Scalable Production of hiPSC-Derived Cardiomyocytes in Stirred-Tank Bioreactors

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Abstract

Stem cell-based technologies lay the basis for pioneering approaches in drug discovery, drug safety testing, and regenerative medicine. The routine application of human induced pluripotent stem cells (hiPSCs) and functional cells derived from hiPSCs in high-throughput applications for drug discovery will require the constant supply of high cell numbers at a consistent, high quality. Two-dimensional systems like T-flasks are widely used for the cultivation of stem cells; however, they are limited in control and scalability. Stirred-tank bioreactors have emerged as promising culture system for large-scale expansion of stem cells and their differentiation into the desired cell type.

Ncardia® is a stem cell drug discovery and development company developing highly predictive human cellular (disease) assay systems for safety and efficacy testing. The company's goal is to accelerate and improve drug candidate selection, reduce drug development costs and ultimately increase drug discovery and development efficiency. Large-scale manufacturing of hiPSC-based cardiomyocytes at high quality is integral for its cardiovascular services. Here we demonstrate the suitability of the Eppendorf DASbox® Mini Bioreactor system for the expansion of hiPSCs and subsequent production of Ncardia's Pluricyte® Cardiomyocytes.

We show that using the DASbox system, hiPSCs were successfully expanded as cell aggregates in a highly reproducible manner. During the production of three different batches, aggregates were of homogenous size and the cells retained key iPSC markers including Sox2, Oct3/4 and Nanog during the expansion phase. More importantly, using the DASbox, a fully controlled production process scalable for large-scale manufacturing of hiPSC-derived cardiomyocytes could be established. Quantification of cardiomyocyte markers (cTNT and MLC2v) revealed the robust and efficient generation of multiple and pure ventricular-like cardiomyocyte batches. Analysis of the electrophysiological properties by multielectrode arrays (MEA) demonstrated the mature phenotype of the cells. The expected response to cardioactive reference compounds further confirmed the functionality of bioreactor-derived cardiomyocytes.

Altogether, the Eppendorf DASbox Mini Bioreactor System allows smooth integration of Pluricyte Cardiomyocytes into high-throughput cardiovascular screening solutions to enhance the assessment of cardiac safety and efficacy of drug candidates.

Stem cell technology for drug discovery

The data presented were obtained by scientists at Ncardia. They tested, whether the DASbox Mini Bioreactor System was suitable for hiPSC expansion and differentiation in a reproducible manner.

Ncardia
Stem cell experts

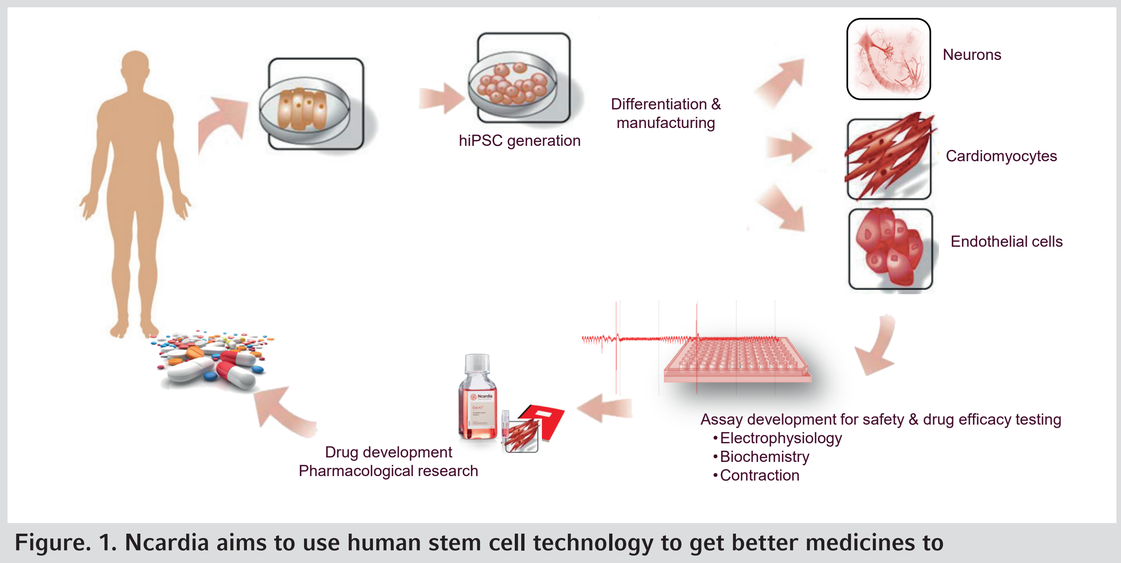


Figure. 1. Ncardia aims to use human stem cell technology to get better medicines to patients faster by improving the drug discovery and development process.

