

Tomoaki Nakayama^a, Manabu Seo^b, Tomoyuki Aratani^a, Toshihiko Hosoya^a
 (e-mail: tomoaki.nakayama@jp.ricoh.com)

a. Drug Discovery Business Department, Healthcare Business Group, RICOH COMPANY, Ltd., Japan
 b. Elixirgen Scientific, United States

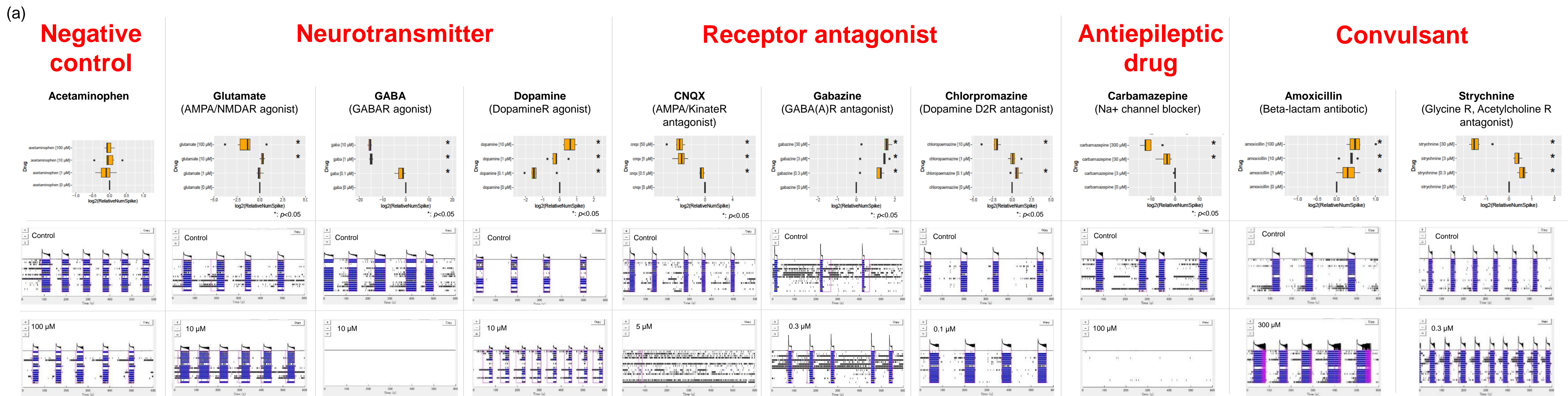
Introduction

Neurotoxicity is a major cause of failures in drug development. Animal models have been insufficient for precise prediction of neurotoxicity because of the species difference. To overcome this difficulty human neurons generated from induced pluripotent stem cell (iPSC) have been extensively tested. A major assay system for this purpose is neuronal networks cultured on microelectrode arrays (MEAs). In this paper we investigated whether Quick-Neuron™ Excitatory (EX-SeV-CW50065) generated with the recently developed quick differentiation method (Quick-Tissue™ technology) are suited to neurotoxicological assays. In addition, we optimized the fabrication and culturing to enable transportation of live-cell MEAs.



