Serum-Free Human iPSC-Derived Cardiomyocytes for Contactless *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]
- iPSC can be differentiated into a variety of cells 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, ax2505) cultured Xeno-Free iPSC-CMs Maintenance Media (Axol, ax2535-500). First 24hrs with 10% FBS, Pen/Strep. Thereafter, serum-free at 37˚C/5% CO_2. Cells were cultured in 3D using GravityPlus™, InSphero. **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. **Plating Efficiency** 1x10^5 cells/cm^2 plated on Matrigel, fibronectin (10µg/ml), vitronectin (10µg/ml) and gelatin (0.1%) in xeno-free iPSC-CMs Maintenance Media (Axol, ax2535-500) with/out 10% FBS in a 12 well plate for 24hrs. **Multi-Electrode Array (MEA)** Cells plated on Alpha MED Scientific's MEA platform. **Optical Control/Calcium Imaging** Cells were cultures in iPSC Cardiomyocyte Maintenance Media (Axol, ax2530). Adenovirus expressing a light-sensitive optical control tool (ChannelRhodopsin 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.

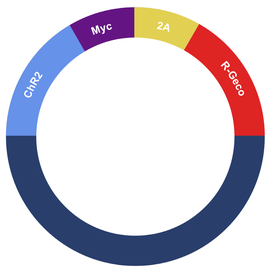
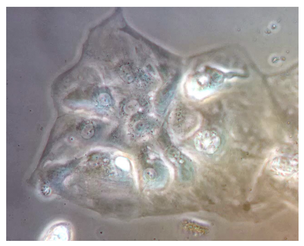
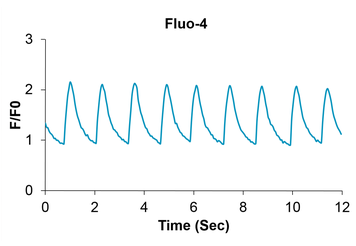


