T1068

Serum-free human iPSC-derived cardiomyocytes for *in vitro* testing

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Overview

We conducted a series of experimental procedures to examine the characteristics and potential applications of human iPSC-derived cardiomyocytes (iPSC-CMs) for cardiovascular research.

Introduction

- Adult cells can be reprogramed using defined factors OCT3/4, KLF4, SOX2 and c-MYC to generate induced pluripotent stem cells (iPSCs)[1-3]
- iPSCs can be differentiated into a variety of cell types including cardiomyocytes, iPSC-CMs
- We show the expression of several cardiomyocyte-selective markers and electrical functioning in our iPSC-CMs
- We developed a simultaneous optical control/calcium imaging approach to demonstrate the application of these cells for drug toxicity testing

Methods

Cell Culture Human iPSC-CMs (**Axol Bioscience**, ax2505) cultured in Cardiomyocyte Maintenance Medium (**Axol Bioscience**, ax2530-500). First 24hrs with 10% FBS, Pen/Strep, thereafter, serum-free at 37˚C/5% CO_2. **Immunohistochemistry** Cells fixed 3% PFA, permeabilized with 0.2% Triton X-100, blocked with BSA. Primary antibody incubation overnight 4˚C. Secondary antibody coupled to Alexa Fluor® dyes (Invitrogen) for 2hrs. **Western Blot** 30µg protein run on 10% SDS-PAGE gel for 70min at 130V and transferred to PVDF membrane. Membranes incubated with primary antibody overnight at 4˚C, washed and incubated with secondary antibody for 1hr. Chemiluminescent imaging. **Manual Patch Clamp** Cells were cultured for 7-14 days post-thaw and syncytial monolayers were patched via perforated patch clamp (100µg/ml gramicidin). **Optical Control/Calcium Imaging** Cells were cultured in iPSC Cardiomyocyte Maintenance Media (**Axol Bioscience**, ax2530-500). Adenovirus expressing a light-sensitive optical control tool (Channel Rhodopsin 2) and a spectrally compatible genetically encoded calcium indicator (R-GECO) (Fig. 1). Cells stimulated with 10msec pulses of 405nm light to induce depolarization, and contraction, which can be followed with 568nm light to visualize the evoked calcium transient.

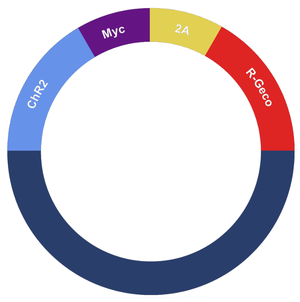
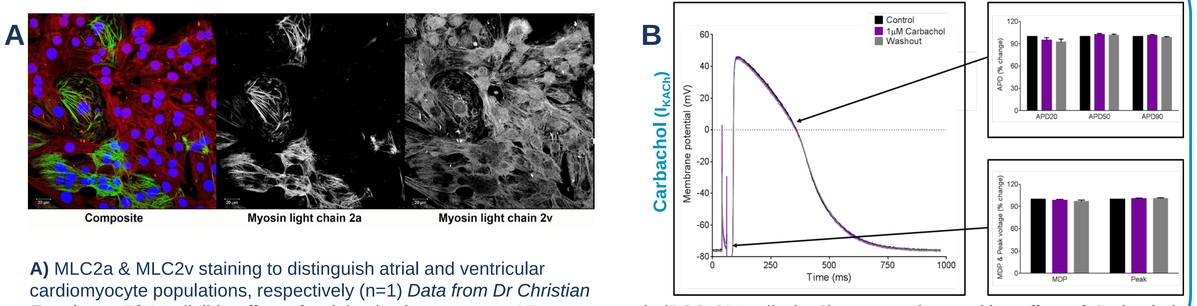


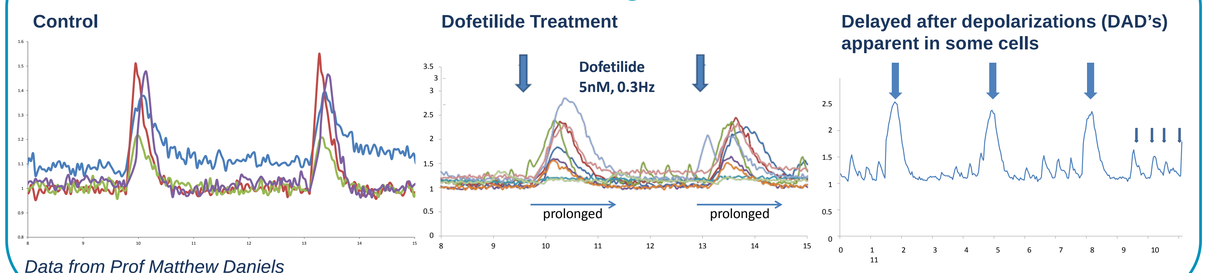
Figure 1: Optical control/calcium imaging vector R_GECO

