Cardiospheres in Action: Multi-cellular 3D systems for more advanced and predictive cardiotoxicity testing

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OVERVIEW

The purpose of this work is to showcase functional cardiotoxicity data from different labs using co-cultures of human iPSC-derived cardiomyocytes (CM), cardiac fibroblasts (CF), and endothelial (EN) cells from the same donor together to generate 3D “cardiospheres”.

INTRODUCTION

The adult human heart is a complex organ containing 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 cultures of cardiac spheroids enhance the maturation and functional activity compared to 2D cultures of cardiomyocytes alone.

METHODS

All cryopreserved iPSC-derived cell types and supporting media formulations were from FUJIFILM CDI. Individual cell types were thawed and combined in defined ratios (65:15:20 of CM:CF:EN) and cultured in ultra-low attachment (ULA) plates. Typically, 5-10K cells per well were used in both 96- or 384-well formats. Cell suspensions formed into spheroids within 48 h and the structures started to contract regularly after 4-5 days. Spontaneous beating of 3D cardio-spheres was investigated using two different methods. First, calcium oscillations were recorded from cells loaded with a calcium-sensitive dye (Calcium 6) on either a FLIPR Penta High-throughput Cellular Screening System (Molecular Devices) or an FDSS/µCell Kinetic Plate Imager (Hamamatsu). Cardiac beating activity was detected via optimal measurements from a voltage-sensitive dye (BeRST) on the VOLTA Scanner (Lumencor).

RESULTS

Baseline activity was recorded after 2 weeks in culture and compared across 3 different sites. Pharmacological modulation was performed with known hERG blockers (dofetilide and E-4031), positive inotropes (isoproterenol, dobutamine, and BayK 8644), ion channel blockers (nifedipine and mexiletine), and other cardioactive drugs (doxorubicin and sunitinib). The results presented here highlight the utility of 3D cardiospheres generated with human iPSC-derived cardiac cell types and showcase the potential for drug discovery or preclinical screening in high-throughput format.

CONCLUSIONS

We have developed application protocols for generating iCell CardioSpheres that are compatible with various platforms including MEA and high content imagers. Future work includes expanded pharmacological validation for assessing drug toxicity and predictivity, as well as expansion into other high throughput “organ-on-a-chip” micro-physiological system (MPS) platforms. There is also interest in generating cardiospheres from disease donor lines and assessing their use for drug discovery.

