Functional Testing of Human iPSC-derived 3D Cardiac Tri-culture Microtissues or CardioSpheres

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Abstract

The adult human heart is a complex organ providing highly regulated processes of pumping blood throughout the body. Heart tissue contains various types of cells, including (but not limited to) cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts. Although cardiomyocytes may occupy about 75% of the total volume, they only constitute 40-50% of the total cell count.

Recent publications show that 3D cell cultures of cardiac spheroids (aka microtissues or "cardiospheres") enhance the maturation and functional activity of cells compared to 2D cultures of cardiomyocytes, thus more closely mimicking actual heart physiology. In this study, we developed a tri-culture spheroid model by mixing human iPSC-derived cardiomyocytes, cardiac fibroblasts, and endothelial cells from the same donor in defined ratios in ultra-low attachment (ULA) plates. Importantly, 3D microtissues were created by directly thawing cryopreserved vials of each individual cell type and culturing them together in an optimized media formulation. Cells formed spheroids within 48 hours and started to contract spontaneously and regularly after 4-5 days. Spheroid formation, size, and morphology was tracked over time using live cell imaging. Additionally, different cell types in the tri-culture system were immunostained using anti-Troponin T (for cardiomyocytes), COL1A1 (for cardiac fibroblasts), and VE-Cadherin (for endothelial cells) antibodies.

Cardiac activity of isogenic 3D cardiospheres was investigated by recording the calcium oscillations on a high-throughput kinetic screening instrument (i.e., FLIPR Penta or FDSS/uCell). Pharmacological modulation was performed by exposing the cells to a set of known small molecule inotropes, including isoproterenol (1-500 nM), dobutamine (0.01-10 uM), and Bay-K 8644 (0.01-0.3 uM) . Importantly, we observed significant increases (approx. 2-fold) in the oscillation rate or peak count (positive chronotropic response) and increased the peak amplitude 1.2-1.5-fold (positive inotropic response) with these compounds. Other drugs, including hERG blockers E-4031 (1-100 nM) and dofetilide (0.1-30 nM) and ion channel blocker verapamil (0.1-10 uM) demonstrated changes in the calcium oscillation patterns consistent with expected mode of action.

The work presented here highlights the utility and biological relevance of using human iPSC-derived cardiac cell types in 3D microtissues compared to 2D cardiomyocytes only as a promising in vitro model for measuring compound effects on human heart tissues in high throughput format.

Methods and Workflow

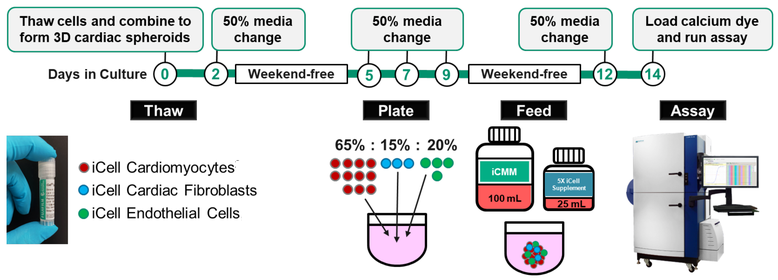

Figure 1. Workflow schematic for generating 3D cardiac spheroids. Human iPSC-derived cell types, including iCell Cardiomyocytes. iCell Cardiac Fibroblasts), and iCell Endothelial Cells from donors 01434 or 11713 were supplied by FUJIFILM Cellular Dynamics, Inc. (FCDI). iCell Cardiomyocytes Maintenance Medium (iCMM), as well as the 5X iCell Cardiac Co-Culture Supplement were also from FCDI. All three cell types were thawed and combined at a ratio of 65:15:20 into ultra-low attachment (ULA) plates (e.g. PrimeSurfuace® 96w or 384w from Sbio). 3D microtissues were maintained for ~14 days in culture until ready for testing. On the day of assay, cells were loaded with EarlyTox™ Cardiotoxicity calcium dye (Molecular Devices) for 2 h and then imaged on a high-throughput cellular screening system, such as the FLIPR Penta or FDSS/µCELL. Spontaneous calcium oscillations were recorded using 30-50 frames per second (fps) that allowed for resolution of complex cardiac waveforms.

