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.

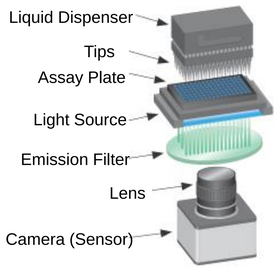
Introduction

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

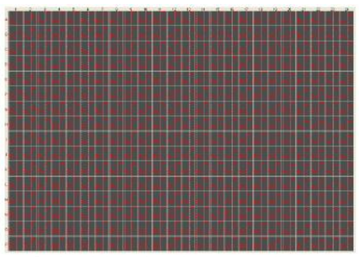
Ultra Sensitive and Uniform Optical Detection

FDSS µCELL



Optical Detection Mechanism

Multiplex High Throughput (HTP) Data Acquisition



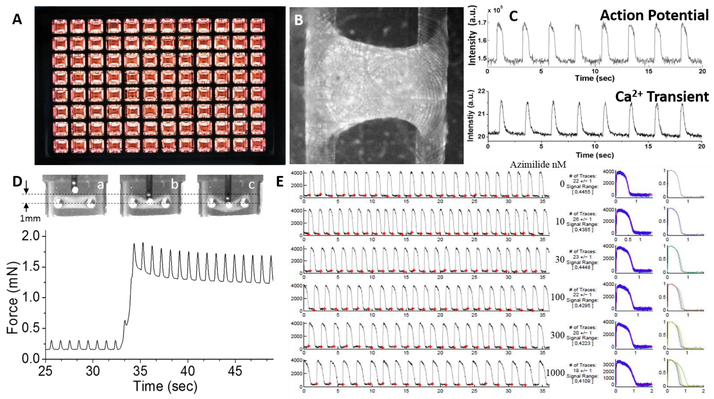
Ca^{2+} signal detection of HEK293 cells growing in 384-well plate in response to Carbachol (100nM)

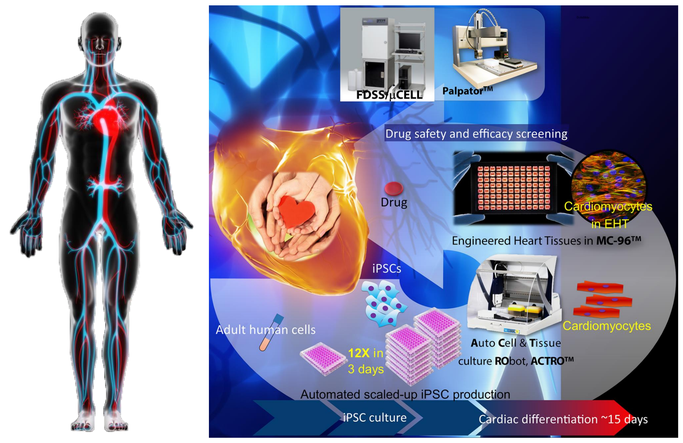
FDSS µCELL is allowing us to achieve:

- Parallel detection of optical signal from each well in microplates (96/384),
- High speed detection (>100 data points per second) of fluorescent signal change using a highly sensitive camera (sensor),
- Maintaining physiological sample temperature during measurements,
- Less than 1 min to acquire Ca^{2+} transients of cardiac myocytes growing in 96- or 384- well plates.

