A New Suite of Media for Cardiotoxicity and Drug Discovery Studies with hiPSC-Cardiomyocytes

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FUJIFILM Cellular Dynamics

INTRODUCTION

- Cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CM) have proven to be a reliable *in vitro* cellular model for safety, toxicology, and drug discovery research.
- iCell® Cardiomyocytes² are a CiPA-validated and widely-tested human iPSC-CM.
- iCell Cardiomyocytes Maintenance Medium (iCMM) contains serum.
- Serum components bind compounds impacting drug delivery, potency, and efficacy.
- With FUJIFILM Irvine Scientific, we have developed two media to enable cardiotoxicity studies under serum-free conditions.
  - iCell® Serum-Free Medium (iCSFM)
  - iCell® CardioTox Assay Medium (iCTAM)

Scheme 1. Decision Tree for iCell Serum Free Medium

MATERIALS & METHODS

- **Cells:** iCell Cardiomyocytes², 01434 (FCDI Catalog # C1016)
- **Plate coating/ECM:** Fibronectin from human plasma
- **Media:**
  - Plated in iCell Cardiomyocytes Plating Medium (iCPM) for 24h
  - Cultured in iCell Cardiomyocytes Maintenance Medium (iCMM) until D7
  - Change to iCell Cardiomyocytes Serum-Free Medium (iCSFM) or iCell CardioTox Assay Medium (iCTAM) on D7 for assay
  - Recommended iCSFM up to seven days (DIV14), iCTAM up to four days (DIV11)

Component	iCMM	iCSFM	iCTAM
DMEM-based	Yes	Yes	Yes
Serum	Yes	No	No
Albumin	Yes	Yes	No
Xeno-free	No	No	Yes

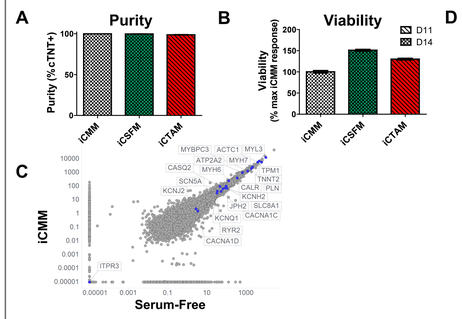
Table 1. *Key differences in formulation between the original iCell Cardiomyocytes Maintenance Medium (iCMM) and new iCell Cardiomyocytes Serum-Free Medium (iCSFM) and CardioTox Assay Medium (iCTAM).*

- **Purity and Viability Analysis:**
  - Cardiomyocyte purity (cTNT) – BD Accuri C6 Plus Flow Cytometer
  - µClear 96-well plates (Greiner Bio-One) – CyQUANT Cell Proliferation Assay on BMG Labtech CLARIOStar
- **Gene Expression Analysis:**
  - Total mRNASeq - Illumina Platform PE150, 250-300 bp insert cDNA library, 20M raw reads/sample (Novogene)
- **Electrophysiology Analysis:**
  - Biocircuit 96w MEA plates (Axion) – Axion BioSystems Maestro Pro
  - RTCA E-Plate Cardio 96 plates (Agilent) – Agilent ACEA xCELLigence RTCA Cardio
- **Metabolic Analysis:**
  - Agilent Seahorse XFe96 Analyzer
  - Seahorse XFe96 FluxPak Mini & Seahorse XF Cell Mito Stress Test Kit

Figure 1. Platforms to Assess iCell Cardiomyocytes² Functionality. ***(A)*** *Cell attachment, field potential and impedance measurements were collected on ACEA xCELLigence system. An image of Cardio-ECR plate w/ 2 electrodes per well shown.* ***(B)*** *Electrophysiology field potential and action potential measurements were performed on Axion Maestro Pro multi-well microelectrode array (MEA) and image of a well from Biocircuit 96-well plate 8 microelectrodes shown.* ***(C)*** *Metabolic activity was measured on an Agilent Seahorse XFe96 Analyzer.*