Methods and Workflow

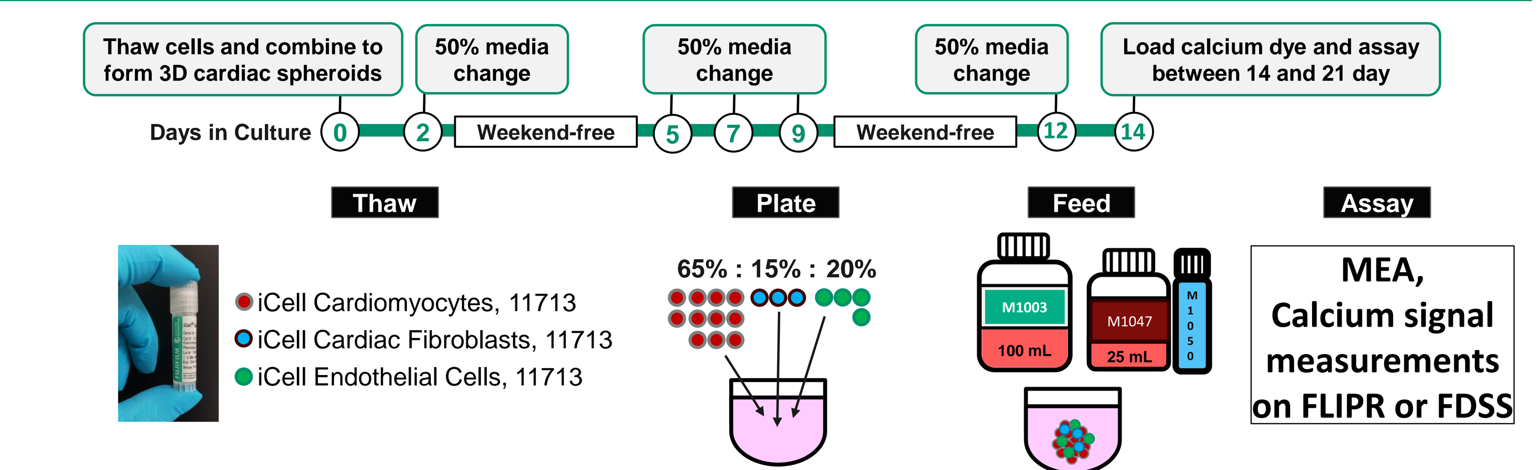

Figure 1. Workflow schematic for generating 3D cardiac spheroids. Human iPSC-derived cell types, including iCell Cardiomyocytes, iCell Cardiac Fibroblasts, and iCell Endothelial Cells from donors 01434 or 11713 were supplied by FUJIFILM Cellular Dynamics, Inc. (FCDI). iCell Cardiomyocytes Maintenance 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.

For an optimized assay protocols please visit www.fujifilmcdi.com for more information.

NEW Cell Type!! iCell® Cardiac Fibroblasts

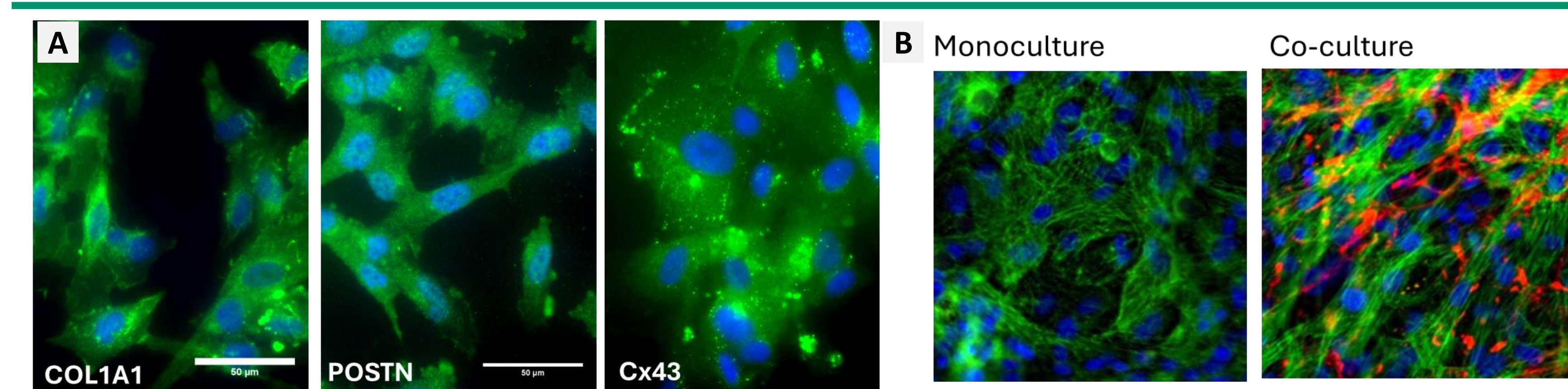

Figure 2. iCell Cardiac Fibroblasts marker characterization. **(A)** Immunostaining for Collagen 1 (COL1A1), Periostin (POSTN) and Connexin 43 (Cx43) is strong in these cells. **(B)** When in co-culture with iCell Cardio-myocytes, iCell Cardiac Fibroblasts (TE-7 red) demonstrated the ability to align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