Bioprocess equipment

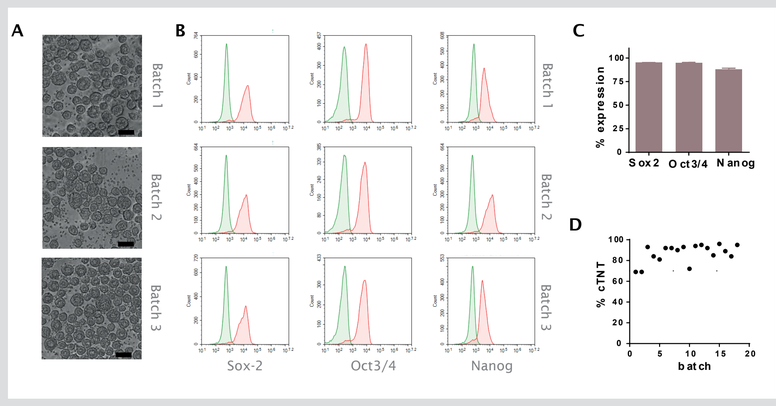
Figure. 2: DASbox Mini Bioreactor System

The DASbox Mini Bioreactor System was used as culture system for the expansion of hiPSCs as cell aggregates and their differentiation into cardiomyocytes.

> Parallel control of up to 24 bioreactors
> Allows monitoring and control of critical process parameters like dissolved oxygen, pH, temperature
> For use with conventional glass bioreactors or BioBLU® Single-Use Vessels
> Rigid-wall, stirred-tank design simplifies culture scale-up

Marker expression in DASbox

Figure 3. Characterization of iPSC aggregates and differentiated cardiomyocytes in DASbox Mini Bioreactors show reproducible stem cell and cardiac marker expression.
A: Phase contrast imaging of iPSC aggregates
B: FACS profiles of iPSC marker expression at the start of pre-cardiac specification phase
C: Quantification of iPSC marker FACS data
D: Percentage of cTNT in cardiomyocytes batches generated with DASbox



Characterization of iPSC-derived cardiomyocytes

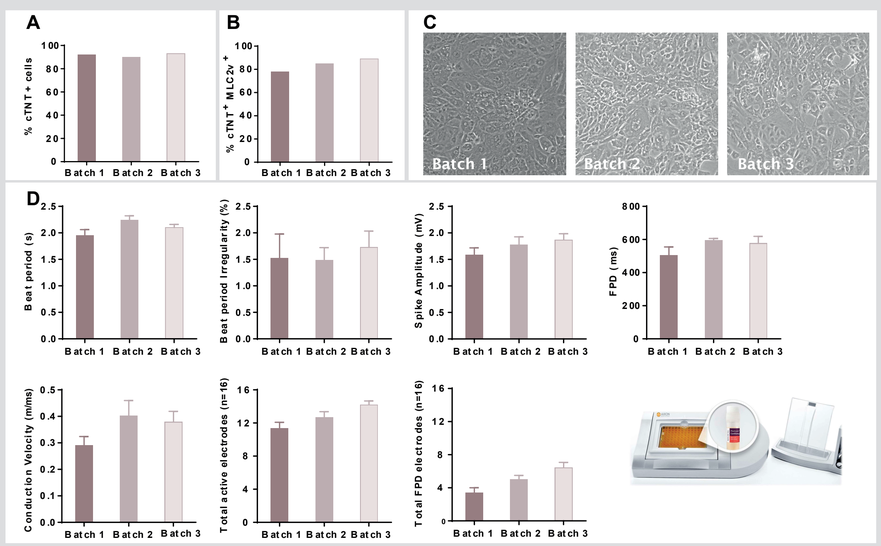
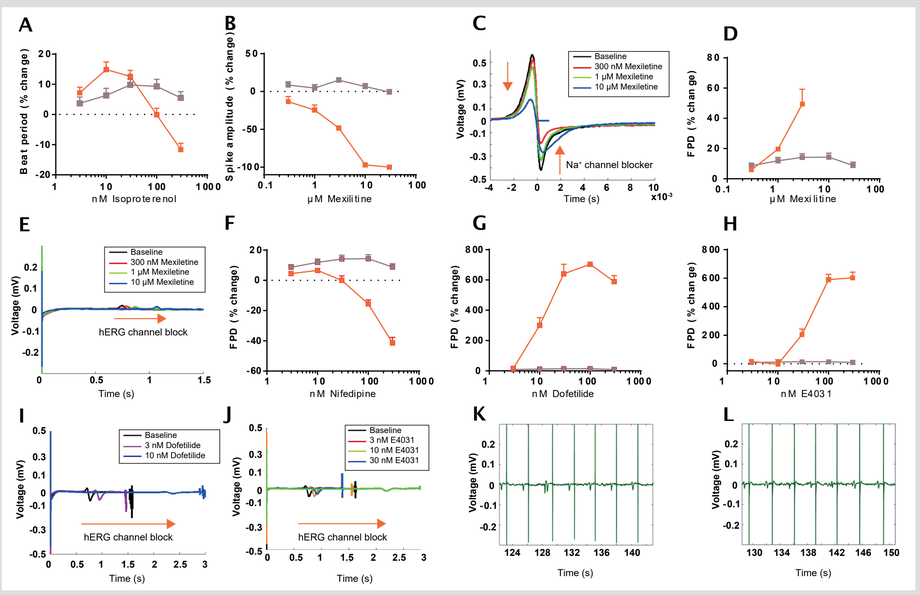