RESULTS

Viability, Purity, and Cardiac Gene Expression Maintained in Serum-Free Media



Gene	FPKM Log10		
	iCMM	iCSFM	iCTAM
Ion channels			
SCN5A	1.9	1.9	1.8
CACNA1C	2.0	1.9	1.7
CACNA1D	0.6	0.5	0.5
KCNH2	1.8	2.0	1.9
KCNQ1	1.4	1.6	1.5
KCNJ2	0.6	-0.6	0.4
Calcium Signaling			
ITPR3	-1.7	-1.1	-5.0
RYR2	1.8	1.6	1.5
ATP2A2	3.0	3.0	2.8
CASQ2	1.5	1.5	1.7
CALR	2.5	2.5	2.5
JPH2	1.9	2.2	2.0
PLN	3.3	3.2	3.3
SLC8A1	2.3	2.3	2.0
Contractile Machinery			
TNNT2	3.6	3.6	3.8
MYH7	3.4	3.5	3.3
MYH6	2.8	2.5	2.1
MYBPC3	3.0	2.9	3.1
TPM1	3.9	3.9	4.0
ACTC1	3.7	3.9	3.6
MYL3	3.5	3.7	3.7

Figure 2. iCell Cardiomyocytes² Maintain High Purity, Viability, and Cardiac Gene Expression When Cultured in iCell Serum-Free Media. *iCell Cardiomyocytes² cultured in iCSFM or iCTAM maintained* ***(A)*** *high cardiac purity by cTNT flow cytometry, and* ***(B)*** *high viability as indicated by CyQUANT Assay at end of recommended workflow: D14 in iCMM/iCSFM; D11 in iCTAM. (A n=3-6; B n=64-90).* ***(C)*** *Total RNASeq gene expression comparing iCell Cardiomyocytes² cultured in serum-containing iCMM to serum-free media at end of recommended workflow to with key cardiac genes are highlighted in blue.* ***(D)*** *FKPM levels of key cardiac genes across medium formulations.*

Comparable Spontaneous Beating and Metabolism Maintained with Serum-Free Culture

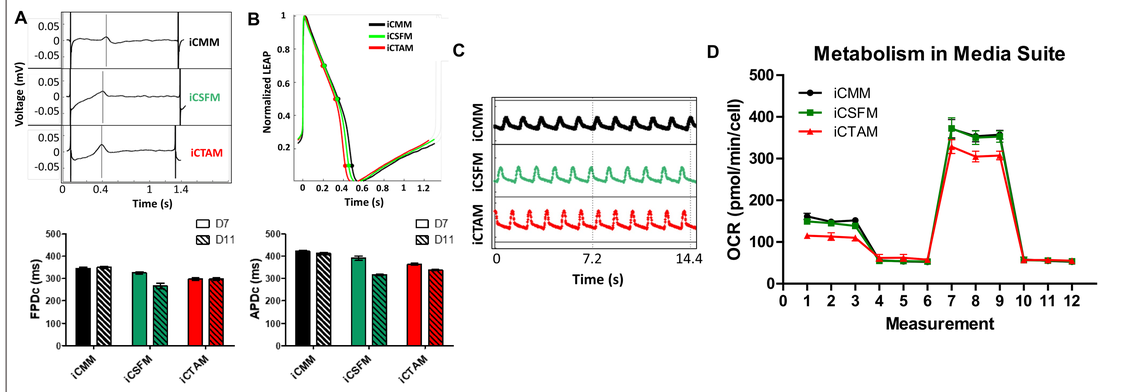


Figure 3. iCell Cardiomyocytes² Maintain Functional Properties Across Platforms When Cultured in iCell Serum-Free Media. *Using impedance and MEA, iCell Cardiomyocytes² cultured in iCSFM or iCTAM maintained comparable cell index, beat rate, beat rate irregularity (data not shown), field potential duration (FPD)* ***(A)*** *and action potential duration (APD, LEAP)* ***(B)*** *to those cultured in iCMM (D7 n=24-48; D11 n=9-18). Representative traces for FPD, APD, and impedance contractility* ***(C)*** *are shown from D7, 4h after media change.* ***(D)*** *iCell Cardiomyocytes² were seeded on a 96 well fibronectin coated Seahorse assay plate (Agilent) and maintained in iCMM until D7, then changed to iCMM, iCSFM, or iCTAM and maintained for two days. On D9 all wells were changed to Seahorse assay media and oxygen consumption rate (OCR) analyzed on an XFe96 Seahorse Analyzer with the XF Cell Mito Stress Test Kit (Agilent).*

