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Application of Rapid iPSC Differentiation to Neurotoxicity Assays

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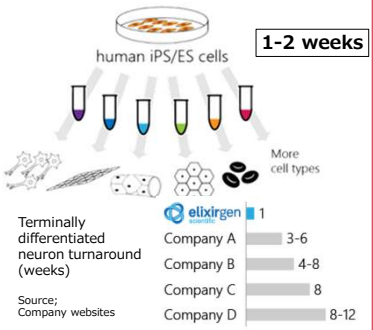
Introduction

The Quick-Tissue™ technology is a transcription factor-based method for rapid differentiation of stem cells (iPSCs or ESCs) into desired cell types. The method generates pure populations of differentiated cells within 1-2 weeks.

To investigate the suitability of Quick-Tissue™ neurons to toxicological assays, we analyzed their pharmacological characteristics using Ca^{2+} imaging.

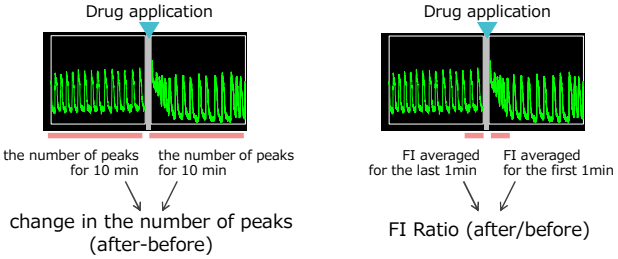


Materials and Methods

Cells: Excitatory neurons were generated using the Quick-Tissue™ technology from iPSCs originating from a healthy donor (Elixirgen Scientific, Inc. #EX-SeV-CW50065). Primary human astrocytes were obtained from Thermo Fisher Scientific Inc.

Spheroid culture: 96-well plates were seeded with the neurons and pre-cultured astrocytes at the density of 8,000 cells/well. Plates were cultured in the BrainPhys medium (STEMCELL technologies Inc.) for 6 weeks.

Ca^{2+} imaging: Spheroids were loaded with a fluorescent Ca^{2+} reporter dye (MOLECULAR DEVICES, LLC.). Fluorescence intensity (FI) was measured with the FDSS6000 system (Hamamatsu Photonics K.K.). Drug responses were analyzed by determining the change in the number of peaks of Ca^{2+} oscillation and the ratio of FI before and after the drug application. Statistical significance of the comparison to 0.1 % DMSO was determined by the Wilcoxon rank sum test.



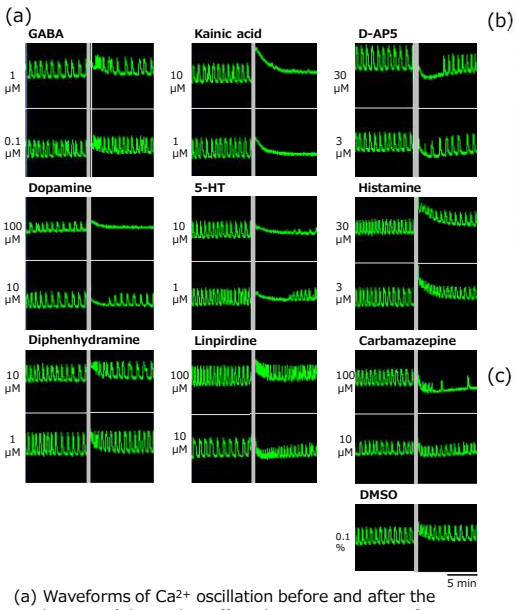
Analysis method

Summary of compounds

Compound	Function	Conc. (μM)
GABA	GABA agonist	0.1, 1
Bicuculline[1]	GABA(A) antagonist	3, 30
Gabazine[1]		10, 100
Pentylentetrazole[2]		100, 1000
Picrotoxin[1]		1, 10
Glutamate	AMPA/NMDA agonist	10, 100
Kainic Acid	Kainate agonist	1, 10
NMDA	NMDA agonist	10, 100
CNQX	AMPA/Kainate antagonist	10, 100
D-AP5	NMDA antagonist	3, 30
Dopamine	Dopamine agonist	10, 100
Chlorpromazine	Dopamine D2 antagonist	10, 100
Haloperidol		1, 10
5-HT	Serotonin agonist	1, 10
Amoxapine	Serotonin H1/2 agonist	3, 30
Buspirone	Mono amine reuptake blocker	3, 30
Bethanechol	Muscarine agonist	1, 10
Pilocarpine[2]		1, 10
Atropine	Muscarine antagonist	1, 10
Strychnine[1]	Nicotine/Gly antagonist	3, 30
Histamine	Histamine agonist	3, 30
Ketotifen[1]	Histamine H1 antagonist	1, 10
Diphenhydramine[1]		1, 10
4-Aminopiridine	Potassium channel blocker	3, 30
Linopirdine		10, 100
Carbamazepine[3]	Sodium channel blocker	10, 100
Phenytoin[3]		10, 100
Valproic acid[3]		100, 1000
Enoxacin[2]	Antibacterial agent	1, 10

1; Convulsant, 2; Epileptogenic, 3; Antiepileptic

Results Ca^{2+} response to major pharmacological compounds



(a) Waveforms of Ca^{2+} oscillation before and after the application of drugs that affect the 6 major types of receptors. (b) Change in the number Ca^{2+} oscillation peaks. The peaks were counted during 10 min before and after the drug application (n=6). (c) Ratio of the fluorescence intensity (FI) measured during 1 min before and after the drug application (n=6).

Summary of drug response

Receptors	Drugs		Response	
GABA	Agonist	all	not determined	
	Antagonist	GABAA	×	
Glutamate	Agonist	AMPA/NMDA	○	Excitatory
		Kainate	○*	Excitatory
		NMDA	○	Excitatory
	Antagonist	AMPA/Kainate	○*	
		NMDA	○	
Dopamine	Agonist	all	○*	Inhibitory
	Antagonist	D2	×	
Serotonin	Agonist	all	○*	Inhibitory
	Reuptake blocker		○	Excitatory
Cholin	Agonist	Muscarine	○	Inhibitory
	Antagonist	Muscarine	○	
		Nicotine/Gly	○	
Histamine	Agonist	all	○*	Excitatory
	Antagonist	H1	○*	
Ion channel	blocker	Potassium	○*	
		Sodium	○*	

The concentration of GABA in this test was lower than IC50 value (2.8 μM) and the data was removed from the analysis.

Functional receptors
Confirmed by the responses to the agonists; Glutamate, Dopamine, Serotonin, Cholin, and Histamine

Functional ion channels
Confirmed by the responses to the ion channel blockers; K^+ and Na^+

Functional Synapses
Confirmed by the responses to the antagonists; Glutamate, Serotonin, Cholin, and Histamine

*: p<0.05

Summary and Conclusions

- Pharmacological analyses of Quick-Tissue™ neurons suggest the presence of functional receptors for all the 6 major neurotransmitters and functional channels for two cations. The results also suggest synaptic connections for Glutamate, Serotonin, Cholin, and Histamine. The neurons also responded to convulsant, epileptogenic, and antiepileptic drugs.
- The fluorescent intensity (FI) and the number for Ca^{2+} oscillation peaks responded to drugs and thus likely constitute reliable drug response markers.
- These results suggest Quick-Tissue™ neurons are suited to neurotoxicity assays and drug screenings.

COI Disclosure Information
We have the following financial relationships to disclose.
Research funds from: Elixirgen Scientific, Inc.

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