Next generation manufacturing technology improves cardiomyocyte assay quality and enables personalized safety assessment

Ncardia
Stem cell experts

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BACKGROUND

Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are widely evaluated as an alternative model for cardiac safety assessment and have the potential to improve drug discovery efficiency through early application in the drug discovery process. The constant supply of high cell numbers of high-quality cardiomyocytes generated by defined, robust, and economically viable culture processes is indispensable for the envisioned application of hiPSCs-CMs throughout the drug discovery pipeline.
We have developed a large-scale manufacturing process for hiPSC-CMs using a combination of stirred-tank bioreactor systems to assess critical process parameters at 15 mL scale, validate conditions at 100-250 mL scale, and manufacture at 1-10 L scale in an automated closed system. We established a controlled process which is robust for manufacturing of reproducible batches of high quality cardiomyocytes from a variety of hiPSC lines.

The developed manufacturing process has resulted in the generation of multiple batches at purity of 87.4 ± 5.9% cTNT in >95% of the cases. The quality and functionality of the cells were confirmed using multi-electrode array (MEA) systems and Ca^{2+} transient profiling (not shown). Bioreactor-derived cardiomyocytes showed clear Ca^{2+} transients (not shown) and reproducible field potential signals with pronounced de- and repolarization peaks which allowed accurate assessment of beat rate and field potential duration. The mean beat period was 2.1 ± 0.14s (intra-well irregularity in each batch was <2%), mean field potential duration was 560 ± 47 ms. The expected and concentration-dependent response to Comprehensive in vitro Proarrhythmia Assay (CiPA) reference compounds confirmed the suitability of the bioreactor-derived cardiomyocytes for safety assessment of drug candidates.

Altogether, we show that we can reproducibly manufacture functional cardiomyocytes derived from a diverse set of hiPSC lines at large scale using a controlled stirred-tank bioreactor system. This hold great promise for drug discovery, providing adequate quantities of cardiomyocytes required for high throughput safety assessment at an early stage, enabling personalized safety assessment, and high-quality assays.