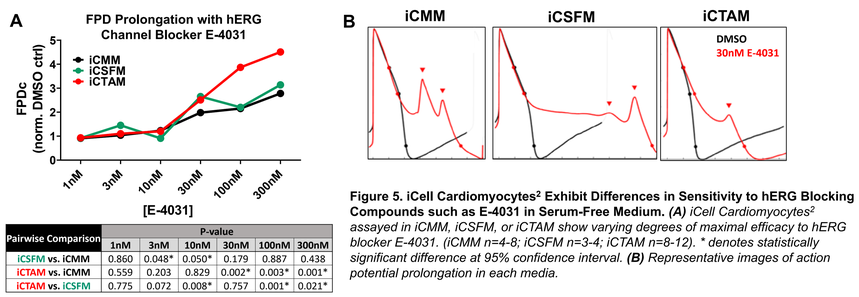
Pro-arrhythmic Risk Assessment in Serum-Free Assay Medium

Comprehensive *in vitro* Pro-arrhythmia Assay (CiPA)

CiPA Subset						APDc (ms)					
Compound	Plasma binding	Cmax	Concentrations Tested			iCMM			iCTAM		
			[Low]	[Med]	[High]	[Low]	[Med]	[High]	[Low]	[Med]	[High]
Azimilide	94%	70nM	10nM	100nM	1µM	497	554	1412	387	452	1339
Sotalol	NA	15µM	1µM	3µM	10µM	467	586	1026	405	459	852
Astemizole	97%	0.3nM	0.1nM	1nM	10nM	459	478	663	391	392	508
Terfenadine	70%	0.29nM	10nM	100nM	1µM	498	547	510	372	474	Q
Ondansetron	70-76%	0.37µM	300nM	3µM	30µM	525	935	1171	433	831	1322
Metoprolol	12%	1.8µM	10µM	30µM	100µM	443	469	572	404	439	518
Nitrendipine	98%	3nM	10nM	30nM	100nM	429	373	284	356	318	237
Ranolazine	61-64%	1.95µM	3µM	10µM	30µM	502	586	695	435	504	596

Figure 4. iCell Cardiomyocytes² Assayed in iCTAM Reliably Predict APD Prolongation when Treated with a Subset of CiPA Compounds. *iCell Cardiomyocytes² were assayed with compounds on D7 after 4h equilibration in respective medium (n=3 per dose). Compounds, with various degrees of serum binding, classified as high (red), intermediate (yellow), or low (green) clinical risk for TdP were tested for action potential duration response. Measurement of AP morphology via LEAP enabled automated detection of arrhythmic events (highlighted in red). (Q=quiescent)*

Cardiomyocytes Cultured in Serum-Free Medium are More Sensitive to hERG Blocker, E-4031



Pairwise Comparison	P-value					
	1nM	3nM	10nM	30nM	100nM	300nM
iCSFM vs. iCMM	0.860	0.048*	0.050*	0.179	0.887	0.438
iCTAM vs. iCMM	0.559	0.203	0.829	0.002*	0.003*	0.001*
iCTAM vs. iCSFM	0.775	0.072	0.008*	0.757	0.001*	0.021*

Figure 5. iCell Cardiomyocytes² Exhibit Differences in Sensitivity to hERG Blocking Compounds such as E-4031 in Serum-Free Medium. ***(A)*** *iCell Cardiomyocytes² assayed in iCMM, iCSFM, or iCTAM show varying degrees of maximal efficacy to hERG blocker E-4031. (iCMM n=4-8; iCSFM n=3-4; iCTAM n=8-12). * denotes statistically significant difference at 95% confidence interval.* ***(B)*** *Representative images of action potential prolongation in each media.*

Action Potential Triangulation Increases with High-Dose Bepridil in Serum-Free Medium

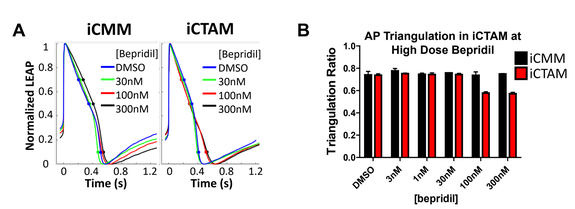


Figure 6. iCell Cardiomyocytes² Exhibit Increased Action Potential Triangulation with High-Dose Bepridil in Serum-Free Medium. *iCell Cardiomyocytes² were treated with bepridil (99% protein bound) in iCMM or iCTAM on D7 4h post media change and assayed on MEA.* ***(A)*** *Action potential morphology does not differ with increasing concentrations of bepridil in iCMM, but APD90 prolongs in iCTAM.* ***(B)*** *This change in AP morphology can be quantified using triangulation ratio* ***(C)****, commonly used as a risk predictor for arrhythmia. iCTAM shows decreased triangulation ratio (increased triangulation) compared to iCMM at high concentrations; suggesting higher sensitivity. (n=4 iCMM, 8 iCTAM)*