Assessing Function of 3D CardioSpheres on MEA

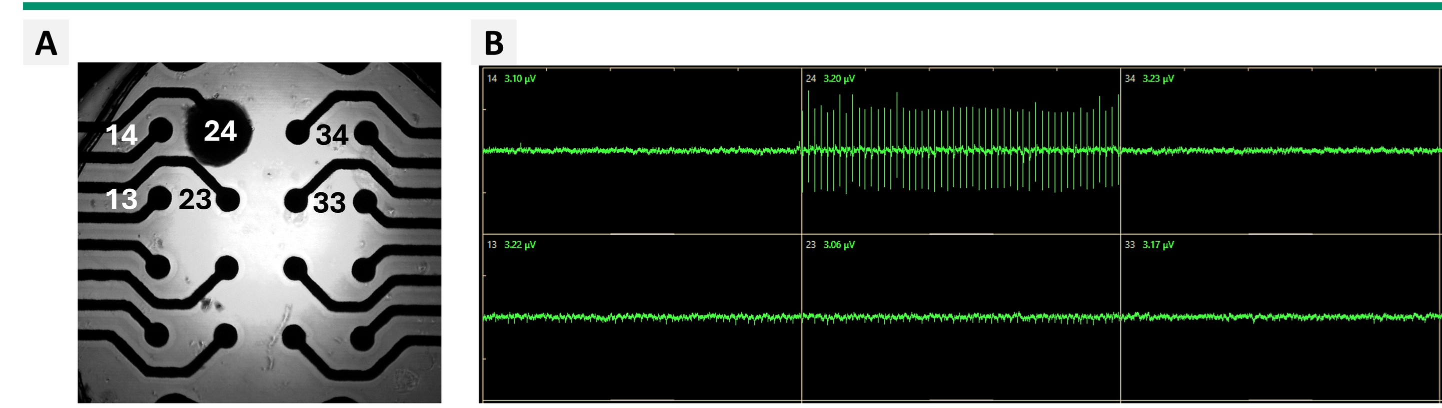

Figure 3. Assessing iCell CardioSpheres function on a SpheroGuide MEA Plate from Axion Biosystems. **(A)** Snapshot of a single CardioSphere transferred into one well of a SpheroGuide MEA plate and positioned above electrode #24. **(B)** Electrical activity from electrode 24 is displayed with robust and uniform spontaneous activity.

3D Cardiac Spheroid Self-Assembly and Rate of Formation in Mono-, Co- and Tri-cultures