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

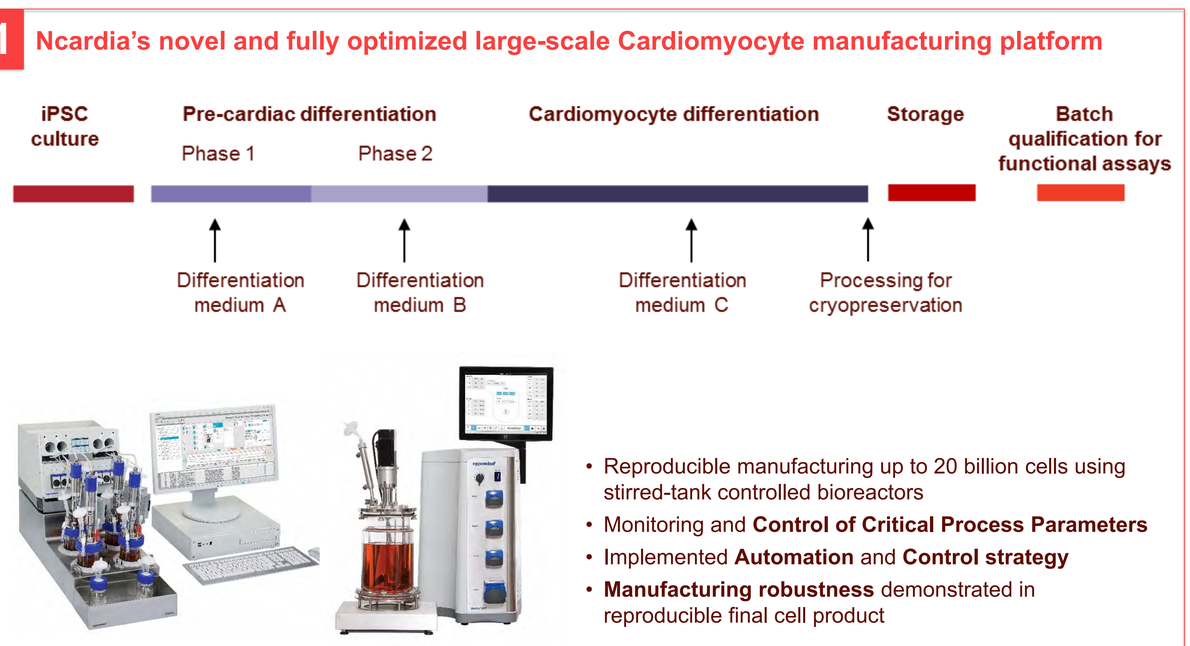


Figure 1. Schematic representation of hiPSC-derived cardiomyocyte manufacturing (top) in fully controlled stirred-tank bioreactors (bottom).

2 hiPSC-cardiomyocyte production in controlled, stirred-tank bioreactors is robust and can easily be applied to any patient iPSC line without further optimization

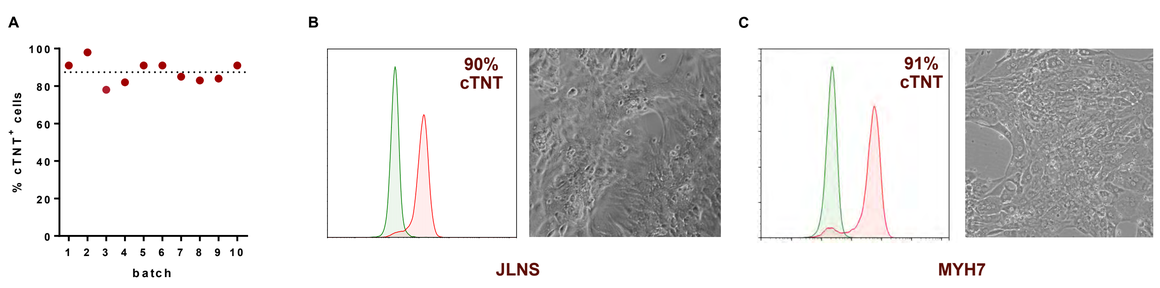
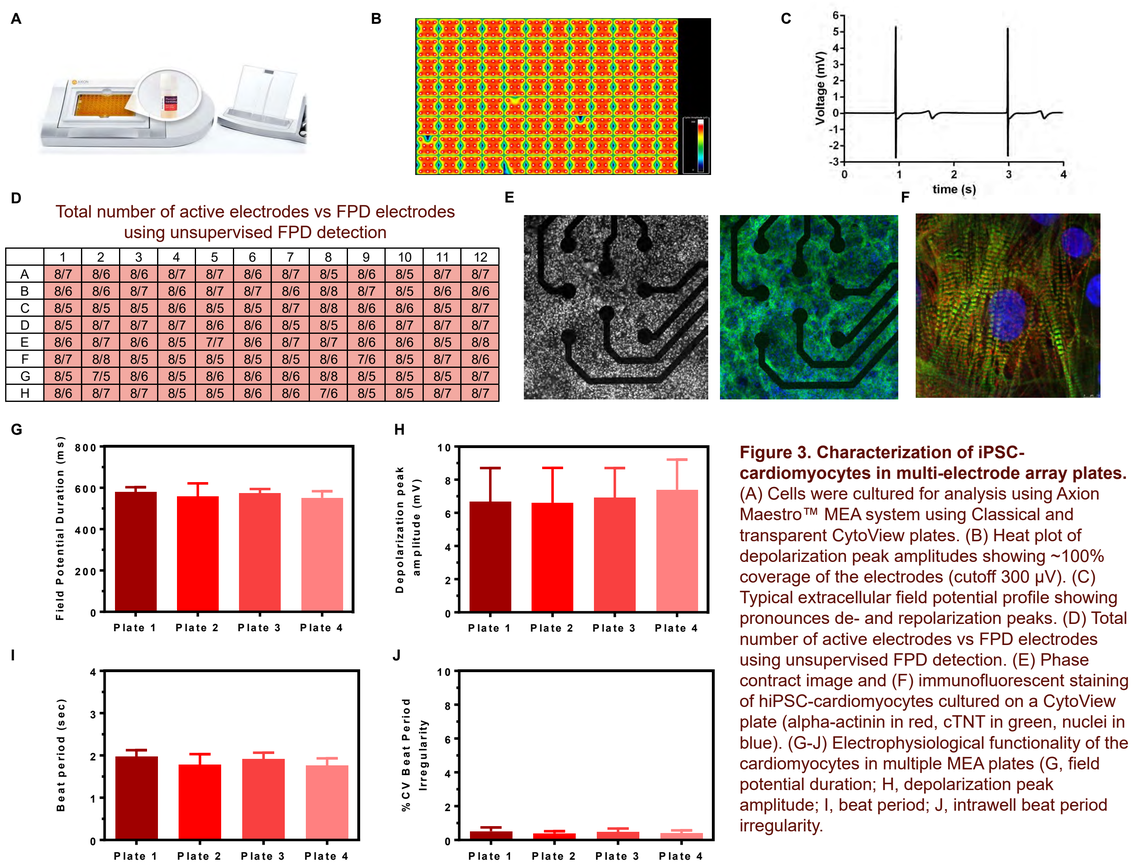


