The Influence of Media on Cardiotoxicity Testing Using Human iPSC-derived Cardiomyocytes in Mono- and Co-culture Models

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Abstract

Background: The FDA Modernization Act 2.0 underscores the urgent need for non-animal-based new approach methodologies (NAMs) for chemical safety assessment. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are emerging as critical tools in in vitro cardiotoxicity studies, with iPSC-derived cardiac fibroblasts and endothelial cells enabling the development of advanced co-culture and 3D cardiac models. However, serum-free, off-the-shelf media that support both acute and chronic toxicity testing across a broad pharmacological range remain scarce.

Objectives: To address this, we developed a suite of cell culture media and supplements, including iCell® Cardiomyocytes Serum-Free Medium (iCSFM), iCell CardioTox Assay Medium (iCTAM), and the iCell Cardiac Co-Culture Supplements. These were specifically designed to maintain cardiomyocyte function, enable flexibility across assay platforms, and support reproducible toxicity testing in 2D (monoculture) and 3D (co-culture) formats.

Methods: Cardiac function was assessed using multiple platforms: microelectrode array (MEA), voltage-sensitive dye (VSD), and calcium flux assays in 2D monoculture. For toxicity testing, standard viability endpoints were employed using iCSFM and iCTAM to evaluate compounds with known cardiotoxic effects. Seahorse XF (metabolism) and impedance assays (contractility) assessed metabolic and functional toxicity profiles. Compatibility with transduction methods (AAV, BacMam) was also tested. Additionally, functional toxicity was further examined through 3D co-cultures incorporating iPSC-CM, cardiac fibroblasts, and endothelial cells.

Results: MEA results demonstrated consistent field potential durations (FPDs), with long-term culture in iCSFM showing slight FPD shortening, while enhancing assay reliability for albumin-binding compounds. A panel of hERG blockers (E-4031, dofetilide, ondansetron) showed dose-dependent effects on cardiac action potential morphology. Metabolic assays revealed decreased maximal respiration with cardiotoxic molecules, while cardiac contractility decreased significantly with chronic idarubicin exposure and increased contraction amplitudes with (S)-BayK-8644. The incorporation of 3D co-culture models showed improved biological relevance, capturing positive inotropic responses and immune response toxicology.

Conclusions: These findings underscore the utility of human iPSC-CM for in vitro cardiac function and cardiotoxicity assessment. Our optimized serum-free media formulations enable robust performance in diverse assay platforms, offering a human-relevant approach for pharmacological and toxicological evaluations. These innovations align with regulatory goals to advance NAMs and reduce reliance on animal testing.

Media, Methods, and Assay Workflows

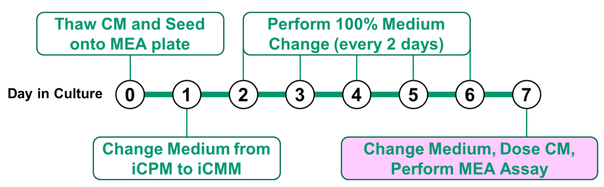


Figure 1: Cardiac MEA assay workflow and suite of cell culture media and supplements. iCell Cardiomyocytes² establish spontaneous and regular beating in culture by Day 4 (CiPA recommended timepoint Day 7). Media can be changed post day 4 to serum free media for acute or chronic assay.

iCell® Cardiomyocytes Maintenance Medium (iCMM)

Definition:
- DMEM-based, contains serum

Duration of use:
- Gold standard for extended long-term culture

Typical uses:
- To model/predict drug responses in a physiological setting

iCell® Cardiomyocytes Serum-Free Medium (iCSFM)

Definition:
- DMEM-based and Serum-free

Duration of use:
- Long-term culture (up to 7 days)

Typical uses:
- To reduce or understand the variability due to drug interaction with known or unknown serum components

iCell® CardioTox Assay Medium (iCTAM)

