Development and Characterization of Non-Human Primate iPSC-Derived Ventricular Cardiomyocytes for in vitro Safety and Toxicity Screening

Ncardia

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Ilse Veldkamp-Baak, Pieter van Loenen, Jessica Koepke, Silke Schwengberg, Timothy Smith, Mariana Argenziano

Ncardia Services BV, Leiden, The Netherlands, support@ncardia.com, www.ncardia.com

Background and Purpose

Human-induced pluripotent stem cell-derived cardiomyocytes (hu CM) are widely used in nonclinical cardiac safety studies but interspecies translation remains a limitation in early-stage pharmacology and toxicology. Non-human primates (NHPs) are physiologically close to humans. are often used as secondary species for in vivo studies, and can provide translational insights. However, there are limited *in vitro* models using NHP cardiomyocytes. We aimed to establish and characterize an *in vitro* platform of NHP iPSC-derived ventricular cardiomyocytes (NHP CM) suitable for translational *in vitro* safety and toxicity applications focusing on consistency, functional responsiveness, and pharmacodynamic sensitivity to known reference compounds.

Methods

iPSCs from cynomolgus macaques were sourced from the German Primate Center[1] and differentiated into ventricular cardiomyocytes in stirred tank bioreactors. Expression of cTnT, α-actinin, and MLC2V was quantified via immunostaining. Cardiomyocyte function was assessed using microelectrode arrays (MEA) and impedance-based contractility assays. The response to cardioactive compounds was compared to human Ncyte vCardiomyocytes (hu CM).

MEA: hu CM were thawed on day –3, NHP CM on day –2, and both were harvested and seeded on day 0. Compound treatments and MEA recordings were performed on day 4 (NHP CM) and day 8 (hu CM).
Impedance assay: hu CM were thawed on day –3 and NHP CM on day –2. Both were seeded on impedance plates at day 0. Acute compound treatment was performed at day 7, followed by 24 h measurements (day 8). Chronic recordings were carried out daily until day 7 post treatment.

Conclusion

NHP CM can be differentiated at large scale, display high structural fidelity, stable function in MEA and impedance assays, and respond to cardioactive compounds. The observed species-specific differences (e.g., arrhythmia signatures, chronic sensitivity) highlight the translational value of the model for *in vitro* tox and safety studies.

1. Tereshchenko, Y.; Esiyok, N.; Garea-Rodríguez, E.; Repetto, D.; Behr, R.; Rodríguez-Polo, I. Transgene-Free Cynomolgus Monkey iPSCs Generated under Chemically Defined Conditions. Cells 2024, 13, 558. https://doi.org/10.3390/cells13060558

Ncyte® NHP-C vCardiomyocytes show high purity and functionality

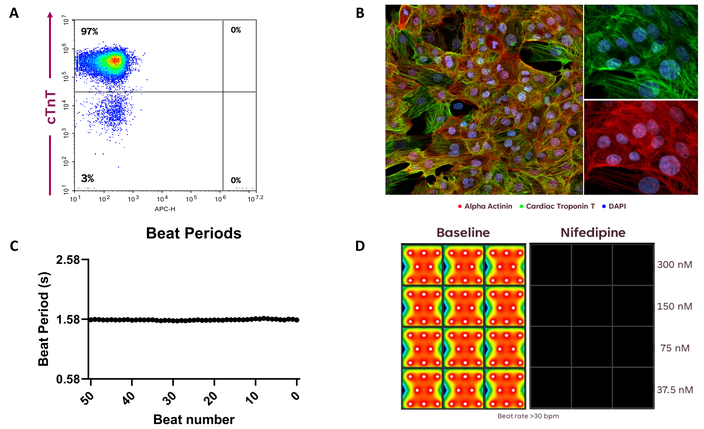

Figure 1. Characterization of NHP CM
(A) Flow cytometry analysis of cardiac Troponin T (cTnT) expression shows high purity with ≥97% cTnT-positive cells.
(B) Immunofluorescence staining of NHP CM demonstrating expression of α-Actinin (red), cardiac Troponin T (green), and nuclear counterstain DAPI (blue).
(C) Representative beat period recordings of NHP CM show stable beating over successive beats.
(D) Functional response of NHP CM to Nifedipine. Baseline electrical activity is suppressed at all doses (37.5–300 nM).

Impedance Assay Reveals Differences in Contractility

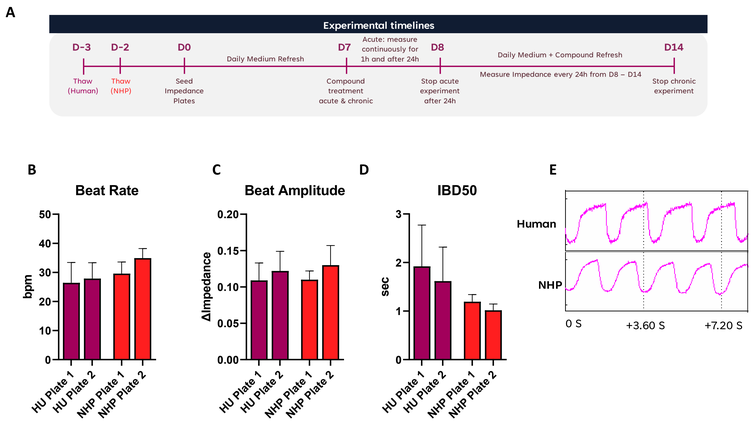

Figure 2. Impedance assay and drug response in NHP CM and hu CM
(A) Experimental timeline for impedance assays
(B – D) Baseline impedance parameter comparison between hu CM and NHP CM (n=96 for hu CM and n=80 for NHP CM). Notice the differences in IBD50 values (inter-beat duration at 50%)
(E) Representative impedance traces of hu CM and NHP CM demonstrate stable and rhythmic beating patterns as well as marked differences in peak shape between species.

