopreserved doses of 1 billion PSC-derived cardiomyocytes

PROCESS DEVELOPED
A manufacturing workflow to produce cardiomyocytes in stirred tank reactors

OUTCOMES
- **Seed train for PSC expansion up to 3 L**
- **20-day differentiation workflow including downstream processing and characterization**

INTRODUCTION

Dr. Michael Laflamme and his team at University Health Network in Toronto, Canada, lead the development of a cell therapy to repair damaged heart tissue. Producing cells at doses required for pre-clinical large animal studies was not feasible using conventional culture methods. CCRM collaborated with Dr. Laflamme to translate a static and partially adherent protocol for pluripotent stem cell (PSC) expansion and differentiation to cardiomyocytes into a scalable and closed-system stirred tank bioreactor platform.

METHODS

PSCs were expanded as aggregates in stirred tank reactors and then differentiated to the cardiac lineage over the course of 20 days, using a 3-stage differentiation protocol based on the pioneering work of Dr. Gordon Keller[1]. Quality control assays were integrated throughout the process to measure batch consistency. Downstream processing incorporated heat shock[2], cryopreservation, thaw and formulation of the cardiomyocyte cell product (Figure 1).

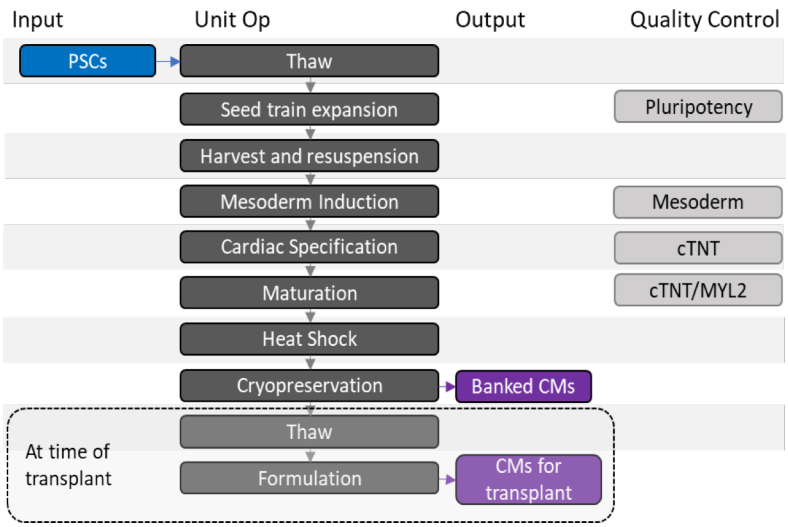

Figure 1. *Manufacturing workflow to produce PSC-derived cardiomyocytes.*

RESULTS & DISCUSSION

PSCs expanded ~6-fold over 5 days at each scale (125 mL, 1 L, and 3 L) and were dissociated to single cells prior to inoculation in the subsequent reactor volume. Throughout the expansion process, PSCs maintained high viability (> 85%) and high expression of pluripotency-associated proteins OCT4, SOX2, SSEA4 and TRA160 (> 90% co-expression), as measured by flow cytometry (Figure 2).

Following expansion, PSCs were dissociated, re-seeded in stirred tank reactors and differentiated to cardiomyocytes. At various stages, cultures were assessed for critical quality attributes using multi-colour flow cytometry panels. Following PSC aggregate formation, mesoderm commitment was induced using Activin A and BMP4 and confirmed by assessing expression of PDGFRα and CD56, after which cardiac specification was achieved by inhibiting Wnt signaling. After 8 days, the majority of cells were positive for cardiac troponin (cTNT), indicating successful cardiac specification. Cells were cultured for an additional 12 days to allow for maturation of the cardiomyocytes. The final cell product contained a mixture of cardiomyocytes, including myosin light chain 2 (MYL2)- positive ventricular cardiomyocytes (Figure 3).

Batches of cardiomyocytes underwent heat shock, dissociation and cryopreservation and detailed characterization. On the day of transplant, doses of 1 billion cardiomyocytes were thawed, formulated, transported to the surgical site and used to engraft a pig model of myocardial infarction.

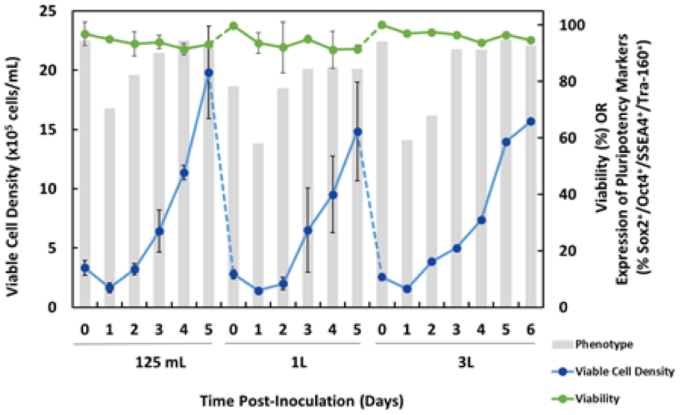

Figure 2. *PSCs expanded over multiple passages in STRs of increasing volume maintained high viability and pluripotency. Dotted lines indicate passaging of cells.*

SUMMARY

We have developed a workflow for the manufacture of PSC-derived cardiomyocytes in stirred tank reactors. This project highlights:

1. PSC expansion in stirred tank reactors
2. Generation of cardiomyocytes at doses required for large animal experiments
3. A manufacturing workflow to support pre-clinical cell therapy studies

REFERENCES

1. Kattman, S.J. et al. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell.* 2011;8(2):228-240.

2. Laflamme, M.A. et al. Formation of human myocardium in the rat heart from human embryonic stem cells. *The American Journal of Pathology*. 2016;167(3):663-671.

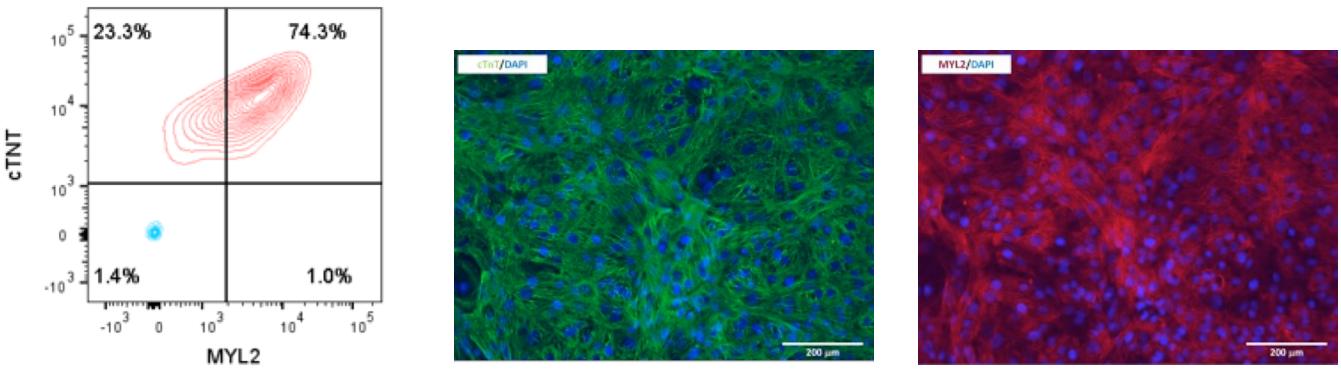

Figure 3. *PSCs aggregates are differentiated to cTNT+/MYL2+ cardiomyocytes over 20 days in STRs.*

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