Definition:
- DMEM-based and Albumin-free (Xeno-free); highly defined

Duration of use:
- Recommended for up to 4 days

Typical uses:
- To reduce variability due to drug interaction with Albumin
- For gene transfection and AAV infection studies

iCell® Cardiac Co-culture Supplements Kit

Definition:
- iCMM Supplement to support cardiac co-culture applications

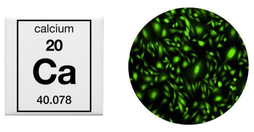
Duration of use:
- Long term cardiac co-culture medium, up to 3 weeks

Typical uses:
- iCell CardioSpheres applications
- Fibrosis assay, to enable activation of iCell Cardiac Fibroblasts by stress

All FUJIFILM Cellular Dynamics (FCDI) cardio media contain DMEM as the base medium. Hence, results can be easily compared across conditions in a media with ion concentrations in the physiological range.

Calcium Waveform Oscillation Assay

FDSS/µCell



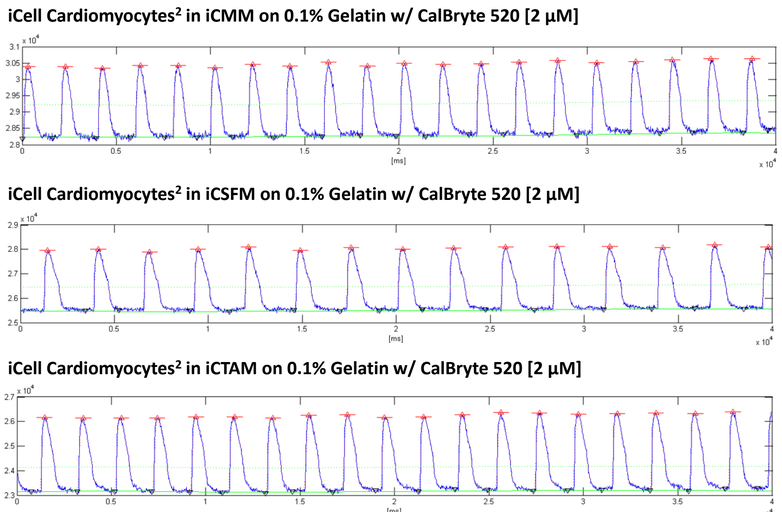
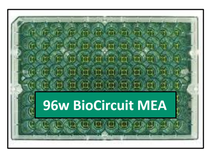
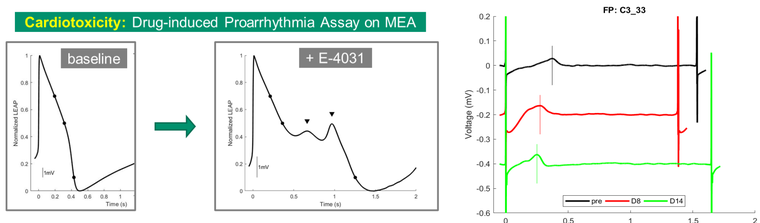
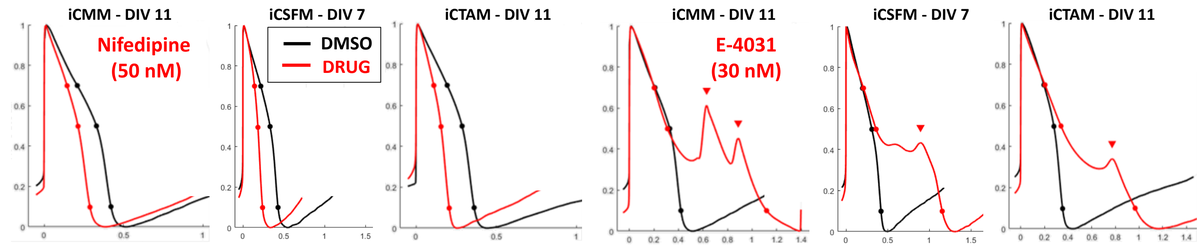


Figure 2. Representative calcium traces from iCell Cardiomyocytes² in serum-free media.

