Alternative Approaches to Enhance the Functional Complexity of Human iPSC-derived Cardiomyocytes in 2D and 3D

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Introduction

Human iPSC-derived cardiomyocytes manufactured by FUJIFILM Cellular Dynamics, Inc. (FCDI) are commercially available under the brand name iCell® Cardiomyocytes² (CM2). Launched in 2015, this product is now the most widely-accepted iPSC-based cardiac cell model in the field for drug discovery and safety/toxicity screening. Importantly, an FDA-based working group launched the Comprehensive in vitro Pro-arrhythmia Assay (CiPA) paradigm and validated the use of iCell CM2 to make meaningful risk assessments of drug cardiotoxicity in a dish. CiPA assays can use either live cell calcium indicators or voltage-sensitive dyes to measure compounds effects on spontaneously beating cardiomyocytes. As a leader in iPSC technology and innovation, FCDI is committed to enhancing the functional utility of iCell CM2 in more complex model systems and novel assay formats. Previously, we have supported the development of application protocols that feature the use of iCell CM2 in calcium assays on the FDSS/µCell from Hamamatsu. Here, we show how iCell CM2 are combined with other technologies to yield an improved human iPSC-derived cardiomyocytes.

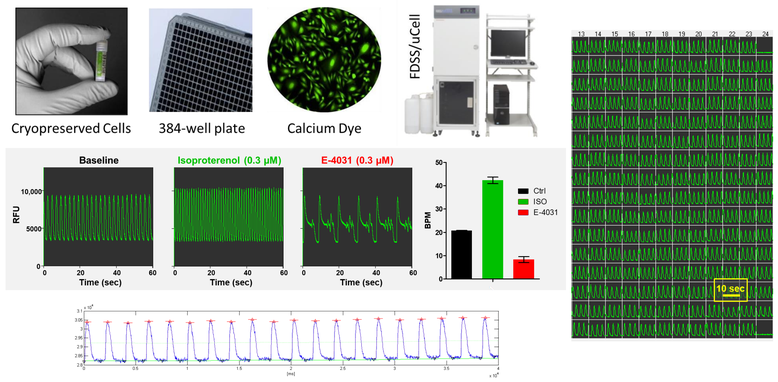


Figure 1. Assay Performance of iCell Cardiomyocytes² on the FDSS/µCell. Cryopreserved iCell CM2 can be shipped on-demand to any laboratory in the world. Protocols for thawing and plating cells in multi-well format (96w or 384w) and then running a calcium assay on the FDSS are available. Robust baseline cardiac activity is consistently obtained by DIV 7 and cells respond appropriately to standard pharmacology, including isoproterenol and E-4031.

ASSAY PLATES: Patterned Surfaces to Enhance Cell Structures

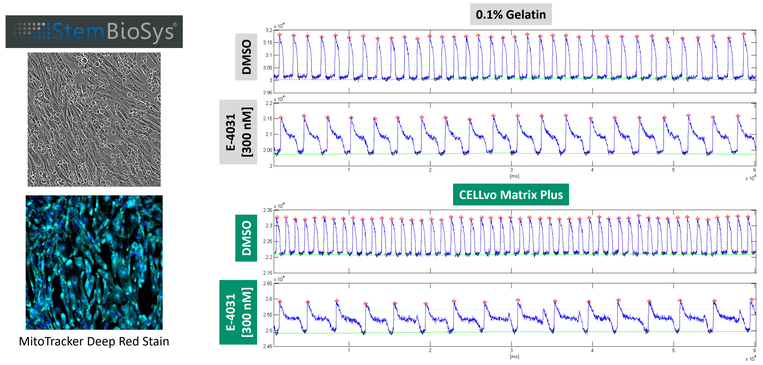


Figure 2. iCell Cardiomyocytes² cultured on CELLvo™ Matrix Plus. iCell CM2 were maintained in iCMM until Day 14 and assayed on the FDSS/µCell to measure voltage with FluoVolt™ Membrane Potential dye. Half the plate was coated with 0.1% gelatin and the other half was coated with CELLvo Matrix Plus. Cells cultured on Matrix Plus do display a faster beat rate and slightly lower amplitude at baseline but show a more sensitive response to treatment with 300 nM E-4031. Brightfield images of iCell CM2 and cells stained with MitoTracker Deep Red Stain (ThermoFisher) illustrate the change in morphology of the cardiomyocytes when cultured on Matrix Plus.

