IN VITRO ASSESSMENT OF EXCITATION-CONTRACTION COUPLING FOR PREDICTING PRO-ARRHYTHMIC RISK IN IPSC-DERIVED VENTRICULAR CARDIOMYOCYTES

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Introduction

Human induced pluripotent stem cell-derived ventricular cardiomyocytes (hiPSC-vCMs) (Axol Bioscience) offer a physiologically relevant model for predictive toxicology screening *in vitro*. The CardioExcyte 96 (Nanion Technologies) is a hybrid screening instrument that simultaneously records cell contractility (impedance) and the extracellular electrical field potential (EFP) in a 96-well plate. Used in combination these tools could help predict the risk of human clinical pro-arrhythmias more accurately.

Here we present data on the optimisation of hiPSC-vCMs on the CardioExcyte 96. We determined seeding parameters and identified the optimal time point for analysis. Excitation-contraction coupling was then assessed in response to three standard reference compounds from the Comprehensive *in vitro* Pro-arrythmia Assay (CiPA) guidelines. The three compounds tested were verapamil, a mixed ion channel blocker acting upon both L-type calcium channels (I_{CaV}) and potassium channels (I_{Kr}); nifedipine, a selective calcium channel (I_{CaV}) blocker; and dofetilide, a selective ion channel blocker for I_{Kr}. Both verapamil and nifedipine exhibit low pro-arrhythmic risk whereas dofetilide is classified as a high risk pro-arrhythmic compound by the Cardiac Safety Consortium. The addition of each of these compounds altered contractility and electrical excitation in the hiPSC-vCMs.

Here we have demonstrated that the CardioExcyte 96, a non-invasive, label-free, high temporal resolution tool may be used in conjunction with Axol hiPSC-vCMs to predict pro-arrythmic risk *in vitro*.

Materials and methods

Cardiomyocyte culture: Human iPSC-Derived Ventricular Cardiomyocytes (ax2505, Axol Bioscience) were thawed on Fibronectin (ax0049)-coated plates according to the manufacturer's protocol, and cultured for the first 24 hours in Cardiomyocyte Maintenance Medium (ax2530) + 10% FBS. The next day cells were switched to serum-free Cardiomyocyte Maintenance Medium. Thereafter, the medium was changed every 2 days.

Plating and recording: After 4 days, cells were dissociated with Axol Unlock (ax0044) and re-plated at 30,000 cells per well on the CardioExcyte 96 Sensor Plate. After one week the following drug compounds were applied: dofetilide (10nM), nifedipine (100nM) and verapamil (200nM).

Immunocytochemistry: Cells were fixed in 3 % PFA, permeabilised with 0.2 % Triton X-100 and blocked with BSA. Primary antibody was incubated overnight 4 ˚C, and secondary antibody coupled to Alexa Fluor® dyes (Invitrogen) applied for 2 hours.

Western blot: 30 µg protein run on 10 % SDS-PAGE gel for 70 minutes at 130 V and transferred to PVDF membrane. Membranes incubated with primary antibody overnight at 4 ˚C, washed and incubated with secondary antibody for 1 hour.

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CardioExcyte 96 technology

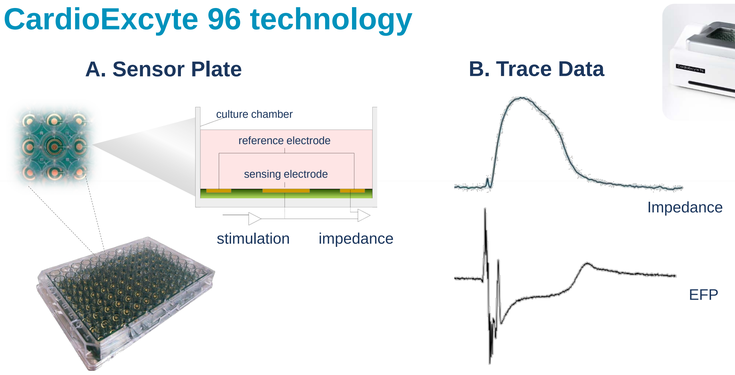


Figure 1: CardioExcyte 96 sensor technology
The CardioExcyte 96 combines impedance and Extracellular Field Potential (EFP) recordings to measure contractility and ion channel activity. *A;* The 96-well sensor plate contains gold electrodes embedded in each well. *B;* Manual overlay of impedance (upper trace) and EFP (lower trace) trace data recorded from an autonomously beating cardiac preparation.

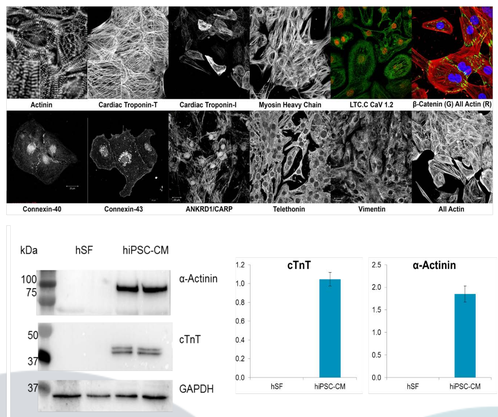
The CardioExcyte 96 (CE 96) is a hybrid system combining impedance and EFP recordings from the same monolayer of cells.

- Recordings are performed at physiological temperature in an incubator, or using the environmental chamber.
- The CardioExcyte 96 can be used in cardiac safety screening on a variety of hiPSC-CMs to assess effects of CiPA-relevant compounds.
- The CardioExcyte 96 provides complementary data to other assays such as automated patch clamping.