Serum-free Medium for Acute and Chronic Toxicity Assessments







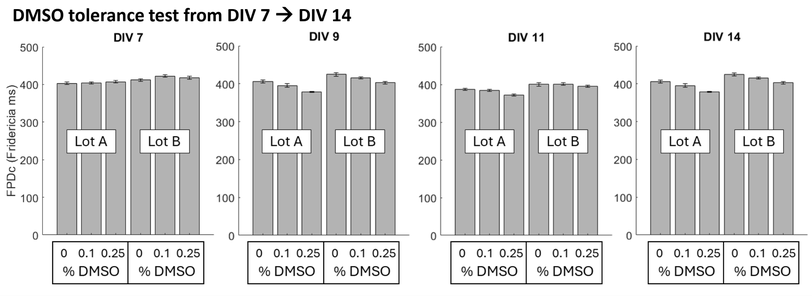
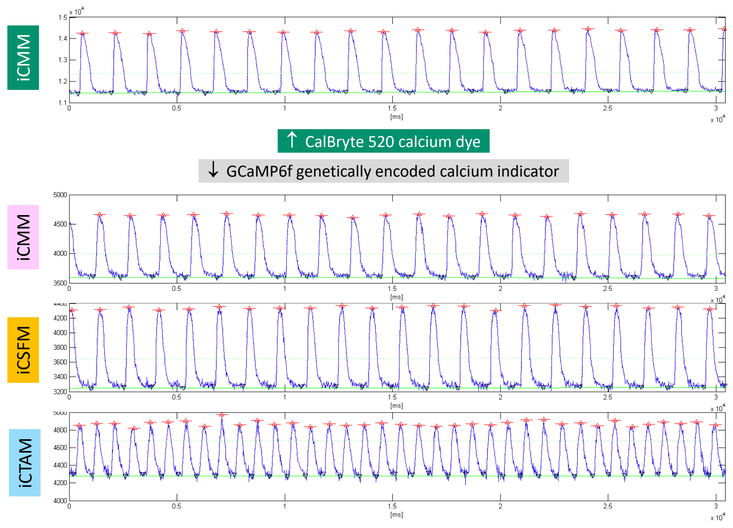
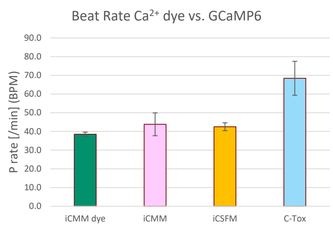
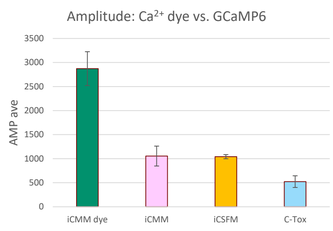


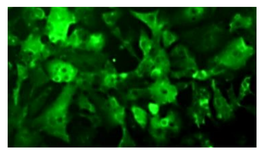
Figure 3. Cardiac MEA assay with iCell CM2 have been validated for measuring drug responses. iCSFM or iCTAM enable acute and chronic toxicity studies for drugs with unknown or high protein binding or low bioavailability. Also, cells can tolerate DMSO at 0.1 or 0.25% for at least 7 days of assay.

Transient Gene Expression: Delivery of Reporter Genes or GOI









Genetically encoded calcium indicator GCaMP6f is expressed in iCell Cardiomyocytes² and can be detected repeatedly on the FDSS/µCell, which is ideal for chronic dosing studies

