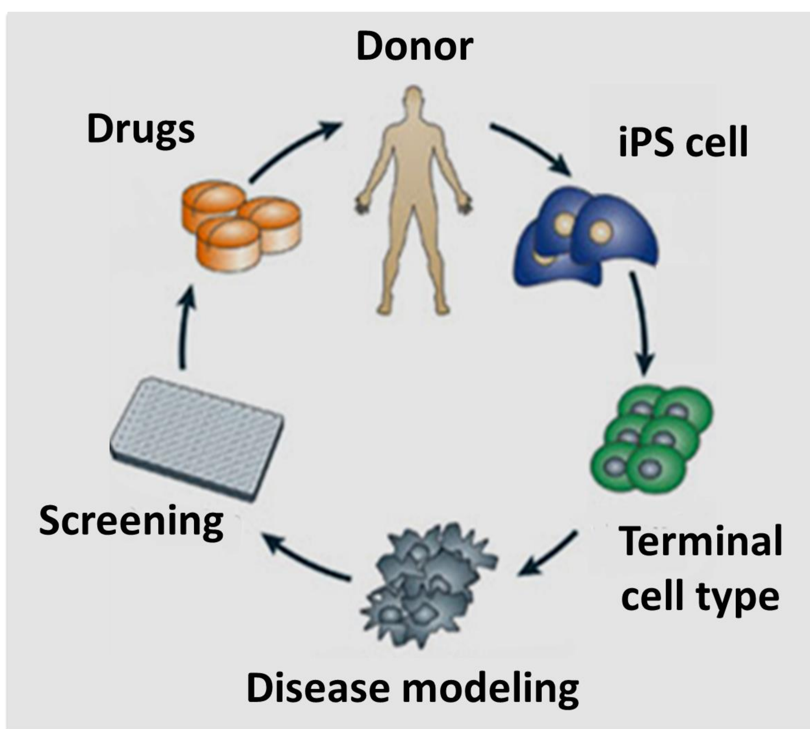
Calcium Handling Assays with Human iPSC-derived Cell Types on the Hamamatsu FDSS/µCell

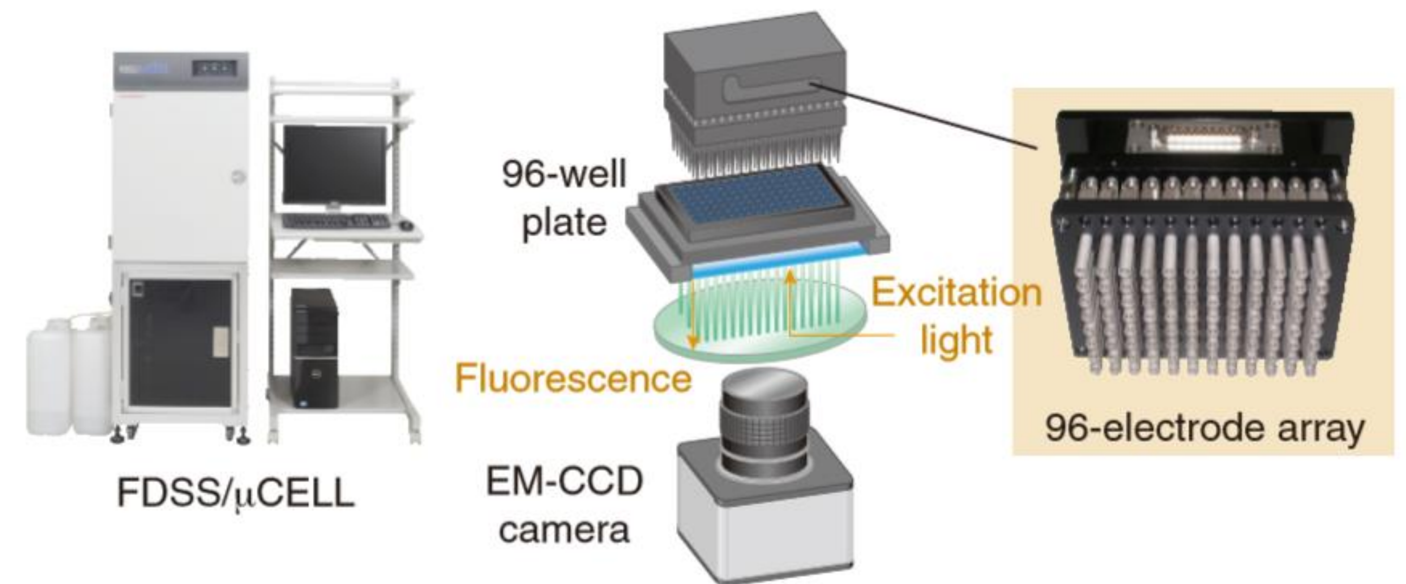
Natsuyo Aoyama[1], Kwi Hye Kim[1], Michael Hancock[1], Kile Mangan[1], Shouming Du[2], and Coby Carlson[1]
[1]Cellular Dynamics International, Inc., A FUJIFILM Company, Madison, WI USA; [2]Hamamatsu Corporation, Bridgewater, NJ USA