Drug Discovery Application

Engineered Heart Tissues with Human iPSC-derived Cardiomyocytes

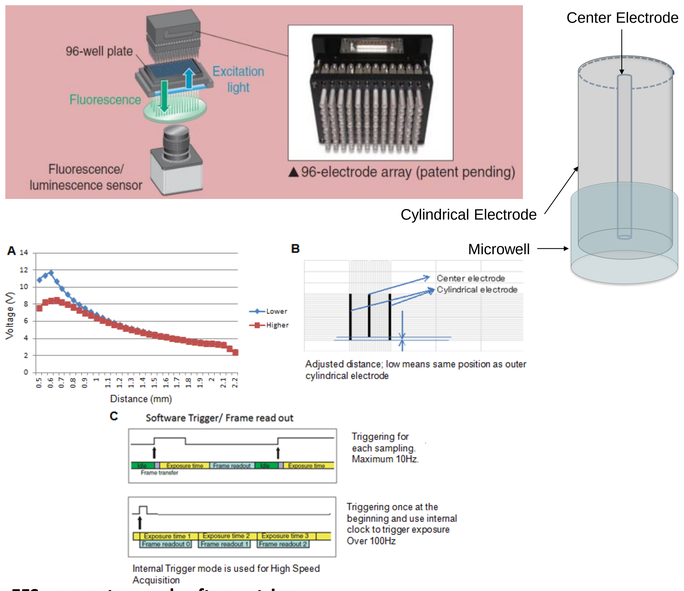




1. Analyzing excitation-contraction coupling of micro EHTs derived from human induced pluripotent stem cells (iPSCs) by measuring action potential, calcium transient, and force of contraction

2. Automated derivation of patient-specific EHTs and their high throughput analysis for precision medicine development

EFS, Electrical Field Stimulation



EFS parameters and software trigger.
EFS system allowed us to electrically stimulate cells growing in all the wells in 96-well plates simultaneously.

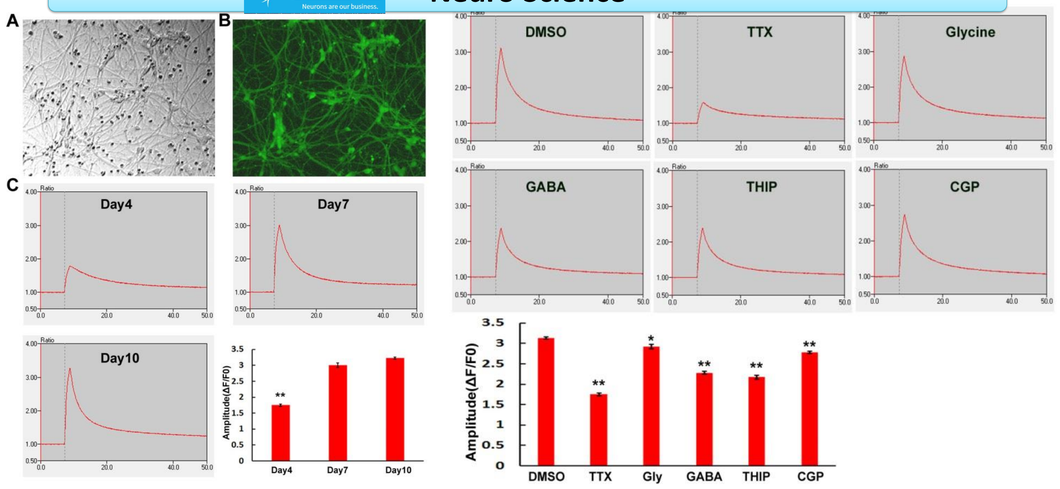
(A) Computational simulations of voltage fields between a center electrode and surrounding cylindrical electrode. X axis is the distance from the center, the center electrode has 1mm diameter. Y axis is the field voltage when applying 10V.

(B) Schematic diagram of center and cylindrical electrodes.

(C) Schematic for comparing data sampling rates between a software triggering each sampling (top) versus triggering once at beginning and use of internal clock to trigger exposure.

Results

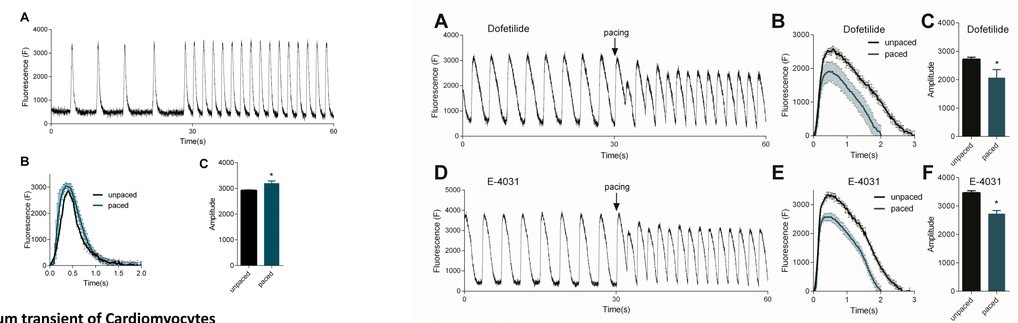
Neuro Science



Optimization of EFS for iPSC-derived motor neurons.
(A) Image of motor neurons showing mature neuronal morphology. (B) Motor neurons loaded with Cal-520 displaying green fluorescence in the cytoplasm, neurites and axons. (C) Calcium change of motor neurons in response to EFS at different time points.