Figure 2. (A) Ncardia's large-scale manufacturing process for hiPSC-derived cardiomyocytes has resulted in the generation of multiple (>10) batches with a mean purity of 87.4 ± 5.9% (D, dashed line indicates the mean). (B, C) Example of cTNT expression (left) and cultures (right) of cardiomyocytes generated from two other hiPSC lines. These lines comprised a patient-derived line with a homozygous mutation in KCNQ1 (JLNS) and one patient-derived line with a mutation in MYH7.

3 Bioreactor-derived hiPSC-cardiomyocytes reveal exceptional stable and reproducible baseline electrophysiological properties in multiple multi-electrode array plates



	1	2	3	4	5	6	7	8	9	10	11	12
A	8/7	8/6	8/6	8/7	8/7	8/6	8/7	8/5	8/6	8/5	8/7	8/7
B	8/6	8/6	8/7	8/6	8/7	8/7	8/6	8/8	8/7	8/5	8/6	8/6
C	8/5	8/5	8/5	8/6	8/5	8/5	8/7	8/8	8/6	8/6	8/5	8/7
D	8/5	8/7	8/7	8/7	8/6	8/6	8/5	8/5	8/6	8/7	8/7	8/7
E	8/6	8/7	8/6	8/5	7/7	8/6	8/7	8/7	8/6	8/6	8/5	8/8
F	8/7	8/8	8/5	8/5	8/5	8/5	8/5	8/6	7/6	8/5	8/7	8/6
G	8/5	7/5	8/6	8/5	8/6	8/6	8/6	8/8	8/5	8/5	8/5	8/7
H	8/6	8/7	8/7	8/5	8/5	8/6	8/6	7/6	8/5	8/5	8/7	8/7

Figure 3. Characterization of iPSC-cardiomyocytes in multi-electrode array plates. (A) Cells were cultured for analysis using Axion Maestro™ MEA system using Classical and transparent CytoView plates. (B) Heat plot of depolarization peak amplitudes showing ~100% coverage of the electrodes (cutoff 300 µV). (C) Typical extracellular field potential profile showing pronounces de- and repolarization peaks. (D) Total number of active electrodes vs FPD electrodes using unsupervised FPD detection. (E) Phase contract image and (F) immunofluorescent staining of hiPSC-cardiomyocytes cultured on a CytoView plate (alpha-actinin in red, cTNT in green, nuclei in blue). (G-J) Electrophysiological functionality of the cardiomyocytes in multiple MEA plates (G, field potential duration; H, depolarization peak amplitude; I, beat period; J, intrawell beat period irregularity.