iPSC Technology



Human cell types differentiated from induced pluripotent stem cells (iPSCs) offer an attractive source of cellular material for both toxicity screening and drug discovery because of the biologically relevant systems they can represent *in vitro*. iPSC technology is a key element for modeling human diseases which are otherwise difficult to explore using conventional cell lines, primary cells, or animal models. Our approach is to develop biologically relevant assays with "normal" cells and then test disease-specific or patient-derived samples for comparison. These systems can then be used to screen for novel therapeutics or to advance existing drugs.

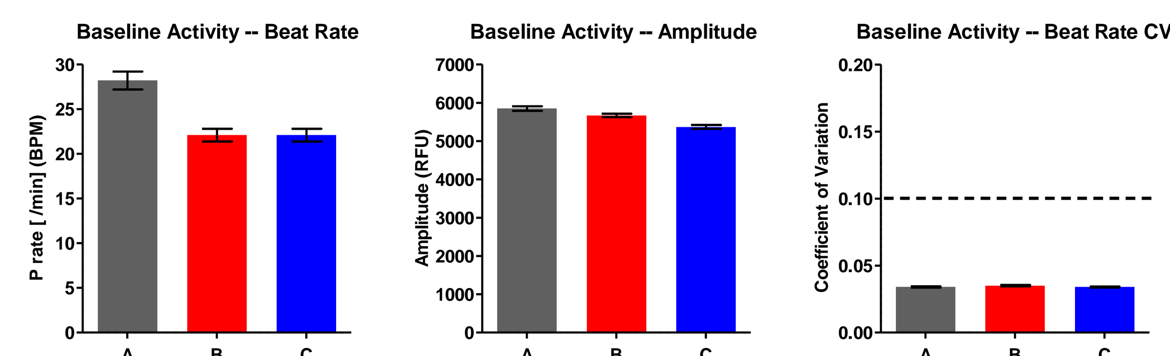
Hamamatsu FDSS/µCell for Calcium Assays

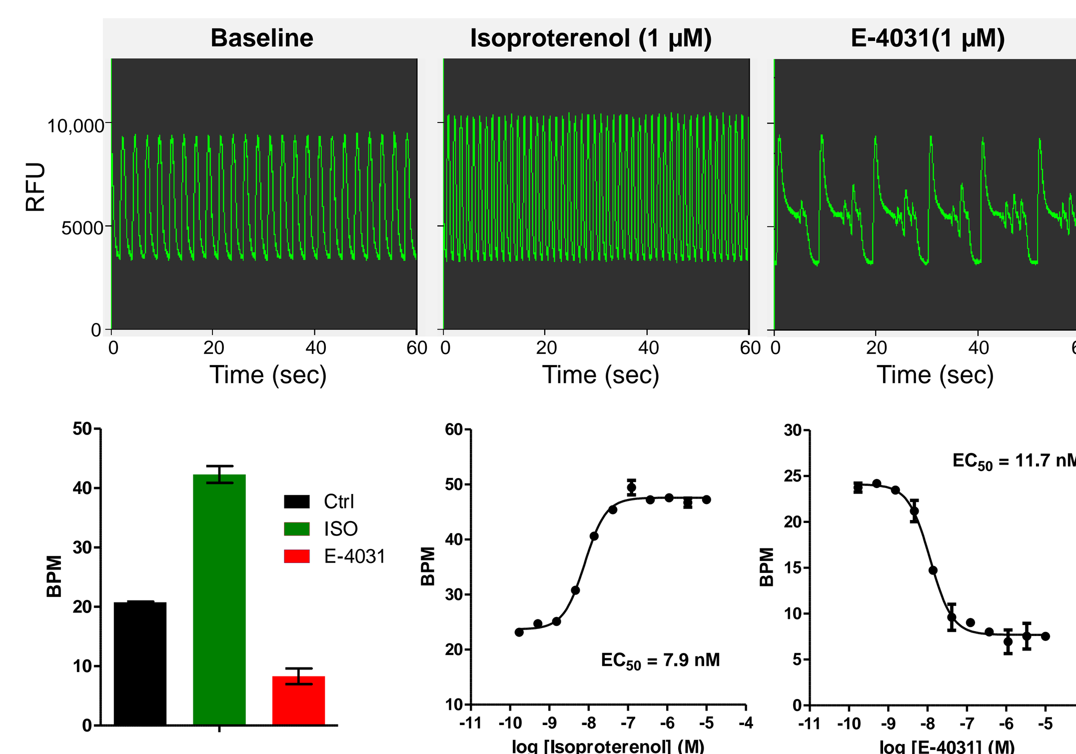