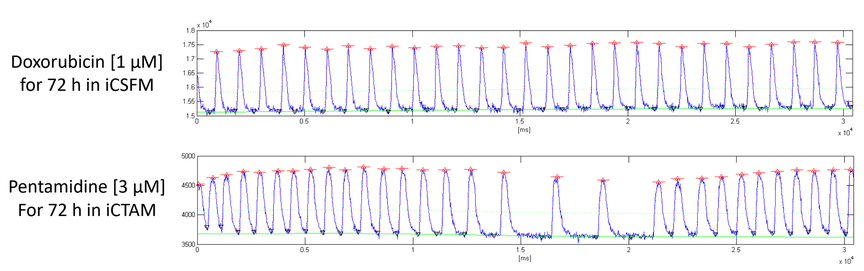
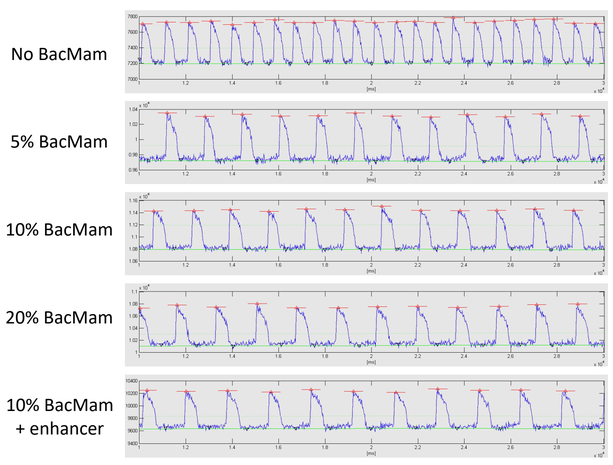


Figure 4. Transduction of iCell Cardiomyocytes² with GCaMP6f on Day 4 and assayed on the FDSS/µCell to measure calcium oscillations after chronic drug treatment in different media on Day 10.



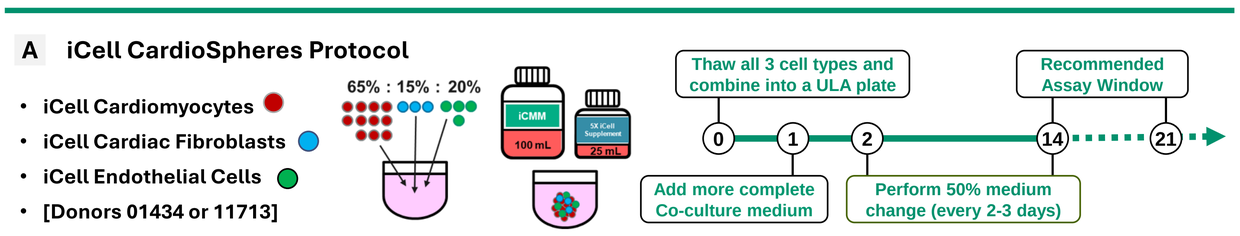
P rate [/min](BPM)		
BacMam	mean	stdev
0%	41.80	4.40
5%	36.43	1.34
10%	36.81	1.41
20%	35.93	1.04
10%+NB	33.53	5.45

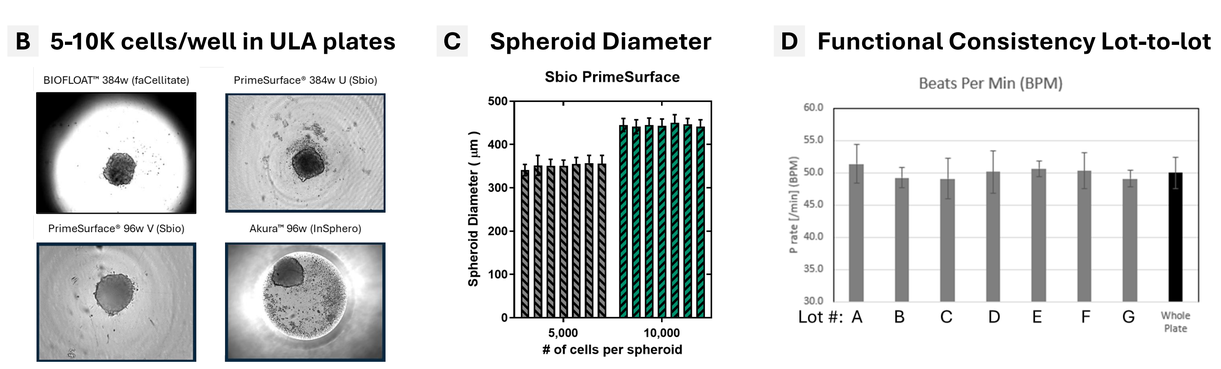
RisingSlope_ave		
BacMam	mean	stdev
0%	2.45	0.84
5%	3.11	0.91
10%	5.06	0.76
20%	4.37	0.74
10%+NB	3.27	0.71