MEA Assay Confirms Stable Baseline and Expected Drug Responses

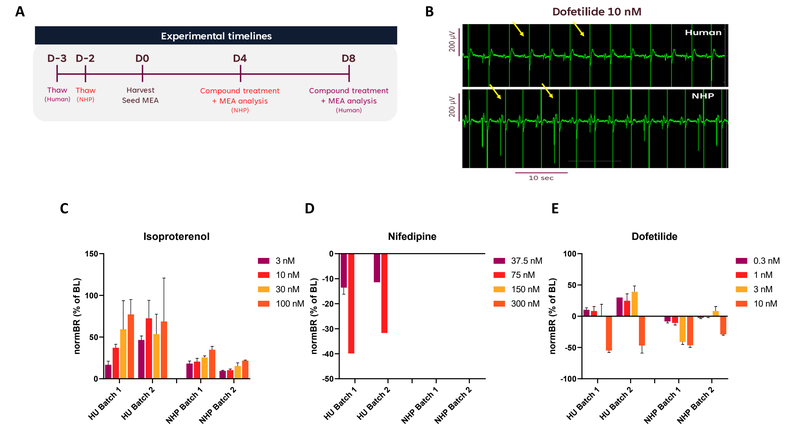

Figure 3. MEA analysis comparison between NHP CM and hu CM
(A) Experimental timeline for MEA analysis
(B) Representative field potential recordings showing the arrhythmic effect of Dofetilide (10 nM) on hu CM and NHP CM
(C) Isoproterenol induced a concentration-dependent increase in beat rate across hu CM and NHP CM, however, the effect size was different
(D) Nifedipine suppressed electrical activity in a dose-dependent manner in hu CM, and induced a complete arrest in NHP CM
(E) Besides inducing arrhythmia (B), Dofetilide caused concentration-dependent changes in beat rate in hu CM and NHP CM

Impedance Assay Highlights Species Differences upon Drug Treatment

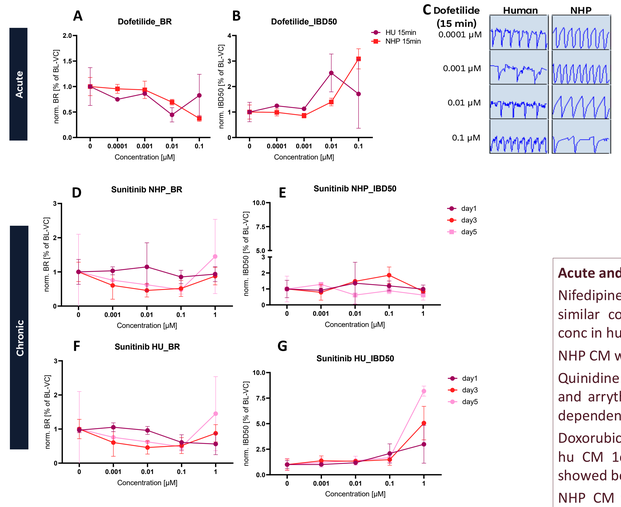

Figure 4 A – C: Acute drug response in NHP CM and human CM
(A-B) Dofetilide induced an increase in IBD50 and a decrease in BR in both hu and NHP CM, but at different concentrations. Representative well graphs highlight arrhythmic events in hu CM already at 0.001 µM, but only at highest concentration in NHP CM. Note the distinct change in the shape of the NHP CM traces.

Figure 4 D – G
(D-G) Chronic effects of sunitinib on NHP CM (D&E) or hu CM (F&G) over 5 days showing changes in beat rate (BR) and IBD50. The increase in IBD50 was much more pronounced in hu CM, but NHP CM showed arrhythmia after 5 days of dosing.
Generally, NHP CM were more prone to arrhythmia, but less prone to cytotoxicity.

Acute and chronic Drug Effects (Data not shown):

Nifedipine induced beat arrest in both species at similar conc, but decrease in amplitude at lower conc in hu CM.

NHP CM were more sensitive to Moxifloxacin.

Quinidine induced conc-dependent increase in IBD50 and arrythmia in hu CM; NHP CM showed a conc-dependent decrease in IBD and fibrillation.

Doxorubicin induced conc-dependent cytotoxicity in hu CM 1d earlier than in NHP CM. Both species showed beat arrest after 2 days at similar conc.

NHP CM were resistant to Blebbistatin, with beat arrest only after 5 days of treatment.

Pentamidine induced cytotoxicity at the highest dose in both species. NHP CM showed arrhythmia and beat arrest, hu CM reacted with an increase in IBD50.

Ncyte® NHP-C vCardiomyocytes open a new era in translational cardiac safety testing

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