The FDSS/µCell is a kinetic plater reader with an integrated dispensing head and imaging-based detector. Whole plate dispensing into 96- or 384-well format enables uniform conditions for data acquisition and simultaneous detection of the kinetics of intensity changes for live cells. An added feature is the 96-electrode array for electrical field stimulation (EFS).

Assessment of Cardiac Beating Activity with iCell Cardiomyocytes^2

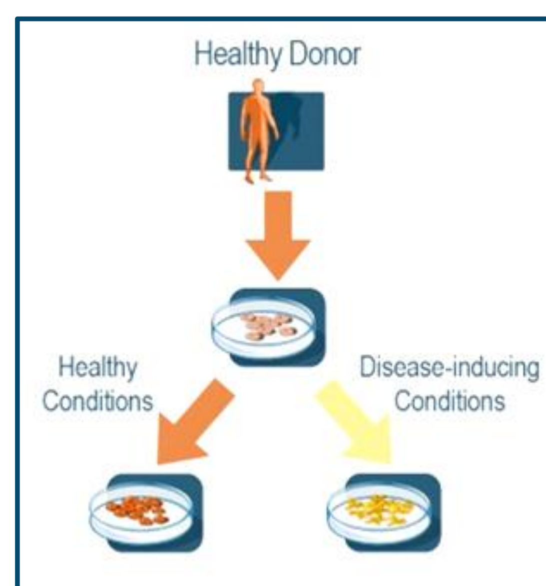
Robust assay performance. iCell Cardiomyocytes^2 display excellent consistency across lots when tested in 384-well format at DIV 7. Various calcium dyes are compatible in the assay, but all example data shown here is with EarlyTox (Molecular Devices). Baseline activity can be visualized (see calcium oscillations to the left) and analyzed for different parameters, including beat rate, amplitude, and variability. For each bar in the graphs below, n≥110 wells and data is presented as mean ± S.D.



