Human iPS cell-derived neurons and their applications for disease model research and development

Brad Hamilton[1], Harumi Kogami[2], Yuichi Okuda[2], Kaishu Shiina[3], Ryu Yamanaka[3], Yutaka Shindo[3], Zachary Yu-Ching Lin[2], Kotaro Oka[3], and Mitsuru Inamura[2]

[1]Stemgent Inc., 51 Moulton Street, Cambridge, MA 02139 USA
[2]ReproCELL Inc., Japan, KDX Shin-Yokohama 381 Bldg. 9F. 3-8-11,Shin-Yokohama, Kohoku-ku, Yokohama, Kanagawa 222-0033, Japan
[3]Department of Biosciences & Informatics, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

REPROCELL
INTEGRATED TOOLS FOR TRANSLATIONAL RESEARCH

Introduction

Human induced pluripotent stem (iPS) cells can proliferate infinitely and differentiate into most cell types in the human body. In addition, human iPS cells are a native cellular source similar to primary cell cultures that can be scaled to support large-scale screening applications. As human iPS cells are able to differentiate into many cell types, these features are attractive for assays to evaluate potential therapeutics and elucidating pathological conditions for therapeutic purposes.

ReproCELL has developed a comprehensive workflow that includes patient specific primary somatic cell isolation, cellular reprogramming, and genetic modification. To evaluate this workflow, we have used iPS cells for directed differentiation to the neural lineage. By regulating the differentiation conditions for these neurons, the proportion of neuronal subtypes can be controlled, and the resulting neurons can be analyzed functionally and phenotypically with MEA assays, ICC, and activity assays.

These derived neurons may also be created bearing Alzheimer's or Parkinson's disease-specific mutations, either by genetic modification, or by using iPS cells reprogrammed from disease patient cells. This comprehensive workflow capability enables us to generate customized disease models that target specific neurological disease requirements.

Materials and Methods

ReproNeuro™ Kit (ReproCELL, Cat. No. RCESD008)
ReproNeuro MQ™ Kit (ReproCELL, Cat. No. RCESDN301MQ)
ReproNeuro DA plus™ Kit (ReproCELL)

Immunocytochemistry

Equipment: CellVoyager™ CV100 (Yokogawa Electric Corporation)

Primary antibody

- Anti-βIII-tubulin (Covance, Cat. Nos. MMS-435P, PRB-435P)
- Anti-Tyrosine hydroxylase (TH) (Abcam, Cat. No. ab75875)
- Anti-Choline acetyltransferase (ChAT) (Millipore, Cat. No. AB144)
- Anti-VGLUT1 (Sigma, Cat. No. V0389)
- Anti-GABA (Sigma, Cat. No. A2052)

Secondary antibody

- Alexa Fluor® 488, (Life Technologies, Cat. Nos. A11034, A10036)
- Alexa Fluor® 546, (Life Technologies, Cat. Nos. A11030, A11055)

MEA Assay

Equipment: MED64-Basic (Alpha MED Scientific Inc.)

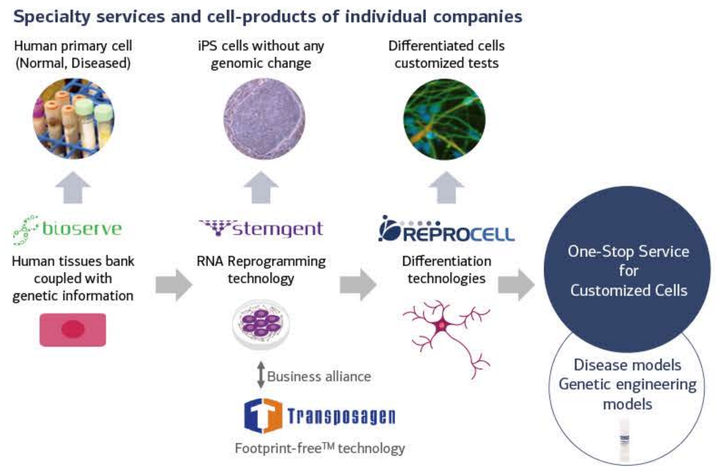
Reagent:

- D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5) (Abcam, Cat. No. ab120003)
- 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (WAKO, Cat. No. 03223121)
- Tetrodotoxin (TTX) (Abcam, Cat. No. ab120055)