Calcium Assay Performance with GCaMP6f

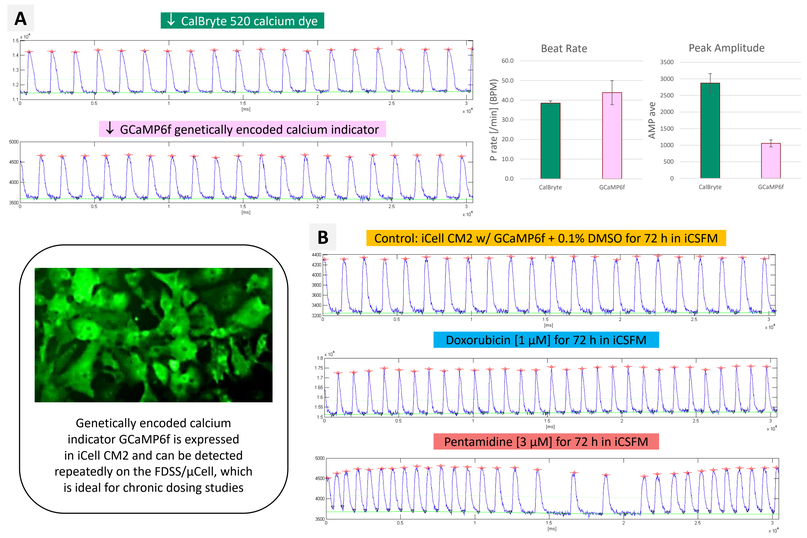
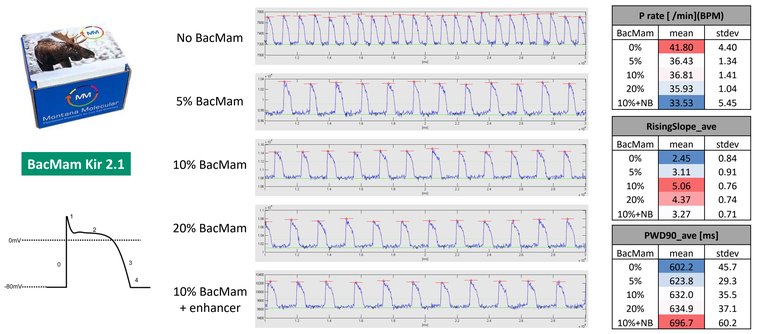


Figure 3. Transduction of iCell CM2 with GCaMP6f enables chronic dosing studies. iCell CM2 were transduced with GCaMP6f (Vector Biolabs) on Day 4 in iCell Cardiomyocytes Serum-free Medium (iCSFM). (A) Baseline calcium oscillations were measured on the FDSS/µCell on Day 7 and compared to the calcium dye, Calbryte™ 520 AM (AAT Bioquest). (B) Next, cells were exposed to different drugs for 72 hours and then assayed on Day 10. Doxorubicin-treatment resulted in a faster beat rate and increased peak amplitude compared to DMSO controls. Chronic exposure to Pentamidine created irregular beating patterns and variable peak heights and widths.

BacMam-mediated Expression of IK1 Channel in iCell CM2



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 4. BacMam-mediated expression of Kir 2.1 in iCell CM2. BacMam technology is an efficient method to deliver cardiac genes that can enhance their functional complexity, such as IK_1 channel. BacMam reagent (Montana Molecular) is al titratable, meaning that increasing amounts of virus result in increased transgene expression. Enhancer solution (e.g., Na butyrate or Trichostatin A) acts by means of HDAC inhibition and does improve BacMam-mediated expression. Only 10-20% of the recommended concentration of Na butyrate was needed in this example. Transduction of cells in iCSFM was performed on Day 6 in culture and assayed on the FDSS/µCell for calcium on Day 10 (data not shown) and voltage on Day 14 with FluoVolt Membrane Potential dye. Increased expression of IK1 channel resulted in a decreased number of beats per minute (BPM), an increased rising slope, and a longer peak width duration at 90% (PWD90). The overall shape of the action potential morphology is certainly altered.

