Large-scale manufacture of hiPSC-derived endothelial cells for drug discovery and cell therapy

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BACKGROUND

The convergence of cell biology and bioprocess engineering is creating fundamental new ways to impact disease. Advancements in induced pluripotent stem cell (hiPSC) technologies have substantially expanded access to many human cell types to accommodate the future demand for such therapies. However, the direct utilization of standard cell manufacturing equipment and methods in the differentiation and manufacture of iPSC-derived cells can face significant challenges in obtaining the necessary production scales, quality standards and high reproducibility between batches for cost-effective cell therapy research and clinical application.

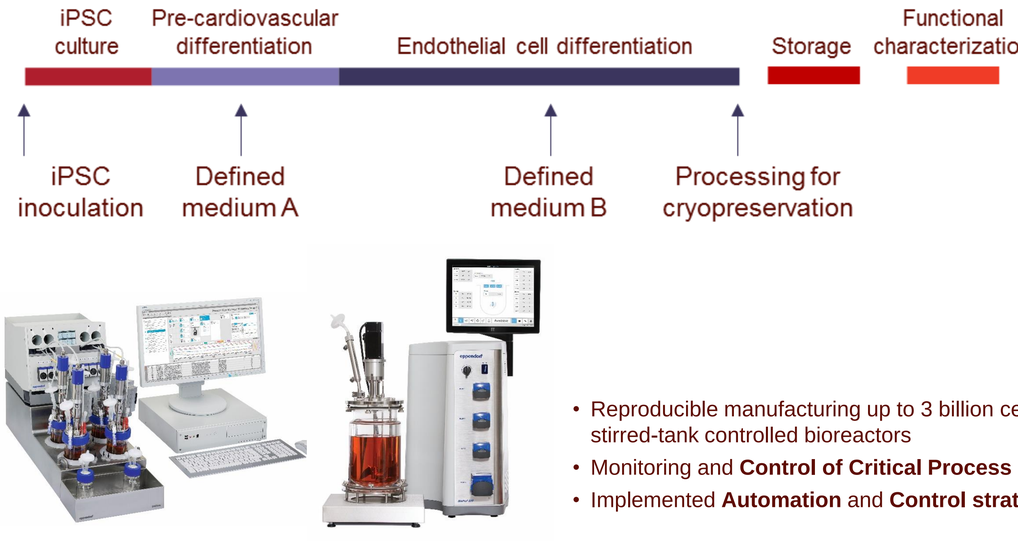
Currently, the development and production of hiPSC-derived cell types is often performed in a small-scale culture, unsuitable for robust generation of a large number of cells. Stirred-tank bioreactors have emerged as promising culture systems for large-scale cell manufacturing from hiPSC sources. These systems allow full automation and conduction in closed systems, resulting in cultures with comparable characteristics from batch to batch. Closed-system, parallel processing with increased automation is also critical to minimize error and contamination from human interaction with cell products.

Ncardia has established a controlled stirred-tank bioreactor platform that is shown to routinely yield high numbers of hiPSC-derived endothelial cells and additional cell models. This scalable technology enables Ncardia to manufacture billions of high-quality iPSC-derived cells, meeting an essential need for effective use in cell therapy, safety and efficacy applications, in terms of volume, safety and affordability. Using a Quality by Design approach, we demonstrate a robust and controlled process for large-scale manufacturing (>1x10^9) of iPSC-derived endothelial cells to a purity of >90% in a serum-free protocol.

The bioreactor-derived endothelial cells are shown to recapitulate angiogenesis in a capillary formation model, which can be used for release-testing and is also compatible with high-content imaging and high-throughput screening.

We demonstrate the implementation of a flexible process development workflow comprised of state-of-the-art bioreactor systems that allows for optimization of processes at 15 mL scale, validation of promising conditions at pilot-scale (100 - 250 mL) and manufacture hiPSC-derived highly functional endothelial cells at a billion scale. This workflow, as part of our large-scale manufacturing platform, enables us to manufacture iPSC-derived cells of the highest quality and purity at any needed scale, meeting the needs of high cell-demanding therapies.