Using iCSFM and iCTAM to Explore Serum-Binding on Chronic Cardiotoxicity

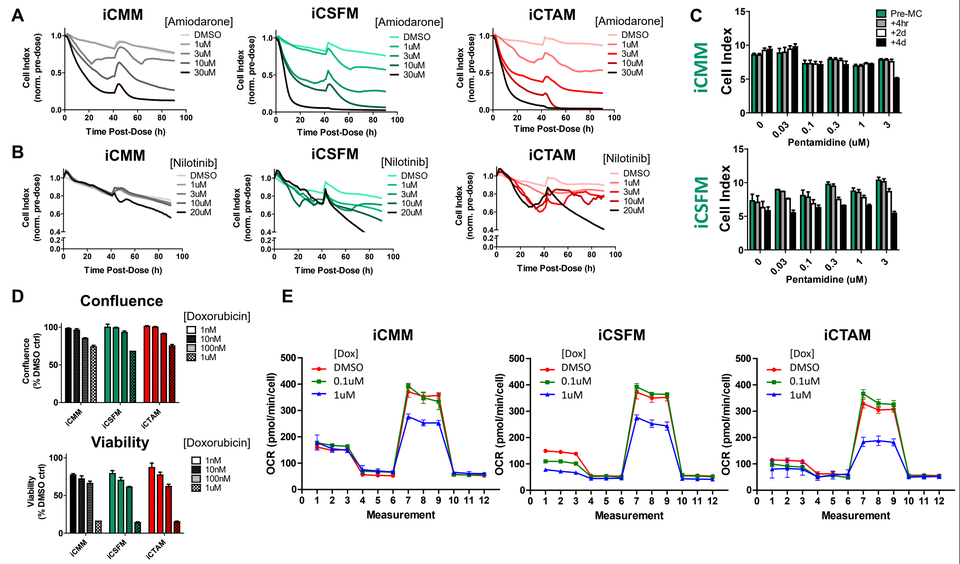


Figure 7. Examples of Chronic Cardiotoxicity Studies to Explore Drug-Binding. *Chronic dosing of iCell Cardiomyocytes² at D7 post thaw in iCMM, iCSFM or iCTAM dosed with* ***(A)*** *amiodarone (96% protein bound) or* ***(B)*** *nilotinib (98%) assayed on xCELLigence shows decreasing cell index over time for higher concentrations. The reduction in cell index in iCSFM and iCTAM at lower doses of both compounds compared to iCMM suggests higher sensitivity to the compounds in the absence of serum (iCSFM) and albumin (iCTAM) (n=4 wells per dose).* ***(C)*** *iCell Cardiomyocytes² were chronically dosed with pentamidine at varying concentrations on impedance system in either iCMM or iCSFM from D7 to D11 post-thaw. Cell Index shows similar decrease in cellular attachment/health as pentamidine concentration increases. Additionally, iCell Cardiomyocytes² were chronically dosed with doxorubicin (75% protein bound) in iCMM, iCSFM or iCTAM and assayed on D11 for confluence and viability (n=3 per dose)* ***(D)****. Increasing concentration of doxorubicin showed decreasing monolayer confluence and viability.* ***(E)*** *Cells cultured in iCMM, iCSFM, and iCTAM were dosed for two days with DMSO control, 0.1µM and 1µM doxorubicin. Cells were analyzed with the XF Cell Mito Stress Test Kit on the XFe96 Seahorse Analyzer. The spare capacity of cells in each media was reduced in all media, as shown by the response to addition of FCCP.*

CONCLUSIONS

- This study highlights two new serum-free iPSC-CM media, iCell Serum-Free Medium (iCSFM) and iCell CardioTox Assay Medium (iCTAM), that enable long- and short-term cardiotoxicity studies in the absence of serum (iCSFM) and albumin (iCTAM).
- iCell Cardiomyocytes² cultured in these media maintain high purity, viability, and cardiac gene expression as compared to those cultured in serum-containing iCell Cardiomyocytes Maintenance Media (iCMM).
- iCell Cardiomyocytes² cultured in iCSFM and iCTAM maintain comparable spontaneous beating and metabolic activity to those cultured in serum-containing iCMM.
- iCSFM and iCTAM offer the option to study serum/albumin binding effects of cardiotoxic compounds, such as pentamidine and doxorubicin, as well as to explore the pro-arrhythmic risk of investigative new drugs.