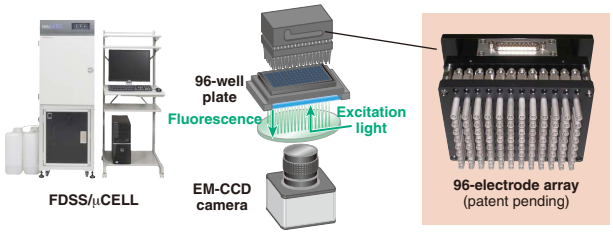
Measurements of Ca^{2+} response in human iPSC-derived peripheral neurons by electric field stimulation in FDSS/μCELL

Sunao Hisada, Natsumi Kato, Masanobu Matsubara, Takuji Kataoka (Hamamatsu Photonics K.K. Systems Division, Hamamatsu City, Shizuoka Pref, Japan)
Ralf Kettenhofen and Felix von Haniel (Axiogenesis AG, Nattermannallee 1, 50829 Cologne, Germany)

Introduction

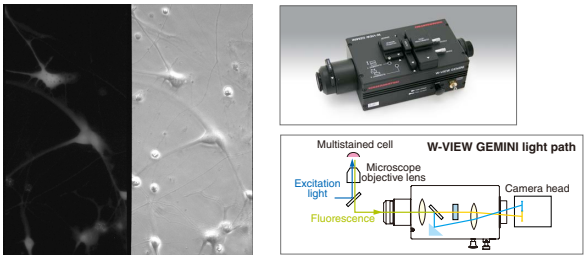
Development of iPSC technology has shown its great potential in disease modeling and drug discovery, as well as in regenerative medicine, such as use of human cardiomyocytes and neural cells for high-throughput screening assays. We recently developed a 96-channel electrode array that is mounted on the FDSS/μCELL, a high-throughput-amenable kinetics microplate reader for cell-based fluorescent and luminescent assays. It adds electric field stimulation (EFS) to all 96 wells in a microplate simultaneously while fluorescent and luminescent signals are monitored. In this study, we observed changes in intracellular Ca^{2+} concentrations in human iPSC-derived peripheral neurons by adding pulsed electric field stimulations. We also monitored effect of the presence of a Ca^{2+} channel blocker on such EFS-triggered Ca^{2+} responses in the peripheral neurons.

Electric Field Stimulation for 96-well microplate: the EFS system

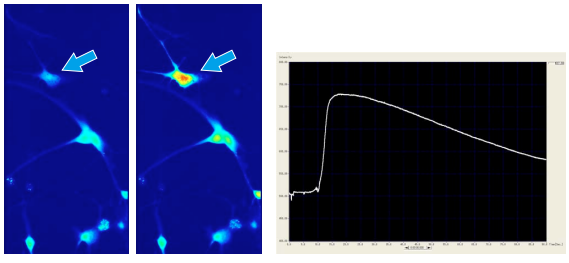


Our developed 96-channel electrode array is used coupled with the FDSS/μCELL. The electric field stimulations are given to all 96 wells in a microplate simultaneously by electrode array that is positioned at the upper side over the microplate. The excitation light is introduced from the bottom side, and fluorescence signals from cells are monitored with an EM-CCD camera.

Microscope observation of human iPSC-derived peripheral neurons



First, we observed human iPSC-derived peripheral neurons (Axiogenesis AG) under the microscope. The above bright-field (right) and fluorescent (Ca^{2+} sensitive dye, left) images were obtained simultaneously using W-VIEW GEMINI (Hamamatsu, A12801-01), an image splitting optics that provides one pair of dual wavelength images onto a single microscope camera.



Next, the peripheral neurons were stimulated by Bradykinin. The increase of intracellular Ca^{2+} concentration was observed (arrow).

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* The FDSS/μCELL EFS system should not be used for optically detecting/monitoring change in transmembrane potential of the cells.

* The FDSS/μCELL EFS system should not be used on any cell or cells in which the user or anyone else has expressed target ion channels.