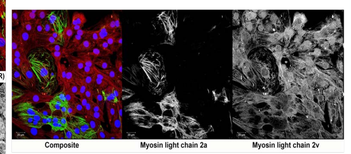
Features	CE 96
Number of channels	96
Impedance and EFP measurements	✓
Pacing	✓
Cell adhesion and spreading assays	✓
Operation in standard incubator	✓
Environmental control (for use outside incubator)	✓
Temporal resolution (Imp/EFP)	1ms / 0.1ms
Dedicated analysis and recording software	✓✓
Integration with CYBERNANO analysis	✓

Characterisation of Axol ventricular iPSC-CMs

A. Protein marker expression



B. Atrial vs. ventricular phenotype quantification



MLC2v (87%) vs MLC2a (13%)
Nodal population not included

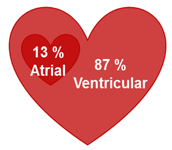


Figure 2: Molecular and physiological characterisation of Axol hiPSC-vCMs
A; *Top* Immunocytochemistry data showed the expression of ventricular cardiomyocyte markers (cardiac troponin-I; 93.5% expression). Data from Dr Christian Zuppinger, University of Bern and Prof Matt Daniels, University of Oxford. *Bottom* Western blot data confirmed that Axol hiPSC-vCMs express more cardiac troponin-T (cTnT) and α-Actinin than human skin fibroblasts (hSFs). Data from Abigail Robertson, University of Manchester. *B;* 87 % of Axol hiPSC-vCMs have a ventricular phenotype determined by MLC2v expression, compared to 13 % expressing atrial MLC2a (n=1). Data from Dr Christian Zuppinger, University of Bern.

Compound assessment

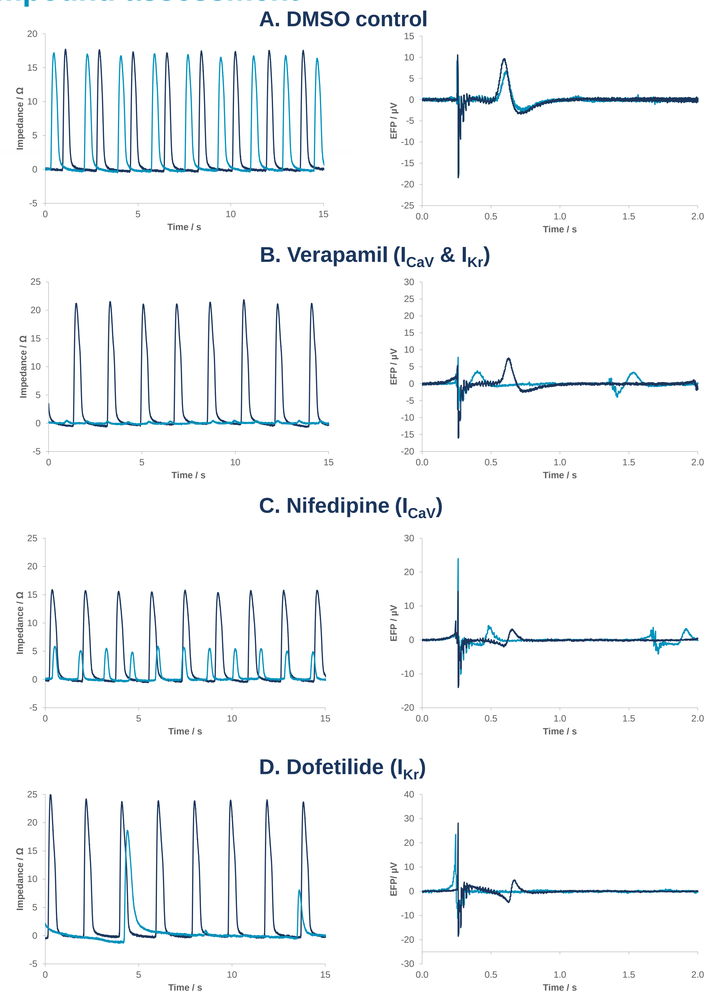


Figure 3. The cardiotoxic effects of cardiac ion channel modulators on Axol hiPSC-vCMs
Axol hiPSC-vCMs showed compound-relevant responses (light blue) to known cytotoxic compounds. *A*; DMSO control, showed no change in EFP but an increase in amplitude. *B*; verapamil and *C*; nifedipine, both reduced the impedance amplitude, increased the beat rate and reduced the EFP. *D*; dofetilide, a selective ion channel blocker for the rapidly activating delayed rectifier potassium channel (I_{Kr}), resulted in arrhythmic events typical of I_{Kr} blockers.

Conclusions

- Axol hiPSC-vCMs show specific compound-relevant responses to known cardiotoxic compounds.
- Impedance and EFP pharmacology shown here confirms the presence of I_{CaV} and I_{Kr} channels in Axol hiPSC-vCMs.
- The use of a dual reading technology that is enabled on the CardioExcyte 96 system, allows for the detection of compound effects on both the contractility and electrophysiological properties of a beating network of hiPSC-vCMs.
- We have shown that the **CardioExcyte 96** (Nanion Technologies) system used in combination with **Human iPSC-Derived Ventricular Cardiomyocytes** (Axol Bioscience) is an effective system for assessing cardiac pro-arrhythmia using compounds from the CiPA validation toolbox.