Atrial vs Ventricular Cells



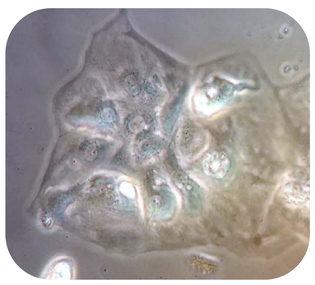
A) MLC2a & MLC2v staining to distinguish atrial and ventricular cardiomyocyte populations, respectively (n=1) *Data from Dr Christian Zuppinger*, **B)** Negligible effect of atrial-selective agent on AP parameters in iPSC-CM cells (n=8) compared to positive effect of Carbachol observed in atrial-derived HL-1 cells. This suggests the majority of cells do not display an atrial phenotype *Data from Metrion Biosciences.*

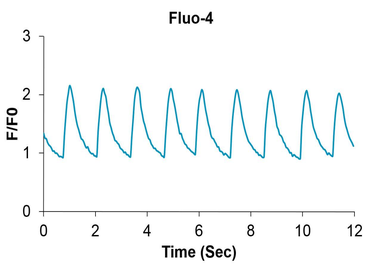
Dofetilide Treatment Prolongs the Calcium Transient



Data from Prof Matthew Daniels

Spontaneously Beating iPSC-Derived Ventricular Cardiomyocytes





Data from Prof Matthew Daniels

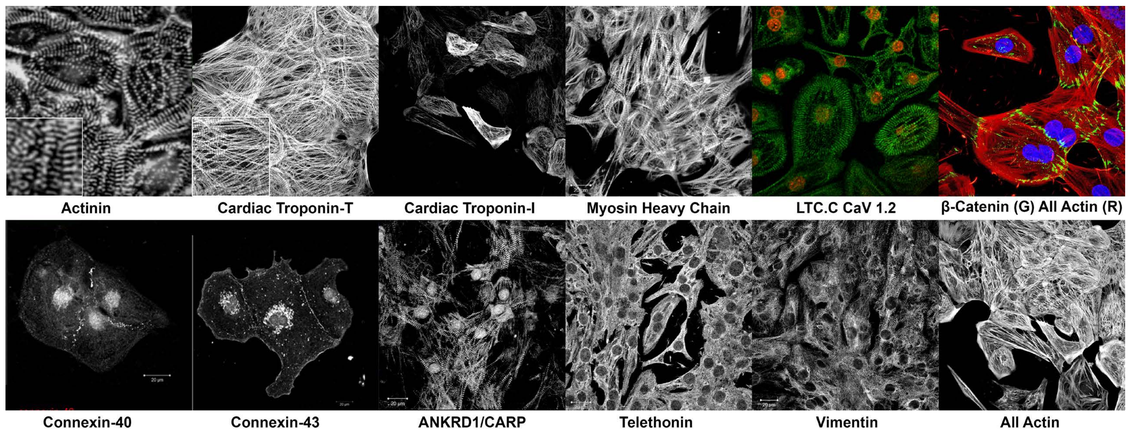
2D Culture

iPSC-CMs spontaneously contract after 10 days in culture.

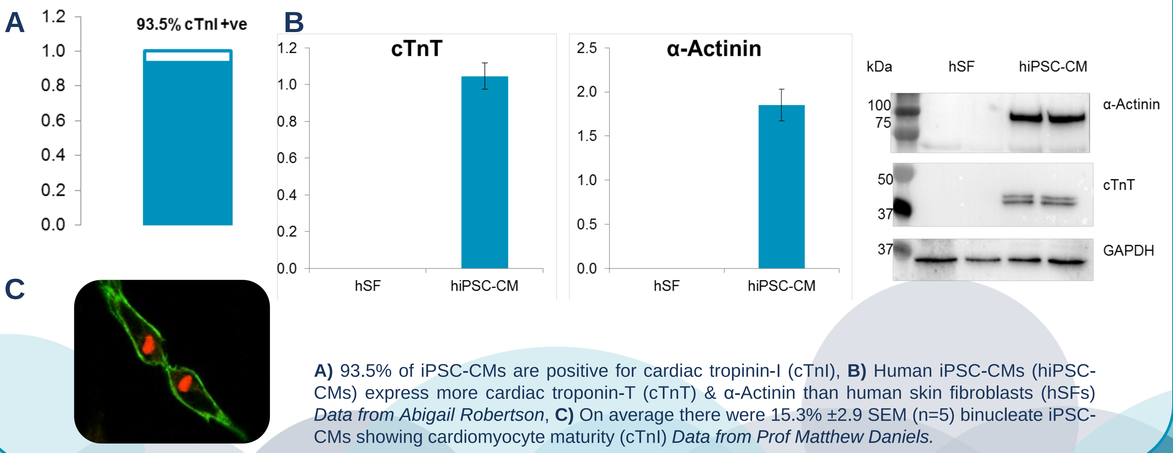
Regular Beating

iPSC-CMs treated with Fluo-4 chemical dye shows the regular beating of iPSC-CMs after 7 days in culture.