4 Expected pharmacological responses in bioreactor-derived cardiomyocytes to reference compounds

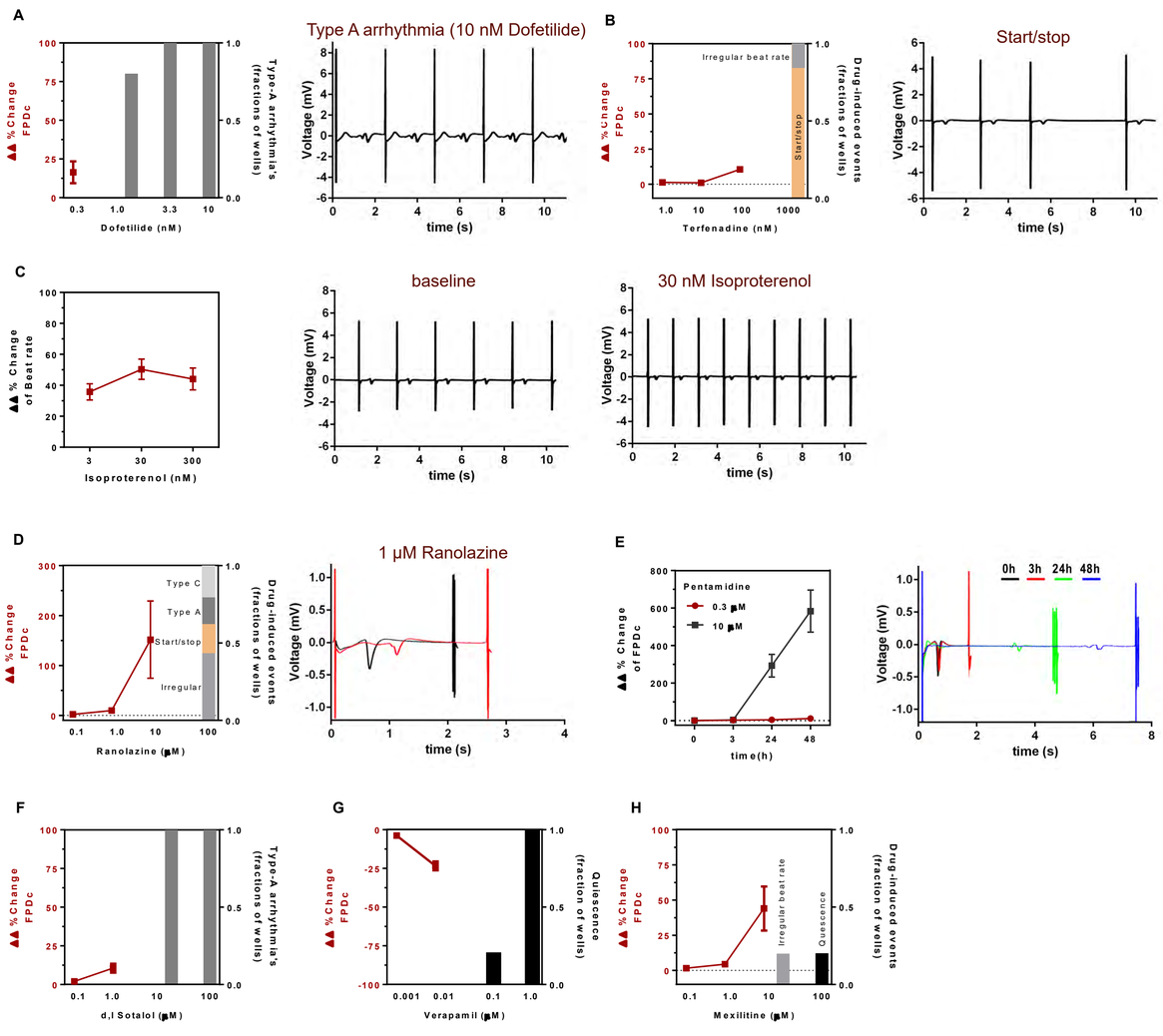


Figure 4. Pharmacological responses in bioreactor-derived cardiomyocytes to reference compounds using Axion Maestro™ MEA. (A) The hERG potassium channel blocker dofetilide prolongs the field potential duration (FPDc) and induces type-A arrhythmia's at higher doses (right). (B) The hERG blocker Terfenadine increases FPDc and causes arrhythmias. Right panel: EFP after 1 µM Terfenadine. (C) Isoproterenol (β-adrenergic receptor agonist) reduces beat period (enhances beat rate; middle panel: baseline EFP; right panel: EFP after 30 nM Isoproterenol). (D). Ranolzine increases FPDc and causes a variety of adverse events at higher concentrations (E) The chronic cardiotoxicant Pentamidine prolongs FPDc after long term treatment. Right panel shows averaged extracellular field potentials (EFPs). (F) The hERG and beta blocker d,l Sotalol increases FPDc and causes type A arrhythmia's at higher doses. (G) The L-type calcium channel blocker Verapamil decreases FPDc and induces quiescence at higher concentrations. (H) Mexiletine blocks the rapid inward sodium current as well as the hERG potasium channels leading to a prolongation of the field potential duration, irregular beating and quiescence with increasing concentrations. Corresponding DMSO concentrations were used as control.

5 Safety profiling and classification of a set of CiPA reference compounds using bioreactor-derived hiPSC-cardiomyocytes

Compound	Risk category	ΔΔ % Change FPDc	Arrythmic events (Type A, B, C, D)	Cmax (µM)	Tested range (µM)
Dilitiazem	Low	↓	---	0.13	0.01 - 10
Mexilitine	Low	↑	---	2.5	0.1 - 100
Ranolazine	Low (FP)	↑	A, C	1.9	0.1 - 100
Verapamil	Low	↓	---	0.05	0.001 – 1.0