Figure 1: Optical control/calcium imaging vector

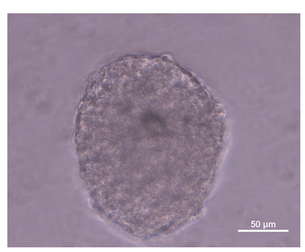
Results

Spontaneously beating iPSC-CMs



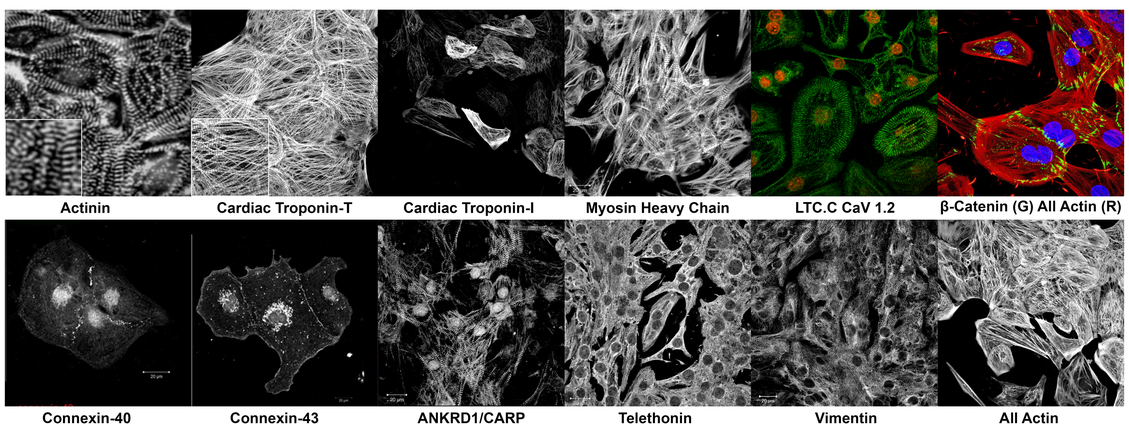


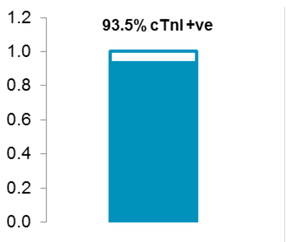
2D Culture



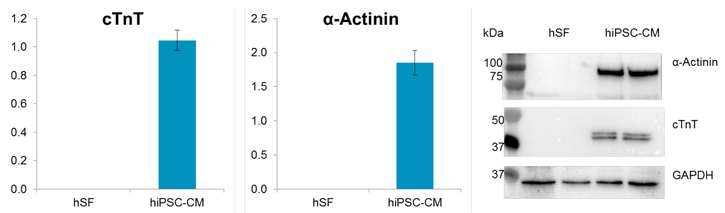
3D Culture

Cardiomyocyte marker expression



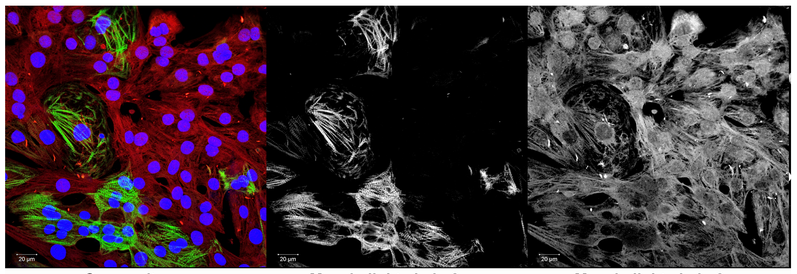


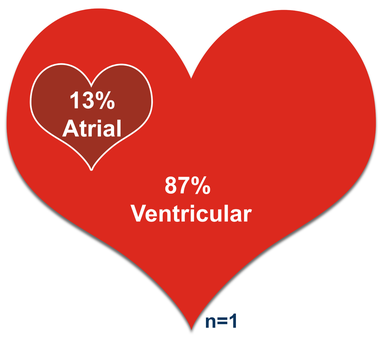
93.5% of iPSC-CMs are positive for cardiac tropinin-I (cTnI)



Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & α-Actinin than human skin fibroblasts (hSFs)

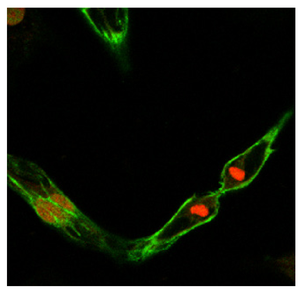
Atrial & ventricular cells





MLC2a & MLC2v staining to distinguish atrial and ventricular cardiomyocyte populations, respectively.

Binucleate cells



On average there were 15.3% ±2.9 SEM (n=5) binucleate iPSC-CMs

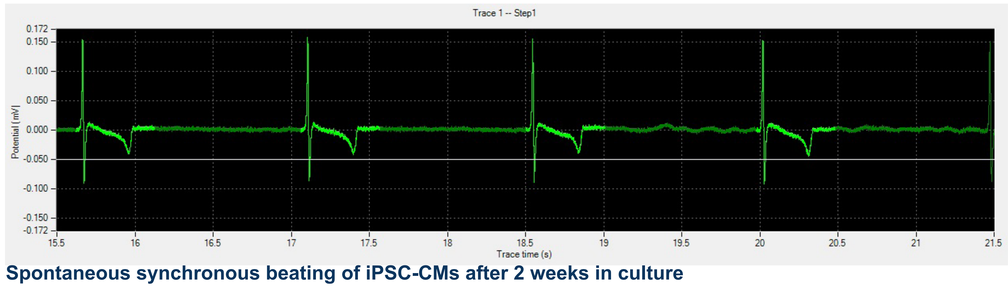
Plating efficiencies on different substrates

Matrigel - Serum
Gelatin + Serum
Fibronectin + Serum
Vitronectin + Serum
Matrigel + Serum

(Hearts denote plating efficiency)

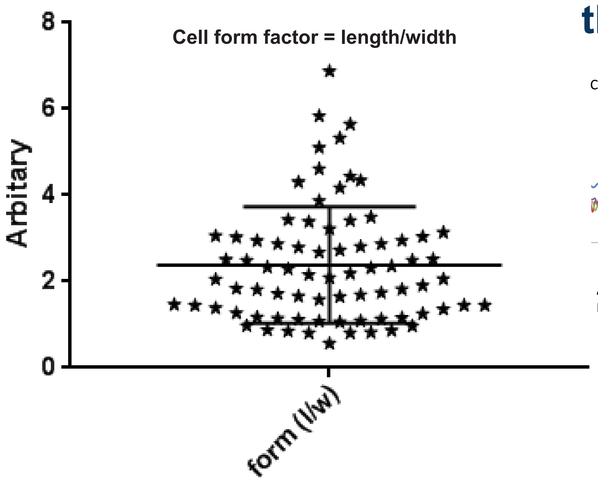
[Matrigel + Serum] was the most efficient substrate for iPSC-CM adhesion. [Matrigel - Serum] was comparable to [Gelatin + Serum].

Multi-electrode array (MEA)



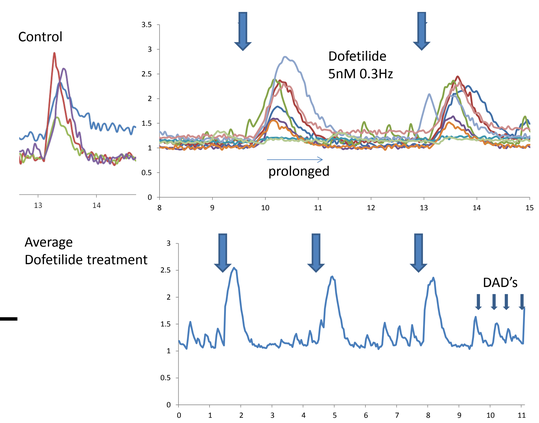
Spontaneous synchronous beating of iPSC-CMs after 2 weeks in culture

Cell form factor



On average iPSC-CMs achieved a cell form factor of 2.5

Dofetilide treatment prolongs the calcium transient



Delayed after depolarizations (DAD's) apparent in some cells

Conclusion

- 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.
- 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*.

References

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.