MPP+ Assay

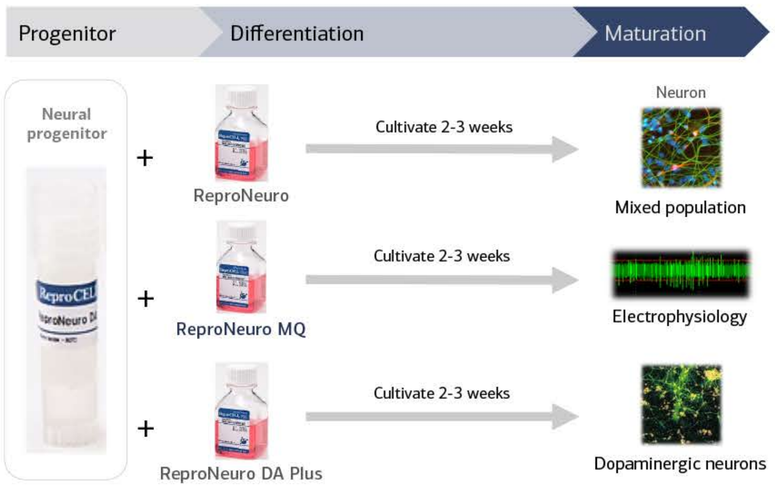
- 1-methyl-4-phenylpyridinium (MPP+ iodide), (Sigma-Aldrich, Cat. No. D048)
- Mag-Fura-2 (Life Technologies, Cat. No. M1292)
- Tetramethylrhodamine (TMRE) (Invitrogen, Cat. No. T-669)
- KMG-301 (ref., Shindo *et al.*, Plos One 6(8):e23684 (2011))

Concept for Human iPS Cell-Derived Neurons



Disease model cell systems can be created by leveraging the technologies of multiple organizations to further translational research. Neurons provide a good example of this system.

Types of human iPS cell-derived neurons used in this study



By varying the final differentiation conditions, a common neural precursor can be differentiated into neurons with different properties. These differentiated neurons are ideal for specific applications.

Characterization of ReproNeuro and ReproNeuro MQ

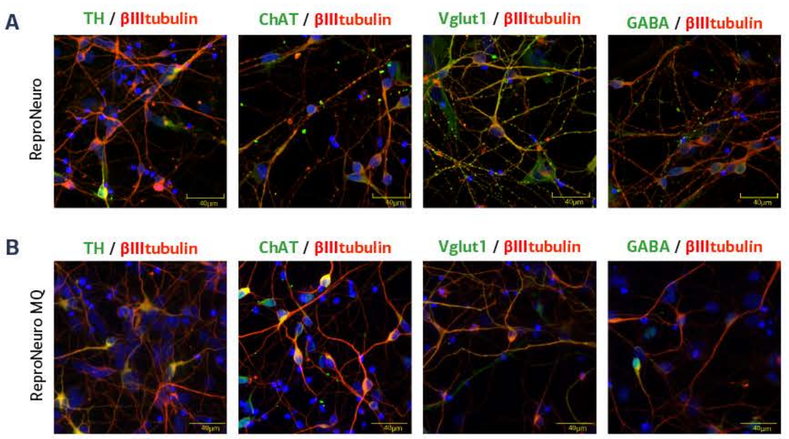


FIGURE 1: ReproNeuro MQ and ReproNeuro contain mixed populations of neural types.
FIGURE 1A: ReproNeuro was stained with neural markers at day 14.
FIGURE 1B: ReproNeuro MQ was stained with neural markers at day 14. Blue: DAPI
TH: Dopaminergic neuron marker, ChAT: Cholinergic neuron marker, Vglut1: Glutamatergic neuron marker, GABA: GABAregic neuron marker, βIIItubulin: neurite formation.

Characterization of ReproNeuro DA plus

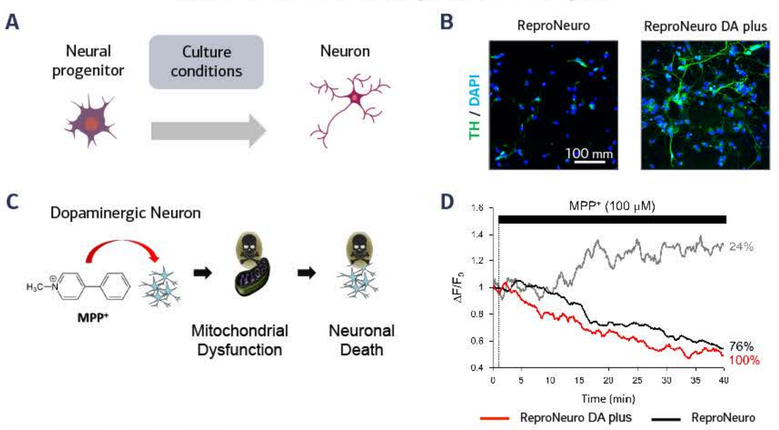


FIGURE 2: ReproNeuro DA plus contains a higher fraction of dopaminergic neurons and has a stable Mg^{2+} response to MPP+ stimulus
FIGURE 2A: Differentiation scheme of ReproNeuro DA plus.
FIGURE 2B: ReproNeuro DA plus and ReproNeuro were stained for TH and DAPI at Day 14.
FIGURE 2C: Assay scheme of dopaminergic neuronal death by MPP+ stimulus. MPP+ induces mitochondrial depolarization and Mg^{2+} outflow from mitochondria into cytoplasm of TH positive neurons. Intramitochondrial Mg^{2+} modulates the fluorescence of KMG310.
FIGURE 2D: KMG301 fluorescence change due to intramitochondrial Mg^{2+} concentration changes in response to 100 mM MPP+ in TH positive neurons. The percentage of each cell type which response to the MPP+ shown on the right.