Chlorpromazine	Medium	=	---	0.0345	0.095 – 3.0
Cisapride	Medium	↑	A	0.0026	0.00317 - 0.1
Ondansetron	Medium	↑	A	0.37	0.03 – 30
Terfenadine	Medium	↑	---	0.0003	0.001 – 1.0
Bepridil	High	↑	C	0.03	0.01 – 10
D,l Sotalol	High	↑	A	15	0.1 – 100
Dofetilide	High	↑	A	0.002	0.0003 – 0.01
Quinidine	High	↑	A	3.0	0.95 - 30

Table 1. Bioreactor-derived iPSC-cardiomyocytes were cultured on Axion Maestro™ MEA plates and subjected to a selection of CiPA reference compounds. Baseline recordings were taken and compounds were applied in a single dose. Drug effects were assessed from recordings 30 min after compound addition. This table summarizes the effects of a selection of CiPA reference compounds that were tested in a blinded fashion during the CiPA phase II validation study. Compounds from the high risk group revealed pro-arrhythmic events at C_{max} concentrations or higher, and thus correctly classify high risk compounds. FP = False positive

CONCLUSIONS

- Ncardia's stirred-tank cardiomyocytes manufacturing process is highly reproducible.
- The process was successfully used, without any single modification, for manufacturing of cardiomyocytes from any other hiPSC line tested enabling patient specific safety and efficacy screening.
- Bioreactor-derived cardiomyocytes showed robust baseline electrophysiological function with clear de- and repolarization peak amplitudes allowing unsupervised FPD detection for easy data analysis.
- Bioreactor-derived cardiomyocytes revealed expected pharmacological responses to a set of CiPA-reference compounds confirming their suitability in cardiac safety assessment.
- This technology is now applied in the new Ncyte CardioPlate™ MEA 96 and Cardio.Acute Safety Service.

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