Electric Field Stimulation (EFS) of human iPSC-derived neuron using Hamamatsu FDSS/μCELL

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Introduction
Hamamatsu developed a 96-channel electrode array system that is mounted on the FDSS/μCELL. It evokes electric field stimulations (EFS) to all 96 wells in a microplate simultaneously while fluorescence/luminescence signals are monitored. Using this instrumental setup, we electrically stimulated human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) to evoke a Ca²⁺ response, transient increase of intracellular Ca²⁺ concentration, which was measured with calcium-sensitive fluorescent dyes.

Materials & Methods
Intracellular Ca²⁺ measurements in neurons using FDSS/μCELL
The human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) were cultured in 96-well microplates (Coster). The cells were incubated with 5 μM of a calcium-sensitive fluorescent dye, Cal-520/AM (AAT Bioquest), with 2.5 mM probenecid (Sigma-Aldrich) and quencher for 1 h at 37 °C in 5% CO₂. The fluorescence images of all wells in a microplate were taken every 9.08 s to capture changes in intracellular Ca²⁺ concentration using FDSS/μCELL (Hamamatsu Photonics K.K.), a kinetic plate reader for cell-based fluorescent assays that acquires fluorescence/luminescent signals of all wells in a microplate simultaneously.

Electric stimulation of neurons using the electrode array mounted on the FDSS/μCELL : the EFS system
Our developed 96-channel electrode array is used with the FDSS/μCELL. The electric field pulses are given to all 96 wells in a microplate simultaneously by the electrode array that is positioned at the upper side over the microplate. The excitation light is introduced from the bottom side, and fluorescence signals are monitored with an EM-CCD camera.

Results ; Intracellular Ca²⁺ concentration changes by EFS in human iPSC-derived neurons
(1) Ca²⁺ response evoked by Electric Field Stimulations

![Image](image-url)

(2) The Ca²⁺ response is inhibited in the presence of a calcium channel blocker, Bepridil

![Image](image-url)

(3) Effects of GABA and an antagonist for non-NMDA receptor, NBQX

![Image](image-url)

Summary
Human iPSC-derived neurons (iCell® Neurons, Cellular Dynamics International) were electrically stimulated by Hamamatsu EFS (Electric Field Stimulation) system mounted on the FDSS/μCELL, while intracellular Ca²⁺ concentration changes were monitored with calcium-sensitive fluorescent dyes. Transient increase of intracellular Ca²⁺ concentration was observed upon EFS. We examined effects of a calcium channel blocker, an inhibitory neurotransmitter, and an antagonist for non-NMDA receptor on such Ca²⁺ response upon EFS.

The results show that the EFS system on FDSS/μCELL is able to evoke the Ca²⁺ response in human iPSC-derived neurons, which would be useful phenomena for characterization of these cells and also in disease-model studies and drug discovery with human iPSC-derived neuronal cells.

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