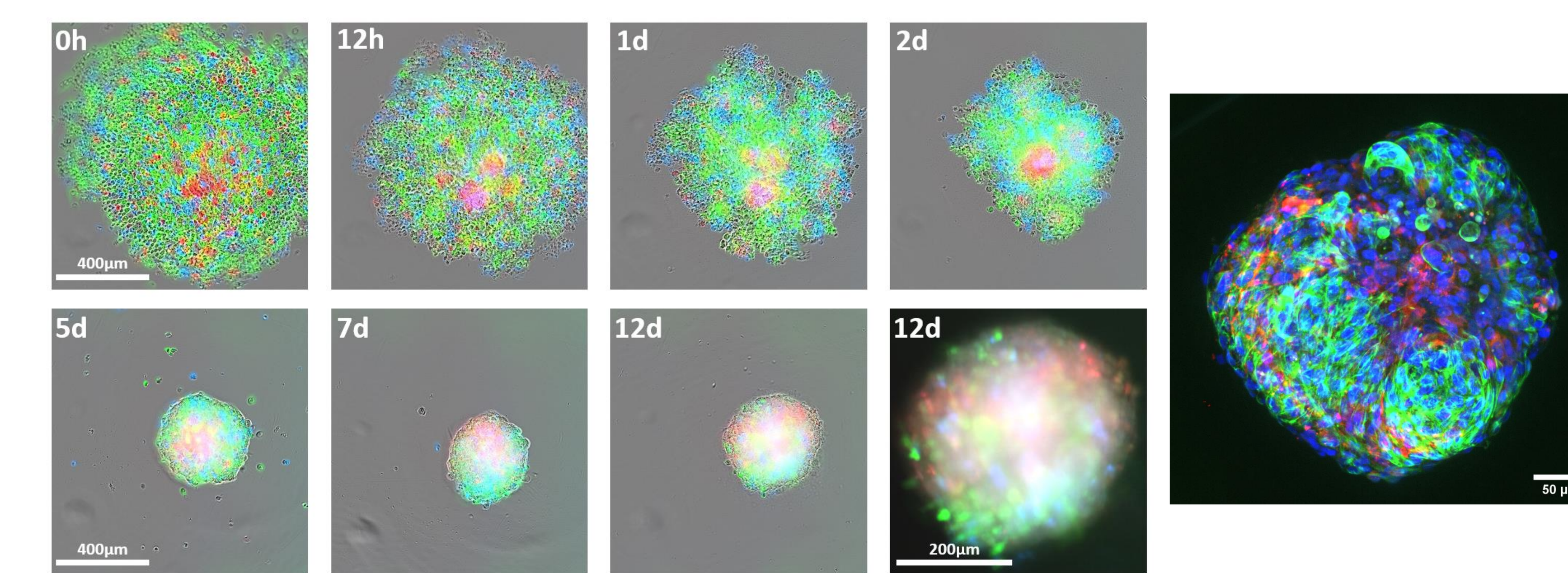

Figure 4. Images of 3D cardiac tri-culture microtissues stained with CellTracker Dye captured over time. The tri-culture microtissues self-assemble into tight spheroids within 4 to 5 days in culture. iCell Cardiomyocytes, 01434 (green), iCell Cardiac Fibroblasts, 01434 (red), and iCell Endothelial Cells, 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.

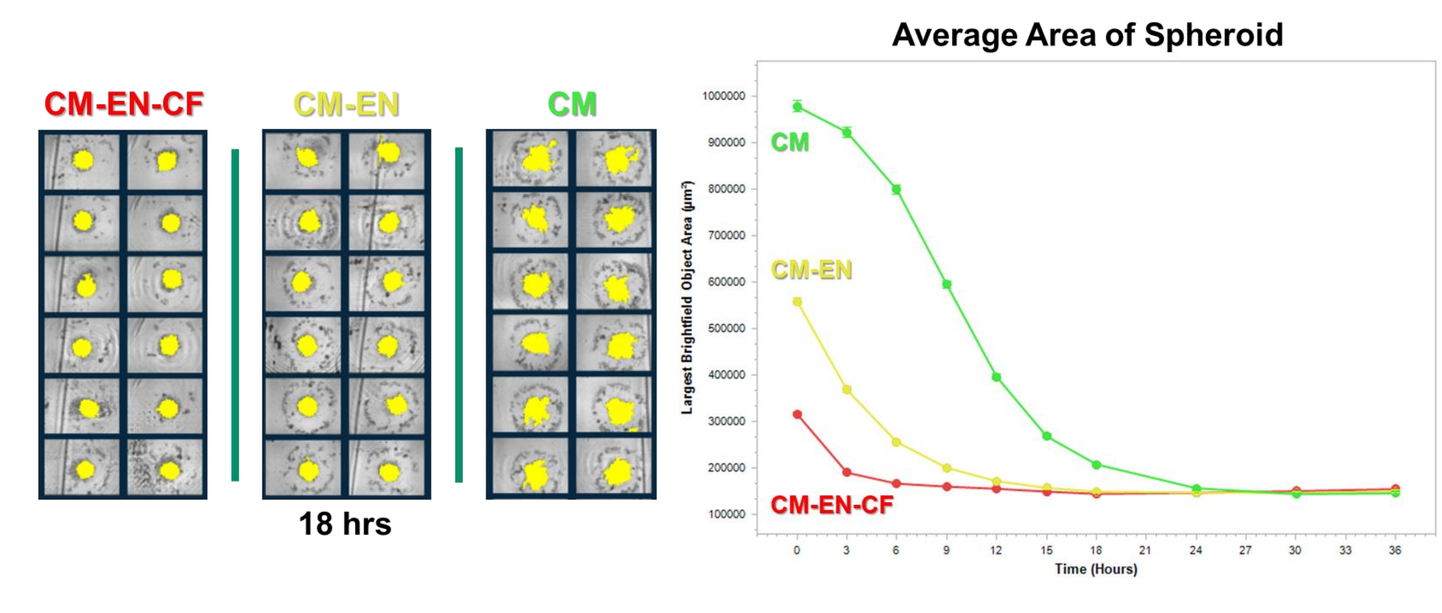

Figure 5. Monitoring spheroid formation on Incucyte. Cultures of either cardiomyocytes only (CM; Green), CM + endothelial cells (EN; Yellow), or CM + EN + cardiac fibroblast cells (CF; Red) were combined in a ULA plate and image analyzed using the spheroid module on the Incucyte SX5. Co-culture with 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 in Multiple Platforms