Figure 4. Phenotypic and functional characterization of three batches of iPSC-derived, cryopreserved cardiomyocytes produced in bioreactors
A: Percentage of cTNT+ and
B: cTNT+/MLC2v+ co-expression in three cryopreserved batches of bioreactor-derived Pluricyte Cardiomyocytes.
C: Phase contrast image of thawed cells, 8 days after culture in Pluricyte Cardiomyocyte Medium.
D: Functionality of the cardiomyocytes (sodium spike amplitude, beat period, beat period irregularity, field potential duration, number of active electrodes per well; >300 µV sodium spike and the number of FPD electrodes; with a detectable T-wave) was assessed in Maestro™ MEA system at different timepoints after plating.

Pharmacological response

Figure 5. Expected pharmacological responses of bioreactor-derived cardiomyocytes to CiPA reference compounds
A: Isoproterenol (β-adrenergic receptor agonist) reduces beat period (enhances beat rate). Mexilitine blocks the rapid inward sodium current as well as the hERG potasium channels leading to a reduction in sodium spike amplitude **(B,C)** and a prolongation of the field potential duration **(D,E)** respectively (**C,E** show averaged waveforms). Nifedipine (calcium channel blocker) causes a shortening of the field potential duration **(F)**. hERG potassium channel blockers dofetilide **(G)** and E4031 **(H)** prolong the field potential duration **(G,H)** and cause flattening of the repolarization peak (**I,J** show average waveforms). Arrhythmia's were observed for both dofetilide (10 nM, **K)** and E4031 (30 nM, **L)**. Corresponding DMSO concentrations were used as control.



High-throughput screening

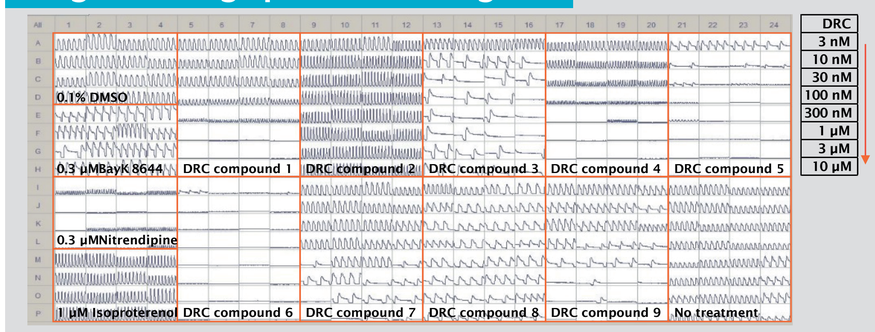


Figure 6. High throughput compound screening in FLIPR calcium transient assay using bioreactor-derived cardiomyocytes.
Bioreactor-derived cardiomyocytes were cultured for 10 days in 384-well plate format. On day 10 cells were treated with dose response curve (DRC) of various cardioactive compounds. 0.1 % DMSO was used as negative control and BayK 8644, Nitrendipine and Isoproterenol were used as positive controls. Effect of compounds 30 minutes post treatment were assessed in FLIPR calcium transient assay. The expected effects of compounds on calcium signal were observed in various concentrations as follows: Isoproterenol increased peak frequency, BayK 8644 increased peak amplitude and peak width, Nitrendipine decreased peak amplitude.

Conclusion and perspectives

> DASbox Mini Bioreactor System was suitable for expansion of hiPSCs and differentiation into hiPSC-derived cardiomyocytes:
> DASbox-derived cardiomyocytes showed excellent baseline electrophysiological function, revealed expected phenotypic responses to a set of reference compounds, and were suitable for high-throughput compound screening in a calcium transient assay.
> Perspective: Further implement bioreactor-derived Pluricyte Cardiomyocytes into high-throughput cardiovascular screening solutions to enhance the assessment of cardiac safety and efficacy of drug candidates.

Acknowledgements

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