Formation and Assay of Tri-culture 3D Cardiospheres

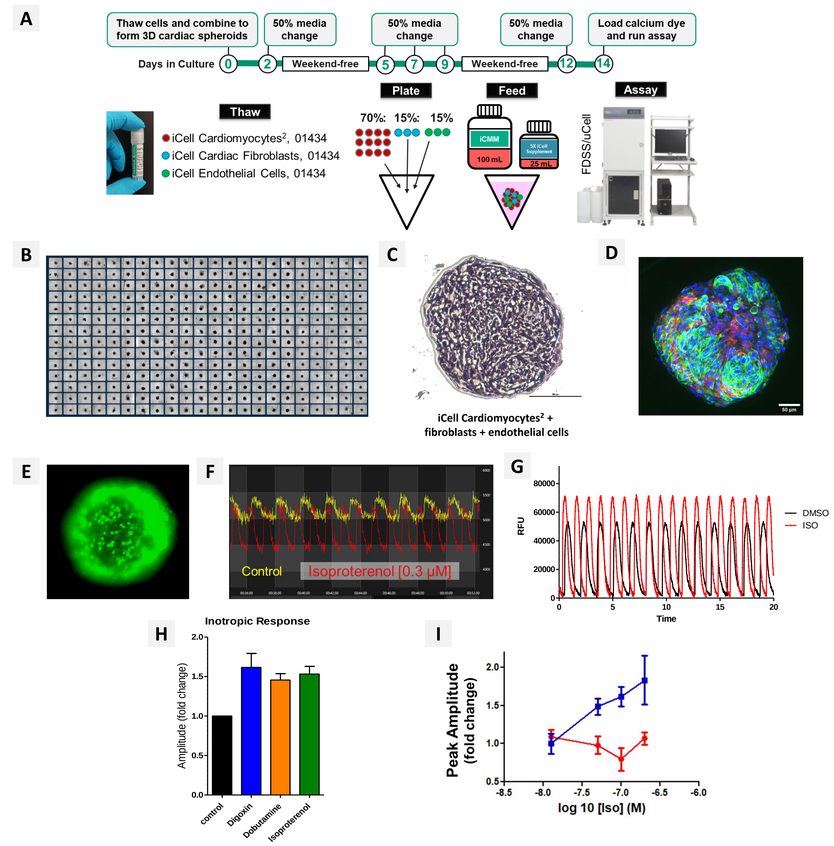


Figure 5. Transition from 2D to 3D cell culture enables complex multicellular systems. (A) Human iPSC-derived cardiomyocytes (iCell CM2), iPSC-derived endothelial cells (iCell Endothelial Cells), and iPSC-derived cardiac fibroblasts (iCell Cardiac Fibroblasts) can be cultured together to form 3D microtissues (or spheroids); (B) "iCell CardioSpheres" are easy to form in 384-well plates and show high uniformity and consistency in every well; (C) Spheroid diameter can be tracked over time and range from 300-400 µm; (D) composite images of 3D cardiospheres with anti-cardiac Troponin T in Green and anti-Te7 in **Red** with Hoechst staining nuclei in **Blue**; (E) cells loaded with fluorescent calcium dyes can be imaged with kinetic and high speed analysis on the FDSS/µCell (Hamamatsu); (F) Baseline recordings of 3D cardiospheres on D14 are shown in yellow, whereas treatment with (G) isoproterenol (Iso, 300 nM) shown in **red** resulted in an increase in peak amplitude & frequency; (H) Positive inotropic response was repeated in a separate experiment with exposure to digoxin, dobutamine, and iso (all 1 µM); (I) Increased peak amplitude is a key feature of 3D cardiospheres and can be a dose-dependent response. All three cell types are isogenic (or from the same iPSC donor).

Summary

iCell Cardiomyocytes² from FUJIFILM Cellular Dynamics are a widely-accepted cell model for drug discovery and safety/toxicity screening. Robust assays to measure cardiac activity and detect cardiotoxicity on the Hamamatsu FDSS/µCell with these cells have been developed previously. To advance the field forward, we have presented various approaches to enhance the functional performance of human iPSC-derived cardiomyocytes in different *in vitro* assay systems. With iCell CM2 as the common tool, we have tested new serum-free assay media for cardiotoxicity assays, evaluated HTS-compatible assay plates modified with relevant ECM material that better mimic a 3D environment for the cells, created 3D co-culture systems with other cell types known to interact with and improve the functional response of cardiomyocytes, and delivered genes via viral transduction to express either a calcium indicator or an important cardiac potassium channel. Evaluation of new technologies is continuously ongoing at FCDI.