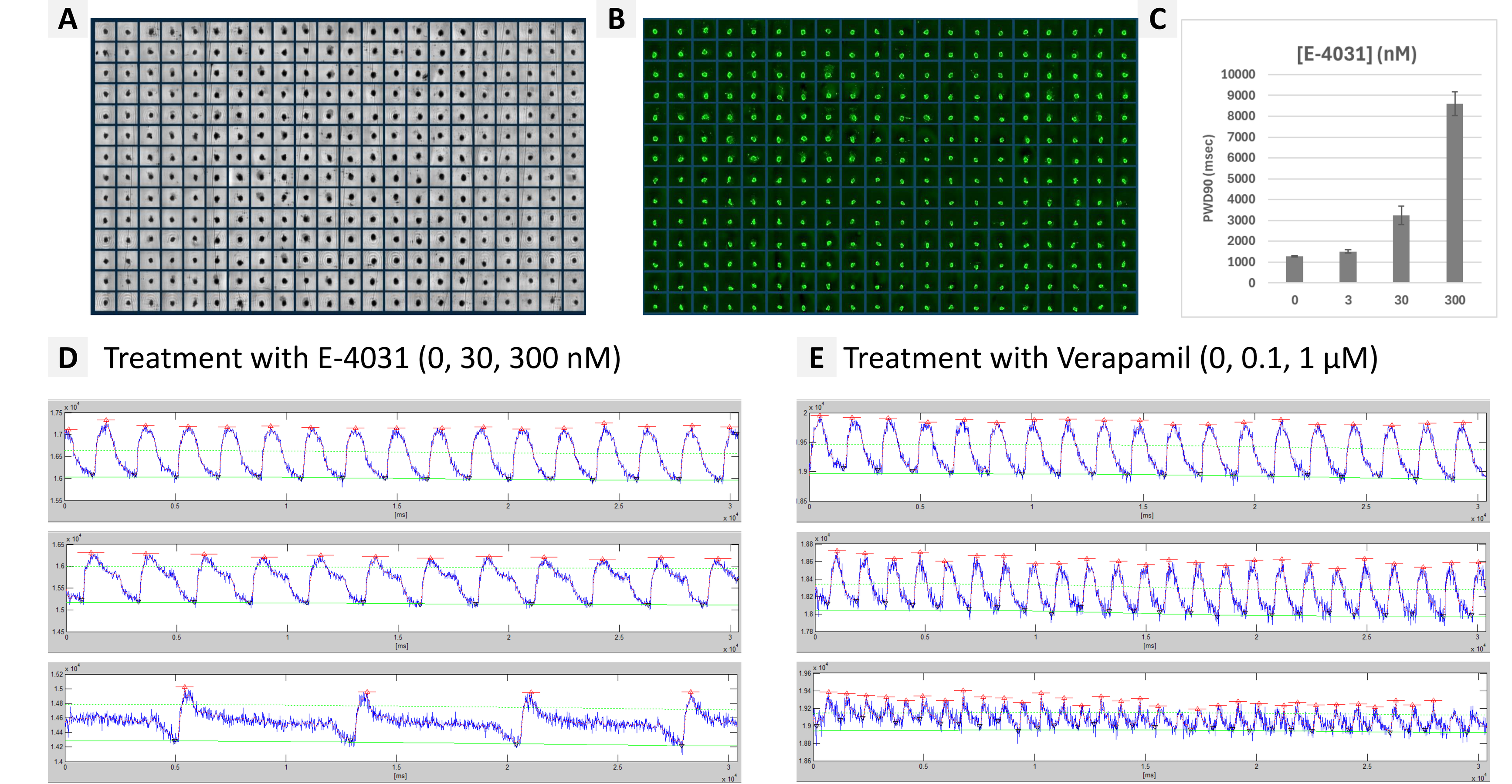

Figure 6. Measuring spontaneous calcium oscillations from 3D cardiospheres. **(A)** One spheroid forms per well of 384w plate. **(B)** Cardiospheres loaded with EarlyTox (or Calcium 6) dye fluoresce green when bound to calcium. **(C)** Spontaneous calcium oscillations are recording on the FDSS/µCell 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.