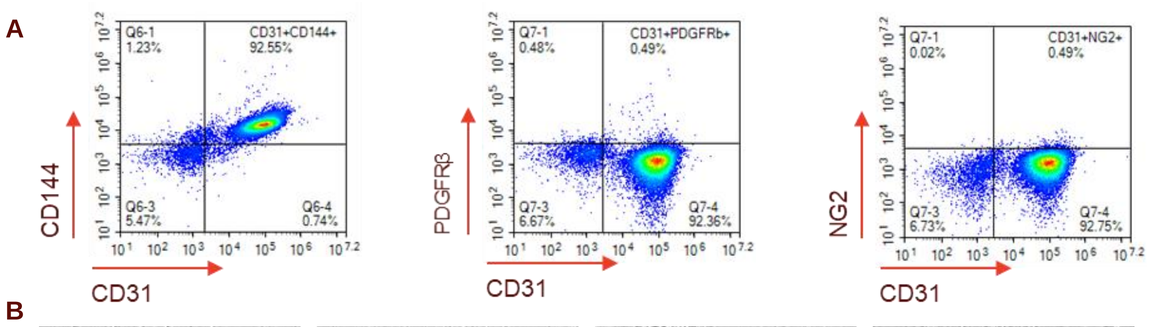
1 Ncardia's novel and fully optimized large-scale iPSC-based manufacturing platform

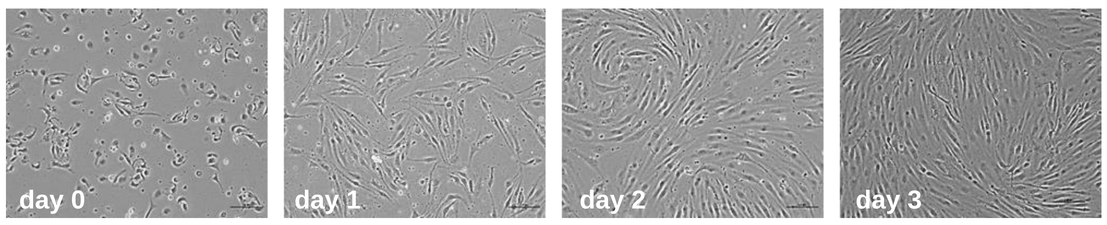


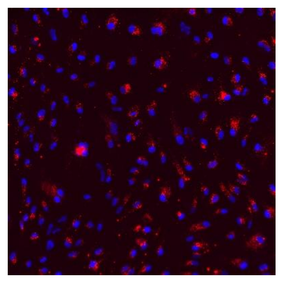
- Reproducible manufacturing up to 3 billion cells using stirred-tank controlled bioreactors
- Monitoring and **Control of Critical Process Parameters**
- Implemented **Automation** and **Control strategy**

Schematic representation of hiPSC-derived endothelial cell manufacturing (top) in fully controlled stirred-tank bioreactors (bottom)