NEW Cell Type!! iCell® Cardiac Fibroblasts

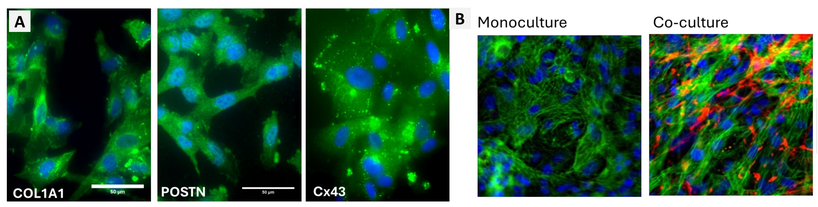

Figure 2. iCell Cardiac Fibroblasts marker characterization. **(A)** Immunostaining for Collagen 1 (COL1A1), Periostin (POSTN) and Connexin 43 (Cx43) is strong in these cells. **(B)** When in co-culture with iCell Cardiomyocytes, iCell Cardiac Fibroblasts (TE-7 - red) demonstrated the ability to align the cardiomyocytes (cTNT- green) better as compared to the structural alignment of iCell Cardiomyocytes in monoculture.

Self Assembly of Tri-cultures into 3D Spheroids

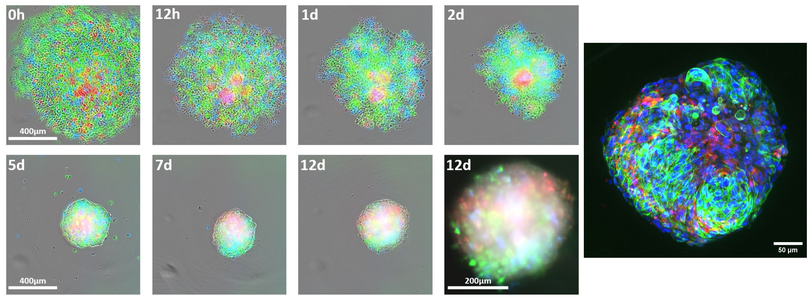

Figure 3. Images of 3D cardiac tri-culture microtissues stained with CellTracker Dye captured over time. The tri-culture microtissues self-assemble into tight spheroids within 4 to 5 days in culture. iCell Cardiomyocytes, 01434 (**green**), iCell Cardiac Fibroblasts, 01434 (**red**), and iCell Endothelial Cells, 01434 (**blue**) were stained with CellTracker Dye for 30 min prior to adding to the ULA plate and were then monitored on an Incucyte. Composite images of 3D cardiospheres were generated after fixation, permeabilization, and staining with anti-cardiac troponin T (green), anti-cardiac fibroblasts (TE-7; red), and Hoechst staining nuclei blue.

Rate of Spheroid Formation of Mono-, Co-, and Tri-cultures

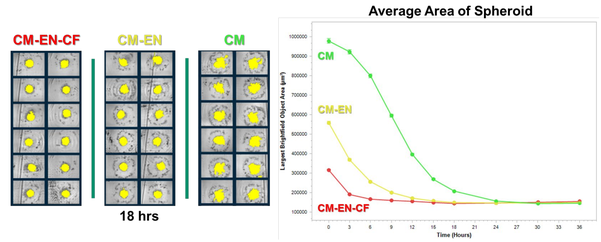

Figure 4. Monitoring spheroid formation on Incucyte. Cultures of either cardiomyocytes only (CM; **Green**), CM + endothelial cells (EN; **Yellow**), or CM+EN + cardiac fibroblast cells (CF; **Red**) were combined in a ULA plate and image analyzed using the spheroid module on the Incucyte SX5. Co-culture with EN and CF results in faster spheroid formation, but the resulting cellular microtissues are the same size after 24 hours.

Functional Cardiac Activity (Ca^{2+} Oscillations) of 3D Spheroids

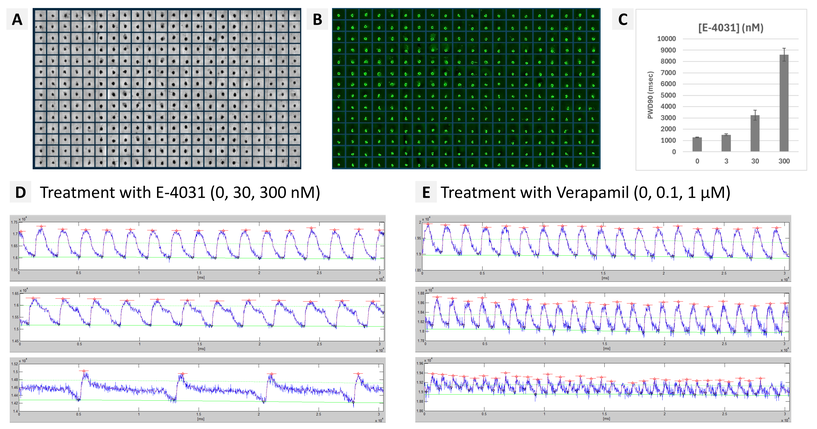

Figure 5. Measuring spontaneous calcium oscillations from 3D cardiospheres. **(A)** One spheroid forms per well of 384w plate. **(B)** Cardiospheres loaded with EarlyTox (or Calcium 6) dye fluoresce green when bound to calcium. **(C)** Spontaneous calcium oscillations are recording on the FDSS/µCell and waveforms +/- compound are analyzed for various parameters, including Peak Width Duration (PWD90), as shown here. **(D)** Duration of calcium transients is increased upon treatment with E-4031 (hERG channel blocker). **(E)** Amplitude of calcium waveforms is decreased upon treatment with the calcium channel blocker, verapamil. Both 30 min post-dose.