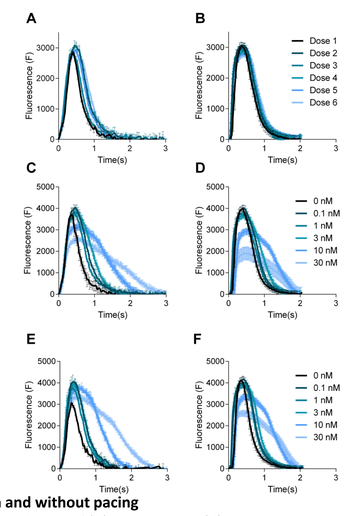
Regulation of neural function by different ion channel blockers and neurotransmitters.
Calcium changes of motor neuron cultures after EFS in the presence of DMSO (absence of any compound), TTX (sodium channel blocker), glycine, GABA, THIP (selective GABA A receptor agonist) and CGP64626 (selective GABA B antagonist).

Cardiac Safety

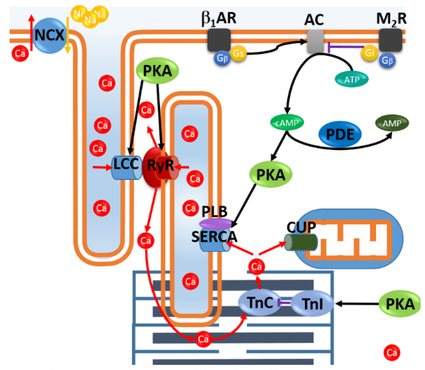


Calcium transient of Cardiomyocytes
(A) Cardiomyocytes unpaced from 0-30 sec and paced from 30-60 sec. (B) Average trace of unpaced and paced cardiomyocytes. (C) Amplitude changes in unpaced and paced cardiomyocytes (unpaced n=4; paced n=29; $p<0.05$).

Effects of dofetilide and E-4031 on cardiomyocytes under electrical stimulation
(A) Cardiomyocytes treated with dofetilide (10 nM) unpaced from 0-30 sec and paced from 30-60 sec at 0.5 Hz. (B) Average trace of unpaced and paced cardiomyocytes treated with dofetilide. (C) Amplitude decreases in paced cardiomyocytes treated with dofetilide (unpaced, n=6-8; paced, , n=27-29; $p<0.05$). (D) Cardiomyocytes treated with E-4031 unpaced from 0-30 sec and paced from 30-60 sec at 0.5 Hz. (E) Average trace of unpaced and paced cardiomyocytes treated with E-4031. (F) Amplitude decreases in paced cardiomyocytes treated with E-4031(unpaced, n=6-8; paced, , n=27-29; $p<0.05$).



With and without pacing
Average traces of (A) unpaced and (B) paced cardiomyocytes vehicle controls. Average traces of (C) unpaced and (D) paced dofetilide (0-30 nM) treated cardiomyocytes from. Average traces of (E) unpaced and (F) paced cardiomyocytes treated with E-4031 (0-30 nM).



Regulation of Excitation Contraction Coupling of Cardiomyocytes
β_1-adrenergic signaling increases intracellular calcium concentration and increase muscle contractility. β_1AR (adrenergic receptor), M_2R (muscarinic acetylcholine receptor), AC (adenylyl cyclase), PDE (phosphodiesterase), PKA (protein kinase A), SERCA (sarcoplasmic reticulum Ca ATPase), PLB (phospholamban), RyR (Ryanodine Receptor), LCC (L-type Ca cannel), NCX (Na-Ca exchanger), CUP (Ca uniporter), TnC (troponin C), TnI (troponin I).

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 physiology based compound screening.

Acknowledgement

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