2 Identity of iPSC-derived endothelial cells manufactured at large-scale

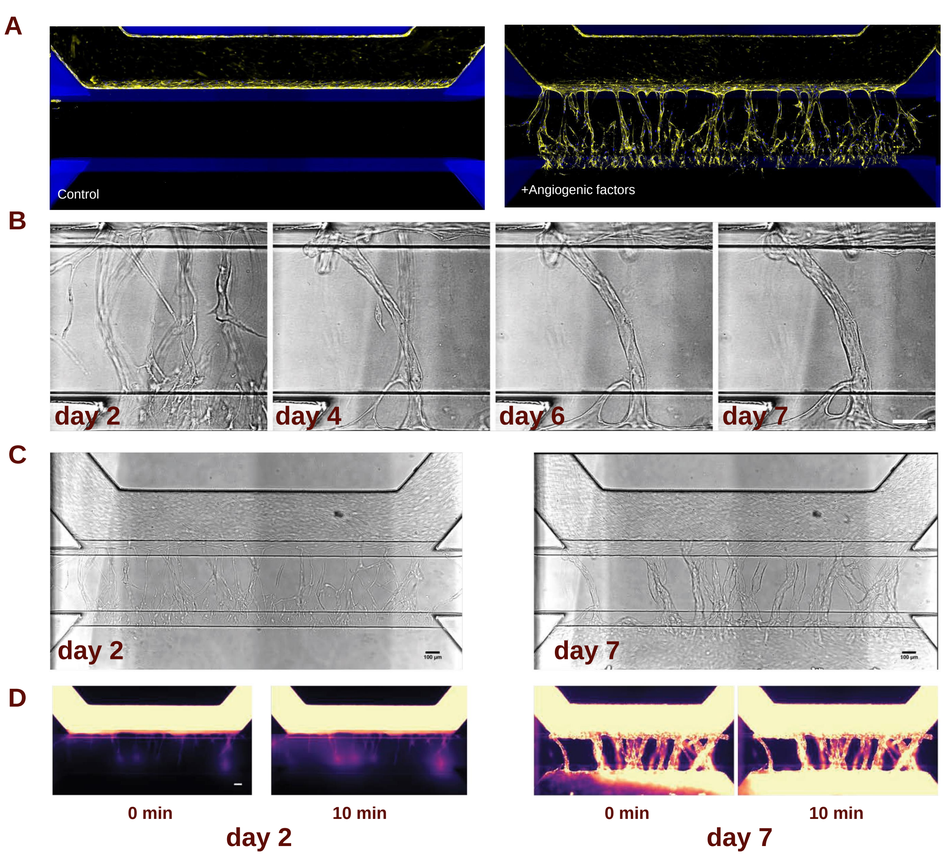






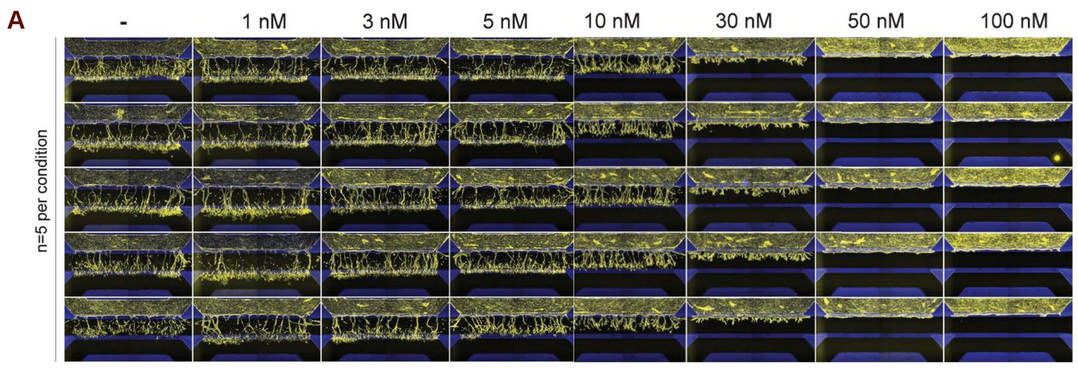
Endothelial cells were manufacturing at large-scale in a strirred-tank controlled bioreactor. A. FACS profiles at cryopreservation representing expression of endothelial cell markers Platelet endothelial cell adhesion molecule (CD31; PECAM-1) and VE-Cadherin (CD144) and mural cell markers Plated-derived Growth Factor Receptor-β (PDGFRβ) and Neuron-glial antigen 2 (NG2). B. Phase contracst images of iPSC-endothelial cells in culture after cryopreservation. C. iPSC-endothelial cells take up acetylated-low density lipoprotein (Ac-LDL in red, nuclei in blue)

3 iPSC-ECs are able to form angiogenic sprouts which stabilize upon anastomosis



A. Microvessels were cultured for 2 days, followed by 2-day exposure to controls (left) or angiogenic growth factors (50 ng/mL VEGF, 2 ng/mL PMA and 500 nM Sphingsine-1-Phosphate; right), fixated and stained for DNA and F-actin. The figure shows a representative example of a single microfluidic unit. B. Close-up of a capillary bed at 2, 4, 6 and 7 days after stimulation. C. Angiogenic sprouts after 2 days (left) and 7 days (right) after stimulation. D. Anastomosis with basal channel triggers pruning and maturation of angiogenic sprouts. At day 2 (left) and 7 (right) microvessels were perfused with 0.5 mg/mL TRITC-albumin solution. After 7 days sprouts are connected to the other side and formed a confluent microvessel in the basal perfusion channel (right). Scale bars: 100 µm

4 iPSC-EC angiogenic sprouts are blocked by pharmacological concentrations of angiogenesis inhibitors



	Migration distance	Capillary area	Nuclei in capillaries	Reference values
Signal window	14.8	11.7	11.7	>1
Z'-factor	0.8	0.8	0.8	>0.5
CoV max signal (0 nM) (%)	13	25	24	<20
CoV min signal (0 nM) (%)	20	70	71	<20

A. Representative images of angiogenic sprouts of iPSC-ECs after 48 hours in combination with various concentrations of Sunitinib. B. Vessels (blue) and nuclei (yellow) segmentation result with complete inhibition (left) and without inhibition (right). C. Quantified sprout length (left), vessel area (middle) and total number of nuclei (right). Sprouting was quantified using Molecular Devices MetaXpress software. D. Assay characteristics of iPSC-ECs with or without Sunitinib inhibiton. E. Quantified sprout length at different Sunitinib concentrations normalized to the positive control.

CONCLUSIONS

- Ncardia has developed a controlled process for large-scale manufacturing of iPSC-derived endothelial cells.
- Manufactured iPSC-ECs are able to reproduce important hallmarks of angiogenic sprouting in vivo, including
  - Tip- and stalk cell formation
  - Gradient-directed angiogenic sprouting
  - Formation of sprouts with perfusable lumen
  - Junction stabilization upon anastomosis
  - Concentration dependent, physiological response to two anti-angiogenic model compounds
- Ncardia's large-scale manufacturing platform enables the manufacture of highly functional iPSC-derived endothelial cells at any needed scale, meeting the needs of high cell-demanding therapies.