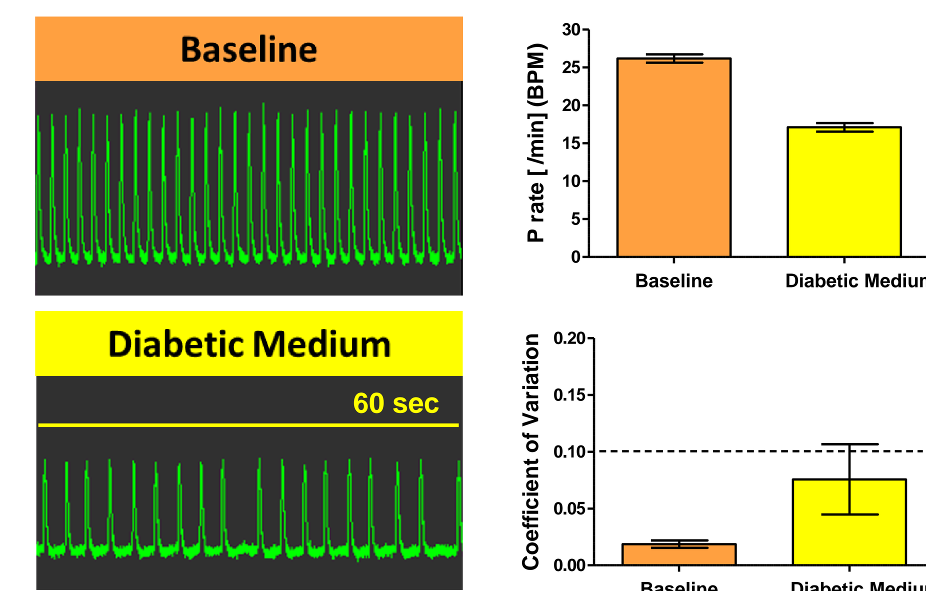


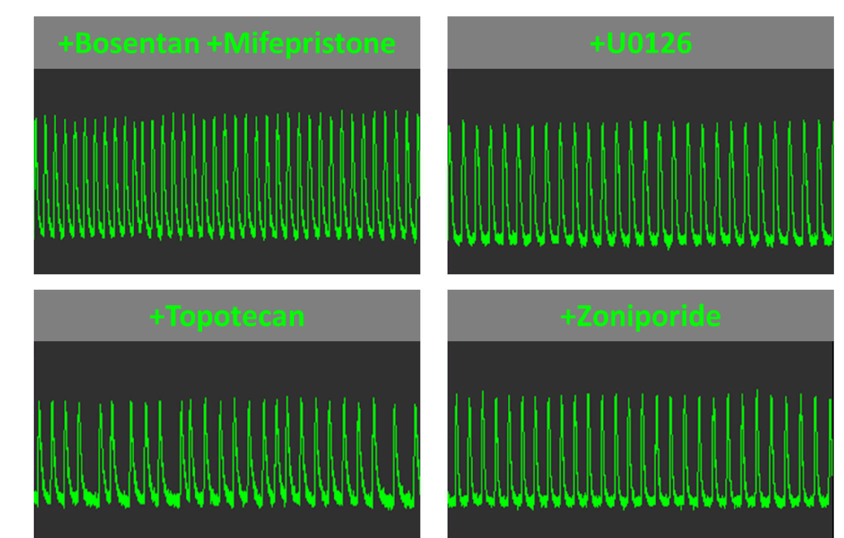
Expected pharmacological response. iCell Cardiomyocytes^2 were cultured in 384-well format according to the CDI Application Protocol for measuring calcium flux on the FDSS/µCell. On DIV 7, cells were treated with compounds, such as isoproterenol (ISO) or E-4031 to modulate cardiac activity. As depicted in the representative cardiac waveforms and analyzed data, cells responded to ISO by an increase in beats per minute (BPM) (approximately 2X) while E-4031 slowed down beating (by over 50%) and made it more irregular.