PWD90_ave [ms]		
BacMam	mean	stdev
0%	602.2	45.7
5%	623.8	29.3
10%	632.0	35.5
20%	634.9	37.1
10%+NB	696.7	60.2

Figure 5. BacMam-mediated expression of Kir 2.1 channel (IK_1) in iCell Cardiomyocytes² is titratable and efficient method to deliver cardiac genes that can enhance the functional complexity.

Cardiac Co-culture: 3D Models and Culture Media





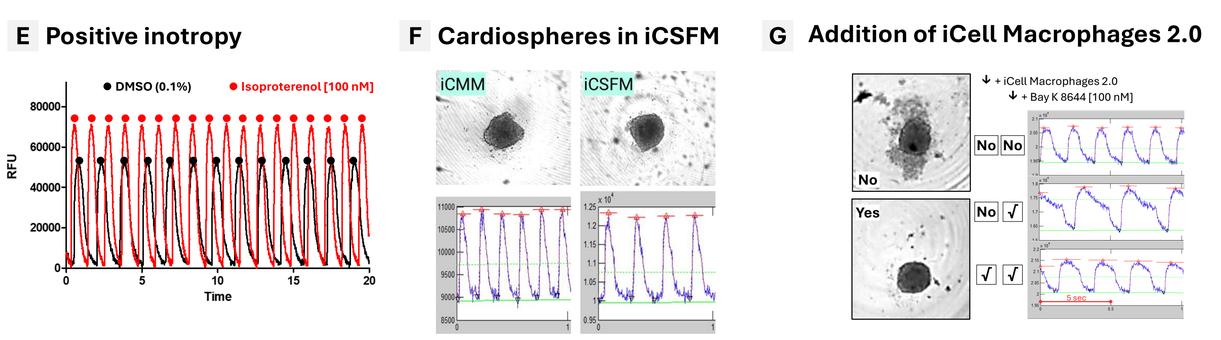


Figure 6. iCell CardioSpheres are easy to generate and amenable to high throughput screens.

A. iCell CardioSpheres can be generated combining the 3 cells times in the specified ratio using complete co-culture medium (iCMM + iCell Cardiac Co-culture Supplements Kit components) following this protocol.
B. Uniform sphere formation from 5-10K cells per well in various ultra-low attachment (ULA) plate types.
C. Spheroid diameter varies depending on number of cells and ULA plate type, but ranges from 350-450 µm.
D. Preparation of cardiospheres from different lots and combinations of cells show similar functionality. In this example, the Beats Per Min (BPM) of cells in 96w plate on DIV 14 averaged ~ 50 bpm.
E. Dosing with compounds like isoproterenol results in a positive inotropic response (↑ peak amplitude).
F. iCell Cardiospheres typically are cultured in iCMM + iCell Cardiac Co-Culture Supplements, but we have demonstrated that supplemented iCSFM also results in functional 3D spheroids. Data is from DIV 14.
G. Increasing the model complexity via addition of iCell Macrophages 2.0 not only cleans up cellular debris, but also the cells modify baseline and dosed Ca^{2+} handling properties.

Summary

iCell Cardiomyocytes² provide an in vitro test system the recapitulates the metabolism and physiology of native human cardiomyocytes. Complementary cell types including iCell Cardiac Fibroblasts and iCell Endothelial Cells are essential for making more complex and biologically relevant cell models. The work presented here highlights the utility and flexibility of using human iPSC-derived cell types in 3D as a promising in vitro model for measuring compound effects on human heart tissues in high throughput format for drug discovery studies.