iPSC-Derived Ventricular Cardiomyocyte Marker Expression

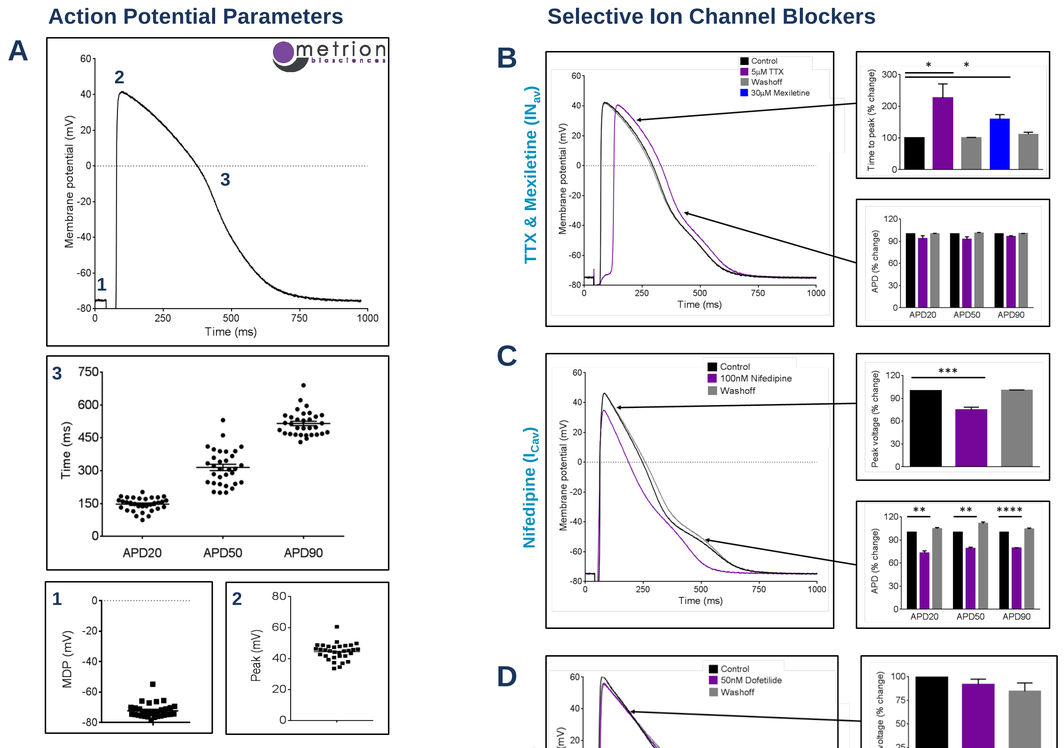


Data from Dr Christian Zuppinger



A) 93.5% of iPSC-CMs are positive for cardiac tropinin-I (cTnI), **B)** Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & α-Actinin than human skin fibroblasts (hSFs) *Data from Abigail Robertson*, **C)** On average there were 15.3% ±2.9 SEM (n=5) binucleate iPSC-CMs showing cardiomyocyte maturity (cTnI) *Data from Prof Matthew Daniels.*

Selective Ion Channel Blockers Show Presence of I_{Na}, I_{Ca} & I_{Kr}



A) AP parameters for untreated control cells (n=32). Recorded from cell syncytium using perforated patch clamp (100 µg/mL gramicidin), **B)** Treatment with TTX & Mexiletine prolonged time to peak, **C)** Treatment with Nifedipine reduced peak voltage & shortened APD20, APD50 & APD90, **D)** Treatment with Dofetilide causes APD90 prolongation as observed with the calcium imaging above. (n=8) *Data from Metrion Biosciences*

Conclusions

- **We identified a range of characteristics in these human iPSC-CMs that confirms their ability to function as a highly-pure population of single beating human cardiomyocytes *in vitro*.**
- **We present a technically simple and scalable platform for cardiotoxicity screening assays, that could be incorporated into the cells directly via genome editing in the future.**
- **Human iPSC-CMs can be cultured under serum-free conditions and as such, offer a platform to investigate the effect of growth factors, cytokines and drugs on the development and functionality of human cardiomyocytes *in vitro*.**

1) iPS cell technologies: significance and applications to CNS regeneration and disease. Okano & Yamanaka. *Mol Brain*, 2014; **2)** Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Takahashi & Yamanaka. *Cell*, 2006; **3)** Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Takahashi *et al*., *Cell*, 2007.