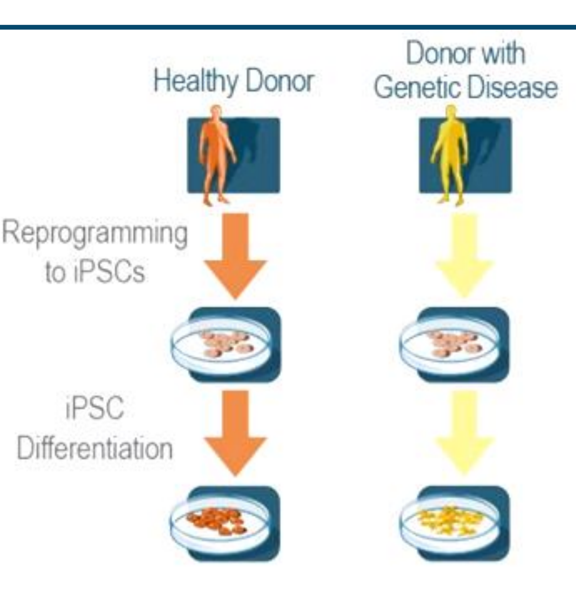
Disease Modeling with iPSC-derived Cardiomyocytes



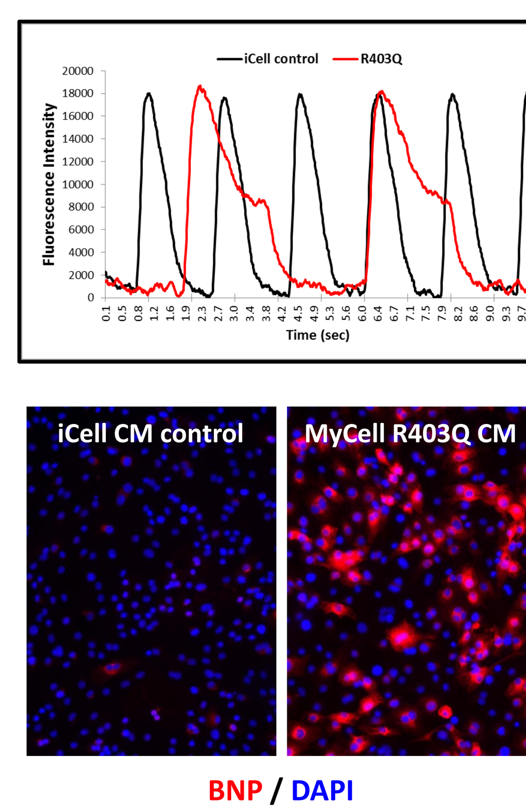
Induced disease modeling. Culturing iCell Cardiomyocytes in a diabetogenic extracellular environment has been shown to induce cardiac hypertrophy and cause a loss of sarcomeric integrity (Drawnel et al. Cell Reports 2014). The effect of such disease-mimicking conditions on cardiac calcium oscillations was demonstrated on the FDSS (see "Baseline" vs. "Diabetic Medium" data). This cellular model establishes an HTS-compatible assay system where compounds can be screened for the reversal of the disease phenotype back to the normal/healthy state. Testing of some of the published "hit compounds" (Topotecan, U0126, and Zoniporide) demonstrated recovery to near baseline conditions.