Electrophysiology of ReproNeuro MQ

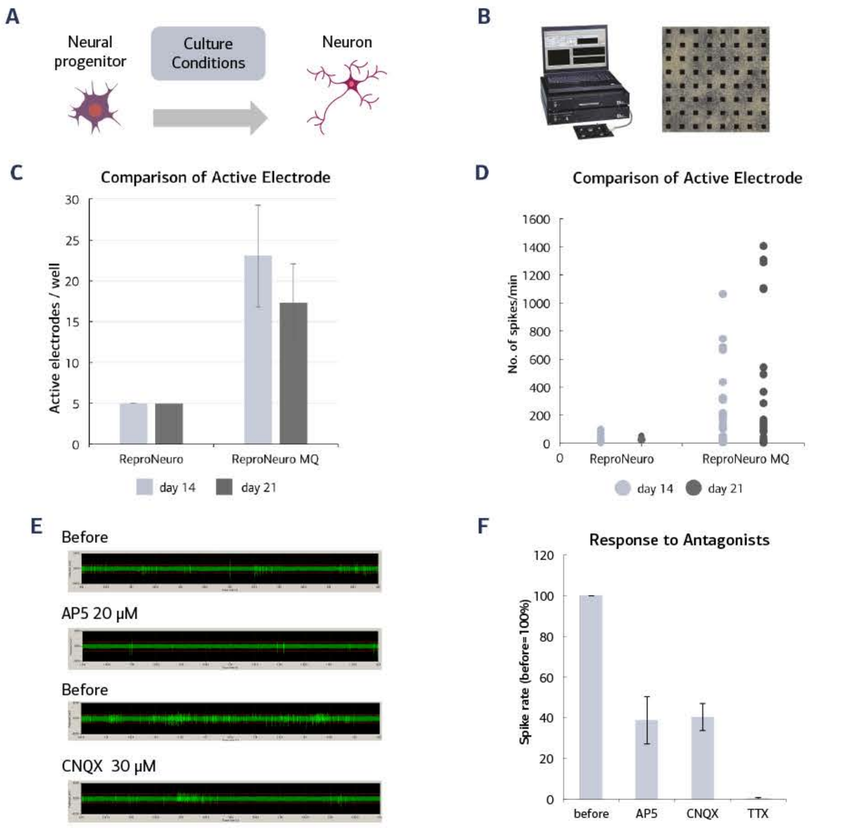
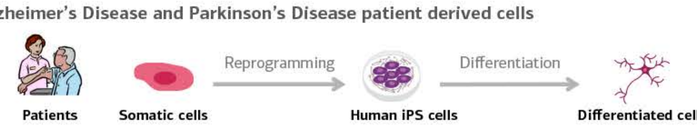


FIGURE 3: ReproNeuro MQ has more spontaneous activity in an MEA assay than ReproNeuro and responds to NMDA and non-NMDA receptor antagonists.
FIGURE 3A: Differentiation scheme of ReproNeuro MQ.
FIGURE 3B: MEA (Multiple Electrode Array) assay system. Human iPS-derived neurons (1×10^5 cells) were seeded directly on the assay area of MEA probes and cultured 21 days.
FIGURE 3C: Number of active electrodes per minute in MEA assay. An electrode was considered active if at least 5 spikes/min were detected.
FIGURE 3D: For a more detailed analysis, the number of spikes per minute was analyzed for each electrode. The data were collected during the second 5 minutes of a 10 minute incubation. Each dot represents a single electrode.
FIGURE 3E: The change of spike pattern after administration of NMDA (AP5) or non-NMDA (CNQX) receptor antagonists.
FIGURE 3F: Spike rate was measured after administration of the NMDA receptor antagonist AP5, the AMPA receptor antagonist CNQX, or the sodium channel blocker TTX. TTX blocks all ion channel-specific spiking.

Ongoing Research

Alzheimer's Disease and Parkinson's Disease patient derived cells



Alzheimer's disease donor information

NO.	AVAILABLE GENE MUTATION ANALYSIS					GENDER	AGE AT BIOPSY
	PS1	PS2	APP	MAPT	APOE		
Alzheimer 1	–	R62H	–	–	–	M	94
Alzheimer 2	–	–	–	–	–	M	82

Disease model cell systems can be created by leveraging the technologies of multiple organizations to further translational research. Neurons provide a good example of this system.

Conclusion

By regulating the differentiation conditions, iPS cell-derived neuron subpopulation composition and function can be controlled for different product characteristics.

Compared to the current ReproNeuro neurons:

- ReproNeuro DA plus neurons contain a higher proportion of dopaminergic neurons with a more robust MPP+ response.
- ReproNeuro MQ neurons have more spontaneous MEA activity and show higher-frequency spikes and greater sensitivity to antagonists.

REPROCELL GROUP COMPANIES
bioserve stemgent reinnervate