Materials and Methods

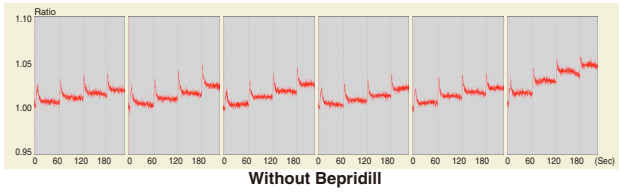
Human iPSC-derived peripheral neurons (Peri.4U™, Axiogenesis AG) were grown in the DMEM high glucose medium (Life Technologies) with supplements in the black bottom clear standard 96-well plates (Corning Coster). 2 mM (final concentration) Ca^{2+} sensitive dye (AAT Bioquest) was added to the wells and incubated for 45min at 37°C in 5% CO_2 atmosphere.

FDSS/μCELL is a kinetic microplate reader with an integrated dispending head and an imaging-based detector. It can do the simultaneous dispensing of reagents into all 384/96 wells in a microplate and the simultaneous kinetics detection of fluorescent or luminescent signals in all wells. The fluorescent images of all wells in the microplate were taken every 0.05 seconds at 37 °C to capture changes in intracellular Ca^{2+} concentration. Peak amplitude and Peak width in the calcium waves were analyzed using FDSS wave analysis software. The pulsed electric field stimulations were given under the conditions described below.

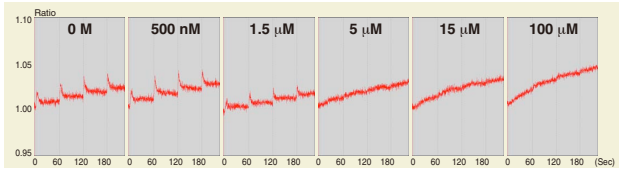
Bradykinin and Bepridill (trade name Vascor) were purchased from Sigma-Aldrich.

Voltage	Pulse width	Number	Frequency
20 V	1 ms	50 times	10/20/30/40 Hz

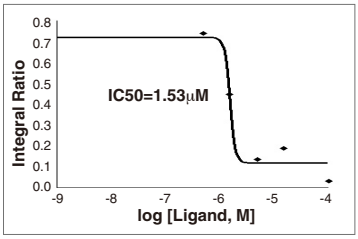
Intracellular Ca^{2+} concentration changes in human iPSC-derived peripheral neurons by adding electric field stimulation (EFS)



Without Bepridill



With Bepridill



Human iPSC-derived peripheral neurons were stimulated with EFS in the absence or presence of Bepridill, a calcium channel blocker. First, we stimulated neurons in the absence of Bedridill (upper panel) in all wells. Then, Bedpiridill was added at each concentration described in each well (middle panel), incubated for 10 min, and the cells were stimulated again with EFS. In the presence of more than 5 μM Bedpiridill, the Ca^{2+} response was not observed.

Conclusions

FDSS/μCELL is a high-throughput screening (HTS)-amenable kinetics microplate reader for cell-based fluorescent and luminescent assays with high speed (over 100 Hz) signal acquisition. Recently, we developed a 96-well electrode array that is used coupled with the FDSS/μCELL, which adds electric field stimulation (EFS) to all 96 wells in a microplate simultaneously.

Using this instrumental system, we measured changes in intracellular Ca^{2+} concentration with calcium-sensitive fluorescent dyes in human iPSC-derived peripheral neurons (Axiogenesis). By adding electric field stimulation, a transient increase of intracellular Ca^{2+} concentration occurred. We also observed effect of a calcium channel blocker, Bedpiridill, on the Ca^{2+} response stimulated by pulsed electric field.

Such EFS function coupled with cell-based fluorescent/luminescent assays, in the high-throughput manner, would be useful in studies with neural-disease-phenotypic cells and in future drug discovery and development.

Reference

Andreas Ehlich *et al.*, Development of ready-to-use human iPSC-derived dopaminergic neurons and peripheral neurons for pre-clinical safety studies.
13th Annual World Pharma Congress held in Boston MA, USA-May,2014