In vitro electrophysiological drug testing using human induced pluripotent stem cell-derived neurons

I. Suzuki\(^1\), A. Odawara\(^1,2\), Y. Shi\(^3\), H. Jiko\(^4\)

\(^1\) Department of Electronics and Intelligent Systems, Tohoku Institute of Technology, Miyagi, Japan,
\(^2\) Japan Society for the Promotion of Science, Tokyo, Japan
\(^3\) Axol Bioscience Inc., Cambridge, United Kingdom
\(^4\) Alpha MED Scientific Inc., Osaka, Japan

**Introduction**

Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for drug discovery and cell-based therapy. We here used a multi-electrode array (MEA) system to investigate the functional characteristics of hiPSC-derived neurons on their long-term spontaneous activity and drug responsiveness over 300 days culture. We demonstrated that hiPSC-derived neurons allowed the culture to be maintained over 10 months with long-term spontaneous activity. After 70 days of culture, we observed synchronous burst firing activity due to synapse transmission within neuronal networks. Addition of the synapse agonist and antagonists kainic acid, bicuculline, CNQX and AP5 induced significant changes of the firing rate in spontaneous firings and electrical evoked responses. Furthermore, we demonstrated that epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and was inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA). High frequency synchronized bursts were evoked over PTZ 100 \(\mu\)M. These bursts were gradually decreased with the increasing dose of anti-epilepsy drug, and disappeared over phenytoin 100\(\mu\)M or VPA 1 mM respectively. These results suggested that long-term electrophysiological measurements in hiPSC-derived neurons using a MEA system may be beneficial for drug screening applications.

**Material & Methods**

(A) Long-term electrophysiological measurement

300 days on the MEA dish

- Multi-electrode array chip 294 days culture on the MEA dish

- \(\beta\)-tubulin III

- Hoechst 33258 50 \(\mu\)m

- Synaptophyslin 50 \(\mu\)m

(B) Human iPSC-derived neurons

Maturation hiPSC-derived neurons after 300 days culture

- Alpha Mad Scientific Inc

- \(\beta\)-tubulin III

- Hoechst 33258 50 \(\mu\)m

- Synaptophyslin 50 \(\mu\)m

- Materials & methods (A) Long-term electrophysiological measurement

- Materials & methods (B) Human iPSC-derived neurons

\(\beta\)-tubulin III

**Result 1 Time course of spontaneous firings**

![Figure 1. Time course of spontaneous firing over 200 days culture, in different culture conditions. (A) The waveforms and lastor plot at 64 electrode. (B) Number of total spontaneous spikes detected vs. the time course. (C) Grids showing the 64 electrodes where colorcoded electrodes detected signals. Electrodess that detected a higher firing frequency are shown in red.](image1)

- Synchronized burst firings were observed over 70 days culture.
- Firing rate and synchronized burst firings increased up to about 200 days

**Result 2 Drug effect in spontaneous firings**

![Figure 2. Drug effect in spontaneous firings over 200 days culture.](image2)

- Bicuculline increased in duration of synchronized burst firings. Kainic acid increased the number of synchronized burst firings. After CNQX and AP5 administration, burst firings disappeared.
- Functional ion channel response at 240 DIV was better than the responses at 100 DIV
- Bicuculline induced high frequency firings. Firing frequency was different by type of synaptics's drug.

**Result 3 Drug effect in evoked responses**

![Figure 3. Effects of drugs in electrical evoked response. (A) The waveforms represent typical changes in the evoked responses after drug administration at 240 culture days in vitro (DIV). (B) Peri-stimulus time histogram (PSTH) of 120 trace data at an electrode. (C) Percentage of duration in a burst and number of spikes in a burst before and after drug administration at 100 DIV and 240 DIV (*\(P < 0.001\)).](image3)

- Effects of synaptic’s drug were detected in evoked responses by electrical stimulation.
- Duration and number of spikes in a burst were different by the type of synaptic’s drug.
- Change of evoked responses were significant compared with evoked responses at 100 DIV.
- AP5 significantly decreased the duration of burst. This results suggest functioning NMDA receptors.

**Result 4 Epilepsy phenomenon and drug effects**

![Figure 4. Evoked epilepsy phenomenon and the effects of anti-epilepsy drugs. (A) Waveform plots in PTZ and VPA administration. (B) Lastor plot in PTZ and Phenyoxin administration. (C) Changes in firing rate and burst count](image4)

- Epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and was inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA).

**Conclusion**

- The hiPSC-derived neurons allowed the long time culture for more than 300 days and the functional maturation using co-culture with astrocyte.
- We have succeeded in the detection of the long-term electrophysiological features and of synaptic’s drugs effects in cultured hiPSC-derived neurons.
- hIPS-derived neurons at 240 days culture were more functional compared with 100 days culture.
- Evoked responses by electrical stimulation are useful to evaluate the effects of drugs.
- Detection of epilepsy phenomenon and effects of anti-epilepsy drug suggest human epilepsy model for drug screening.

**Long-term electrophysiological measurement using multi-electrode arrays enabled drug screening and toxicological assay.**

**Reference**

3. Contact information: i.suzuki@tohitech.ac.jp