A Translational in-vitro platform of Non-Human Primate iPSC-derived ventricular cardiomyocytes for functional profiling

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Background and Purpose

Induced pluripotent stem cell (iPSC)-derived cardiomyocytes are increasingly used to support gene and cell therapy development, including vector optimization, transgene evaluation, and long-term functional assessment. While human iPSC-derived cardiomyocytes are widely available, non-human primate (NHP) models remain essential for translational in vivo studies due to their closer physiological and genetic similarity to humans. However, there is a lack of robust, scalable in vitro NHP cardiomyocyte platforms that enable functional characterization and repeated assessment of electrical and contractile behavior over time in culture.

Here, we report the development and characterization of a non-human primate iPSC-derived ventricular cardiomyocyte (NHP-C vCM) platform designed to support translational gene and cell therapy research.

Methods

Cynomolgus macaque iPSCs 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 multiwell microelectrode array (MEA) recordings and impedance-based assays to assess electrophysiology, contractility, and temporal functional dynamics. Acute and chronic compound exposure paradigms were applied to compare functional responsiveness between NHP and human iPSC-derived vCM across electrical and mechanical readouts

Conclusion

NHP iPSC-derived vCM can be robustly manufactured at scale and display stable structural identity, reproducible electrophysiological behaviour, and dynamic contractile function. Integrated MEA and impedance profiling reveals both conserved and species-specific functional characteristics between NHP and human cardiomyocytes, particularly under extended conditions. This platform provides a translationally relevant in vitro model to support mechanistic studies, functional assessment, and cross-species interpretation in gene and cell therapy development.+

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

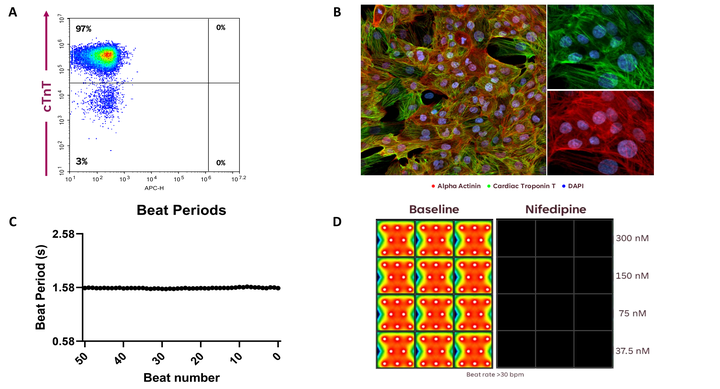

Figure 1. Characterization of NHP CM

(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

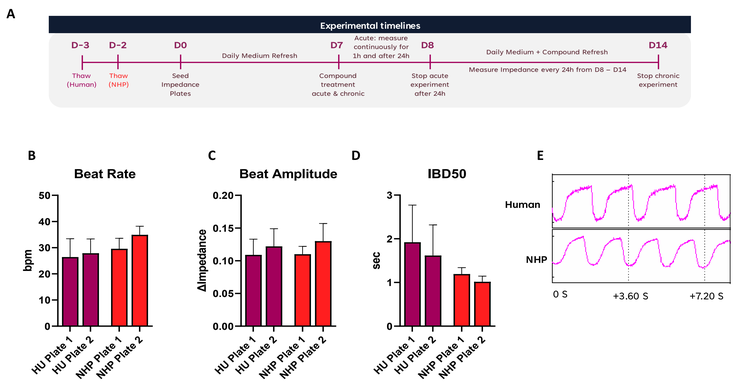

Figure 2. Impedance assay and drug response in NHP CM and hu CM

(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

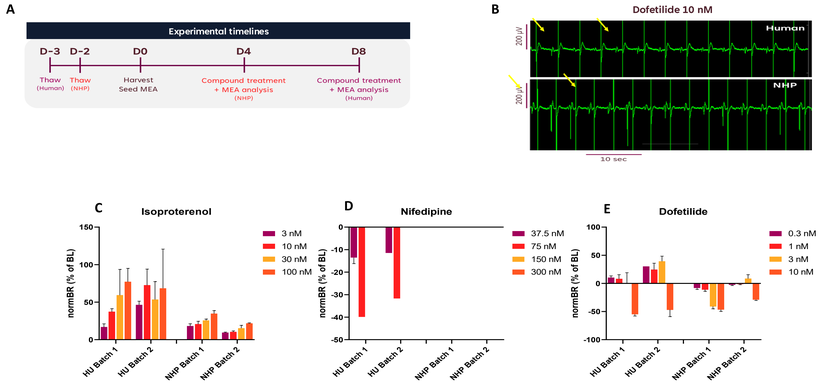

Figure 3. MEA analysis comparison between NHP CM and hu CM

(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 smaller in NHP CM.

(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

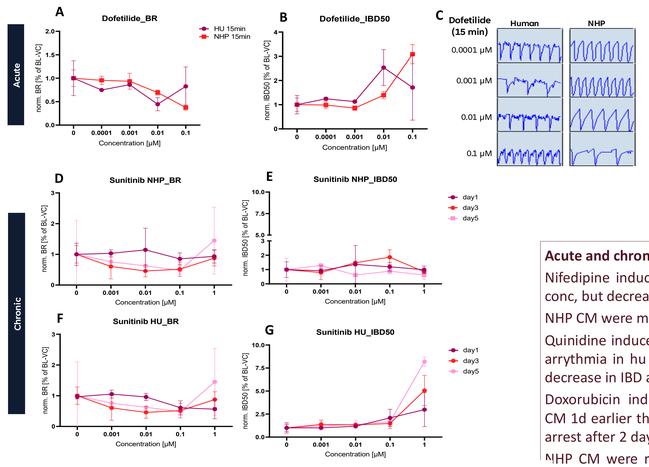

Figure 4 A – C: Acute drug response in NHP CM and human CM

(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 nly after 5 days of treatment.

entamidine induced cytotoxicity at the highest dose in oth species. NHP CM showed arrhythmia and beat rrest, 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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