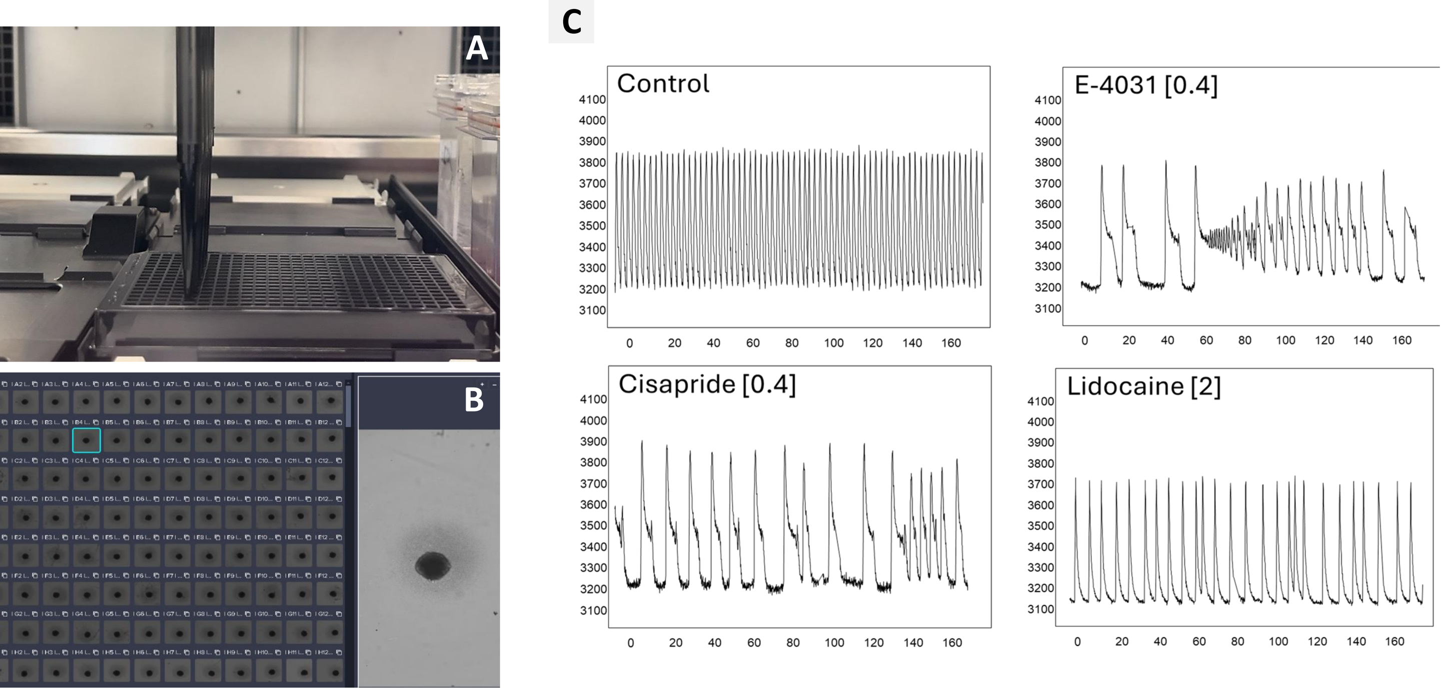

Figure 7. Automated media exchanges using CellXpress.ai and Ca^{2+} assay with 3D CardioSpheres. **(A)** View of the CellXpress.ai system performing media exchanges in a 384-well plate. Hands-free and rate-controlled liquid handling reduces errors rate and saves time for other tasks in the lab. **(B)** Transmitted light image (10X magnification) of iPSC-derived 3D cardiac microtissues cultured in a 96-well plate. A single CardioSphere forms per well in a highly consistent and reproducible manner. **(C)** Calcium oscillations measured after treatment of iCell CardioSpheres with hERG blockers (E-4031 and Cisapride) or Na^{+} channel blocker (Lidocaine). Ca^{2+} waveforms were recorded by kinetic calcium imaging using the FLIPR instrument and analyzed using PeakPro2 software. Representative traces for each compound demonstrates pattern different from control signal. Drug concentrations are in µM.

Summary and Conclusions

iCell Cardiomyocytes from FUJIFILM CDI provide an in vitro test system that 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 3D cell culture. These 3D spheroids are compatible with hands-free and rate controlled liquid handlers. Together, these technologies offer a promising in vitro model for measuring compound effects on human heart tissues in high throughput format for drug discovery studies.