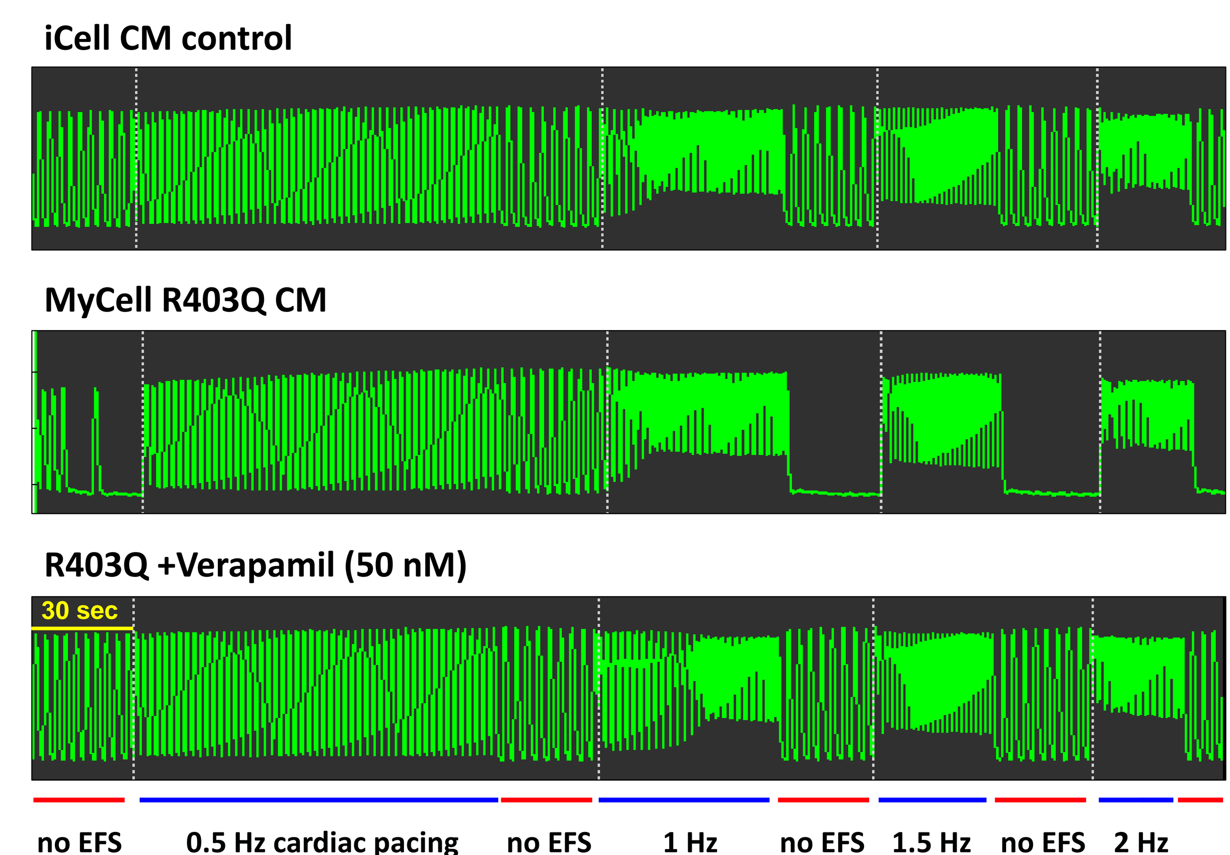




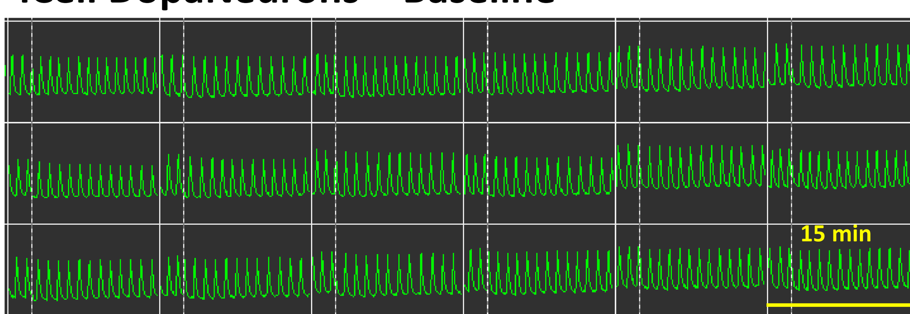


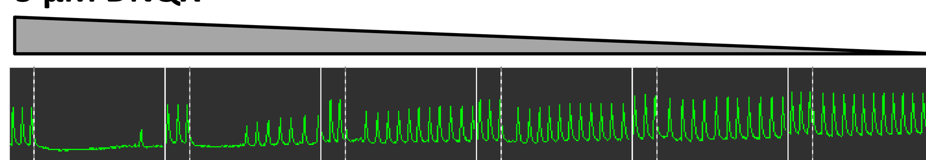
Innate disease modeling. The R403Q mutation in the β-myosin heavy chain (MYH7), is associated with a severe phenotype of hypertrophic cardiomyopathy (HCM). We generated patient-derived MyCell MYH7 R403Q cardiomyocytes with similar viability, purity, and plating efficiency to that for the "wild-type" iCell control cells. Additionally, we have previously demonstrated that these MyCell R403Q CMs express higher levels of BNP (by high content imaging) and display a slower and more irregular beat rate (by xCELLigence). Here, we demonstrate that a distinct phenotype of altered Ca^{2+} transient waveforms is observed. Moreover, when these cells are paced by EFS on the FDSS with increasing frequencies, they cannot recover from mechanical overload like the control CMs can. Culturing the R403Q CMs in the presence of verapamil, however, enables the mutant cell line to beat regularly after cardiac pacing. Together, these data illustrate another example of how an iPSC-derived disease model could be used for drug discovery.

