High Throughput Phenotyping of Electrically Stimulated Human iPSC-derived Cardiomyocytes and Neurons by Fluorescence Imaging

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Abstract
Measurement of electrical activities in excitable cells is typically performed by electrophysiological recording of individual cells. Attempts have been made to measure the activity of multiple cells simultaneously by imaging-based analysis in order to achieve high throughput recordings. However, it is hindered by the speed in image acquisition and the heterogeneous cell populations. By using the highly enriched motor neurons and cardiomyocytes that are differentiated from human induced pluripotent stem cells, we have demonstrated a high-throughput (HTP) phenotyping of the human cells by combining their electric field stimulation (EFS) and ultra-rapid (>100 data points per second) image acquisitions of entire 96/384-well plates. We found that the activities both neurons and cardiomyocytes and their response to EFS and chemicals are readily discerned by our fluorescent imaging-based HTP assay. Therefore, the HTP device enables physiological analysis of human iPSC-derived samples, highlighting its potential application in understanding disease mechanisms and discovering treatments.

Introduction
Techniques of measuring the action potential using electrodes was established more than 50 years ago. This approach requires significant technical investment to analyze action potential (AP) of biological samples changing so rapidly. In addition to techniques using substrate-integrated microelectrode arrays (MEAs), optically measuring AP is an alternative to techniques using electrodes and has been applied successfully to analyze electrophysiology of various sample types including whole hearts. The optical imaging has several advantages over traditional electrophysiological measurements: 1) simultaneous recordings of many samples, 2) electrodes causing cell damage, 3) measurement of multiple forms of activities, including AP firing and transient, synaptic activity, gap junction activities, and other subcellular signals. These advantages of the optical imaging assays are critical for HTP screening of compound libraries for drug discovery. Voltage signals in neurons last ~1-5 ms and is confined to the plasma membrane, leading to low signal-to-noise ratio (SNR). Optical signal for AP in CM also suffers from its low SNR. Depolarizing membrane potential leads to Ca^{2+} influx through voltage gated calcium channels, and calcium transients are significantly longer in duration (especially in neurons) than that of voltage fluctuations. Therefore, quantification of free Ca^{2+} changes can be used as a reliable proxy for neural and cardiac activity.

Assay System
Ultra Sensitive and Uniform Optical Detection

EFS, Electrical Field Stimulation

EFS parameters and software trigger.

Results

Regulation of neural function by different ion channel blockers and neurotransmitters. Calcium changes of motor neuron cultures after EFS in the presence of DMSO (absence of any compound), TX (tetrodotoxin), glatiramer acetate (GA), and (GABA A receptor agonist) and CGP37888 (selective GABA B antagonist).

Cardiac Safety

Calcium transient of Cardiomyocytes

Effects of dofetilide and E-4031 on cardiomyocytes under electrical stimulation

Summary
1. Robust signal to noise ratios of the Ca^{2+} transient measured by optical signal analyses in HTP format.
2. Good dynamic range of amplitudes and durations of Ca^{2+} transients is well suited for HTP analysis.
3. EFS enabled further improvement of reproducibility of cardiac Ca^{2+} transient profiles and neuronal stimulation without use of chemical stimuli.
4. Observation of Ca^{2+} transient before and after EFS stimulation also allows us to observe cardiac arrhythmia phenomena in vitro.
5. The HTP assay system can be used in development of precision medicine using patient-specific model system that mimic healthy and disease state of human cells and organs, i.e., disease model in micro-wells for physiological based compound screening.

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