A Translational in-vitro screening platform using Non-Human Primate iPSC-derived ventricular cardiomyocytes

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Background and Purpose

High-throughput functional screening increasingly relies on iPSC-derived cardiomyocytes to support compound screening and safety assessment. While human iPSC-derived models are widely implemented, non-human primate (NHP) models remain essential for translational decision-making, yet scalable and reproducible NHP in vitro cardiomyocyte platforms suitable for screening are limited. Here, we describe a non-human primate iPSC-derived ventricular cardiomyocyte (NHP-C vCM) platform designed for large-scale manufacturing and high-throughput functional screening.

Methods

Cynomolgus macaque iPSCs[1] were differentiated into NHP-C vCM at large scale using stirred-tank bioreactor systems and cryopreserved for post-thaw functional analysis. Cardiomyocyte identity and ventricular specification were confirmed by flow cytometry and immunofluorescence for cTnT, α-actinin, and MLC2V. Functional characterization was performed using 96-well microelectrode array (MEA) recordings and impedance-based assays to assess electrophysiology, contractility, and temporal functional dynamics.

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

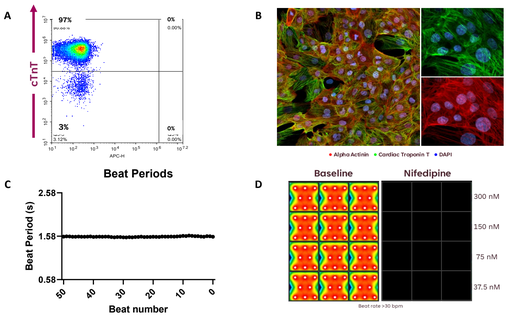


Figure 1. Characterization of NHP CM

(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).

MEA Assay Confirms Stable Baseline and Expected Drug Responses

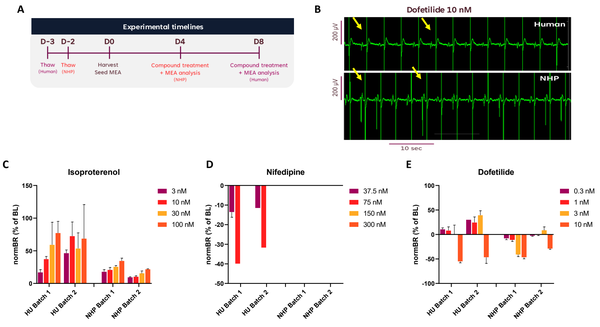


Figure 3. MEA analysis comparison between NHP CM and hu CM

(A) Experimental timeline for MEA analysis

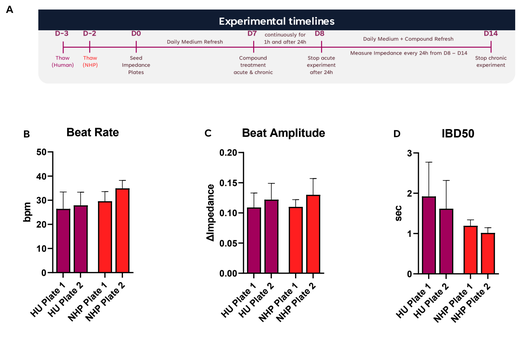
(B) Representative field potential traces showing the effect of 10 nM Dofetilide on hu CM and NHP CM

(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 Reveals Differences in Contractility



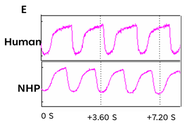


Figure 2. Impedance assay and drug response in NHP CM and hu CM

(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.

Impedance Assay Highlights Species Differences upon Drug Treatment

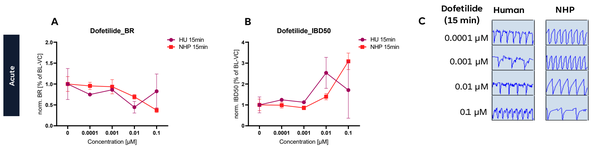


Figure 4 A – C: Acute drug response in NHP CM and human CM

(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.

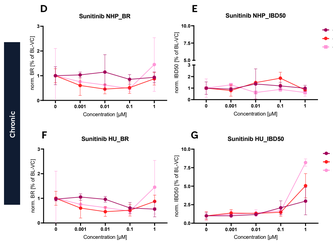


Figure 4 D – G : Chronic drug response in NHP CM and human CM

(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 (Impedance assay, data not shown):

Nifedipine induced arrest in both species at similar conc, but decreased 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.

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.

This platform enables scalable, reproducible, and long-term high-throughput functional screening using NHP cardiomyocytes, supporting cross-species comparison and more informed interpretation of screening data in advanced discovery workflows.

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.

Ncyte® NHP-C vCardiomyocytes open a new era in translational cardiac in vitro screening

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