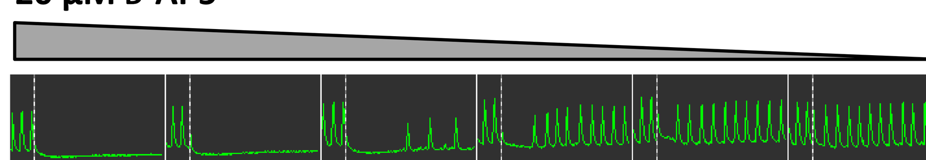




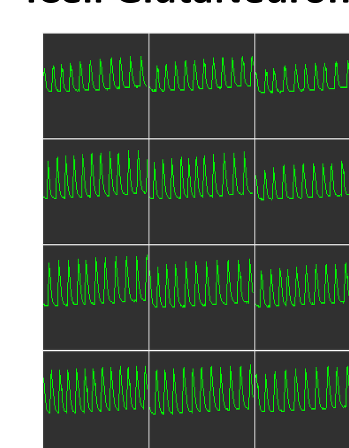
Spontaneous Ca^{2+} Oscillations in Neuronal Cells

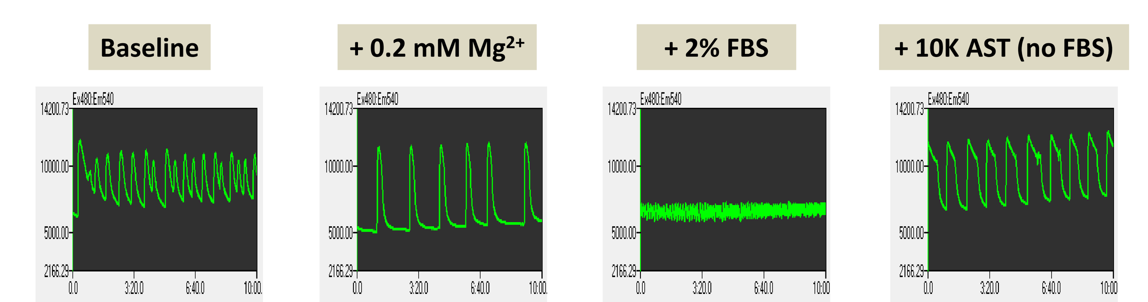






Consistent signal from well-to-well. iCell DopaNeurons are cultured in complete BrainPhys neuronal medium (STEMCELL Technologies) until DIV 14 and then assayed in Mg^{2+}-free buffer to develop spontaneous Ca^{2+} oscillations. The assay signal can be disrupted with antagonists of the AMPA and NMDA receptors (DNQX and AP5, respectively) suggesting that these "bursts" are synaptically-driven events that are being measured. Development of this functional endpoint nicely complements other assay technologies we utilize (e.g. MEA) to assess synchronous networks of iPSC-derived neuronal cultures.





It is not one size fits all. iCell GlutaNeurons can also be assayed in BrainPhys on the FDSS. However, we have done considerable assay development to identify what factors influence the assay signal. As shown above, assay media, FBS, and co-culture with astrocytes all affect the baseline phenotype.

Summary

- CDI is developing numerous iPSC-derived cell types, including cardiomyocytes, neurons, and skeletal myoblasts, that can be assayed for calcium handling on the FDSS/µCell
- Our approach is to develop biologically relevant assays with "normal" cells and then test disease-specific or patient-derived samples for comparison ("disease-in-a-dish")
- A spectrum of "validated" assays and "working" disease models available at CDI is presented
- For technical information or additional questions, please contact support@cellulardynamics.com