Positive Inotropic Response = More Mature Cardiac Phenotype

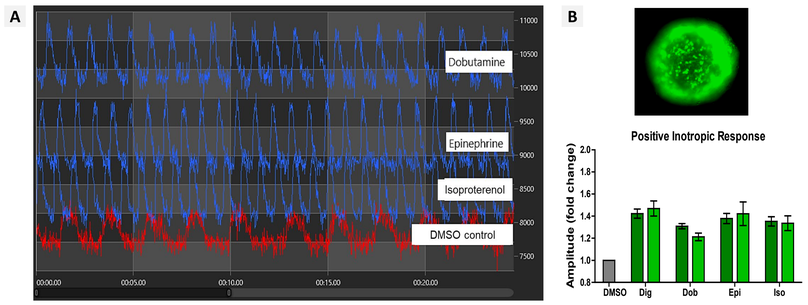

Figure 6. Tri-culture 3D spheroids show a positive inotropic response. **(A)** Baseline recordings on D14 are shown in **red**, and exposure to either dobutamine (Dob, 2 µM), epinephrine (Epi, 10 µM), or isoproterenol (Iso, 100 nM) shown in **blue** resulted in an increase in peak amplitude (1.3- to 1.5-fold) & frequency (~2-fold). The compound digoxin (Dig, 1 µM) was also tested but not shown. **(B)** The positive inotropic response was quantified in the graph with all data double-normalized to the signal pre- and post-drug treatment and then to DMSO control wells on the same plate. This response has been demonstrated across different iPSC donors (01434 and 11713) with all three cell types each being from the same iPSC line.

Impact of Adding iPSC-derived Macrophages?

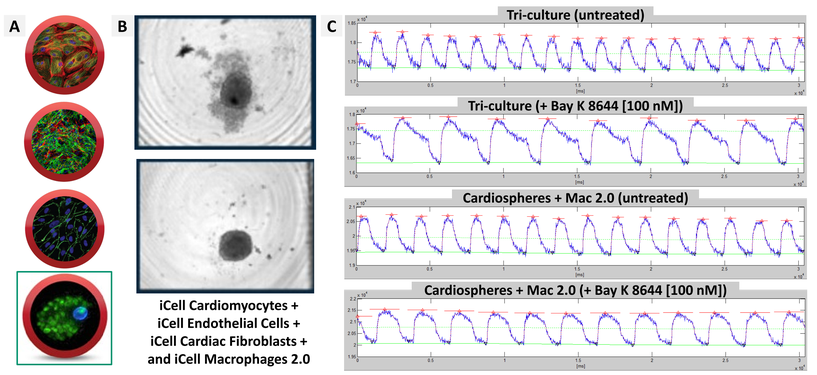

Figure 7. (A) Multiple human iPSC-derived cell types to generate complex co-cultures are available from FUJIFILM CDI, including iCell Macrophages 2.0. Formation of iCell Cardiospheres was performed as usual (5,000 cells per well of 384w ULA plate from Sbio). On Day 10 in culture, iCell Mac 2.0 (approx. 2,500 cells per well) were added to the cell culture plate and maintained in tri-culture medium until Day 14. **(B)** Spheroids were imaged on the Incucyte and it was evident that wells containing macrophages were much "cleaner" than wells with only CM, EC, and CF, which do contain some debris from unincorporated or non-viable cells. **(C)** Beyond phagocytic function, macrophages are regarded as an integral cellular component of the heart, as they are involved in electrical activity. Therefore, 3D cardiac spheroids ± macrophages were evaluated via calcium assay ± treatment with the calcium channel activator Bay K 6844. While these results are preliminary, it was evident that the baseline cardiac activity was different with 3 cell types vs. 4 cell types in culture. Also, the response to Bay K 6844 was attenuated in the presence of macrophages. Efforts to further understand these results and observations are ongoing. Next steps will include macrophage polarization studies and incorporation of an immune component to the cellular toxicity of 3D cardiac microtissues.

Summary and Conclusions

iCell Cardiomyocytes from FUJIFILM CDI 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 (and now even iCell Macrophages 2.0) 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. Optimized culture media and assay protocols are also available. Please visit www.fujifilmcdi.com for more information.