Results

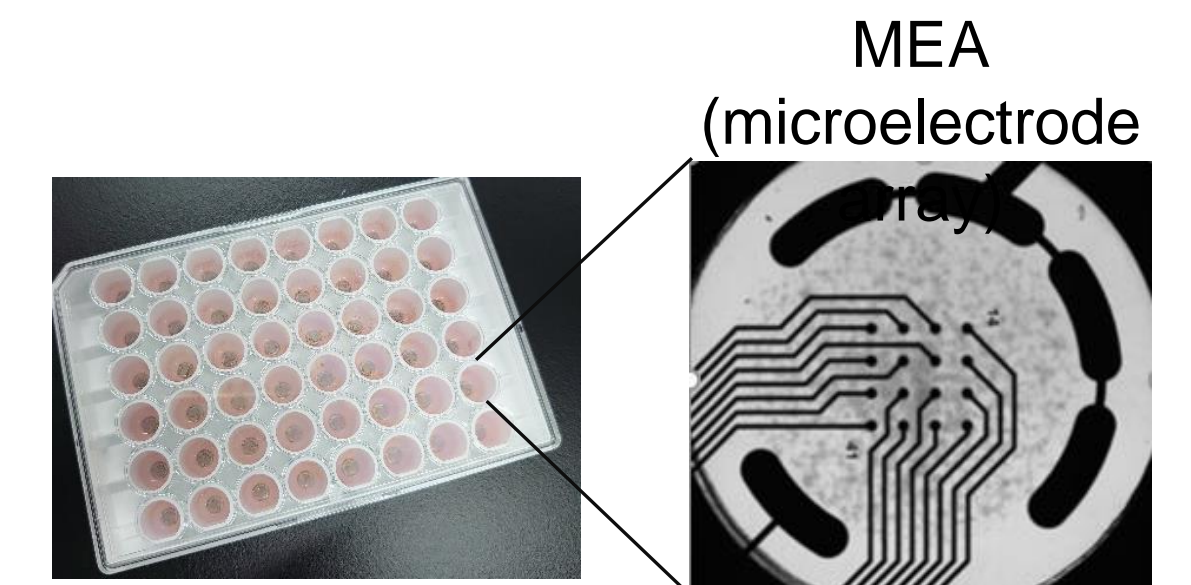
1. Reliable pharmacological responses of Quick-Neuron™ Excitatory



(b)

Mode	Receptors / Ion channels	Chemicals	Testing density	Changes of spike number	Ref. IC50
Negative control	-	Acetaminophen	1-100 μM	No change	-
Neuro Transmitter	Glutamate receptors	Glutamate	1-100 μM	↗	2.3 μM
	GABA receptors	GABA	0.1-10 μM	↘	2.8 μM
	Dopamine receptors	Dopamine	0.1-10 μM	↘	0.122 μM (D1) 2.76 μM (D2) 1.66 μM (D5)
	Muscarine receptors	Pilocarpine(HCl)	0.1-10 μM	↗	18 μM(M1,3) 4.5 μM(M2)
Receptor Antagonist	Histamine receptors	Histamine	0.3-30 μM	↗	24 μM
	AMPA, Kainate receptors	CNQX	0.5-50 μM	↘	0.92/6.1 μM
	GABA(A) receptors	Gabazine	0.3-30 μM	↘	0.2 μM
	Dopamine D2 receptors	Chlorpromazine	0.1-10 μM	↘	0.363 μM
	NMDA receptors	D-AP5	0.5-50 μM	No change	4.1 μM
Antiepileptic Drug	GABA(A) receptors	Picrotoxin	0.1-10 μM	↗	2.4 μM
	Histamine H1 receptors	Ketotifen	0.1-10 μM	↘	-
	Sodium channels	Carbamazepine	3-300 μM	↘	131 μM
Convulsant	beta-lactam antibiotic	Amoxicillin	1-100 μM	↗	100 μM- 10 mM*
	Glycine receptors, Acetylcholine receptors	Strychnine	0.3-30 μM	↘	17-40 nM
	Potassium channels	4-AP	0.3-30 μM	↗	147/117 μM

Device : Maestro Cytoview 48 well plate, Axion Biosystems
 Neurons : Quick-Neuron™ Excitatory, Elixirgen Scientific
 Astrocytes : Human astrocytes, ThermoFisher
 Medium : Neurobasal plus based medium
 Culture : DIV51
 Evaluation : Relative change of number of spikes



Human iPSC-derived neuron generated with transcription-factor-induced differentiation of iPSCs were co-cultured with human astrocytes. The cells were plated onto MEA plates (48 wells, 16 electrodes/well, Axion BioSystems). (a) (Top) The spike numbers generated in response to the indicated compounds (three concentrations) were divided by that generated in response to the control compound (0.1% DMSO). (Middle and bottom) Raster plots. (b) Summary of the results.

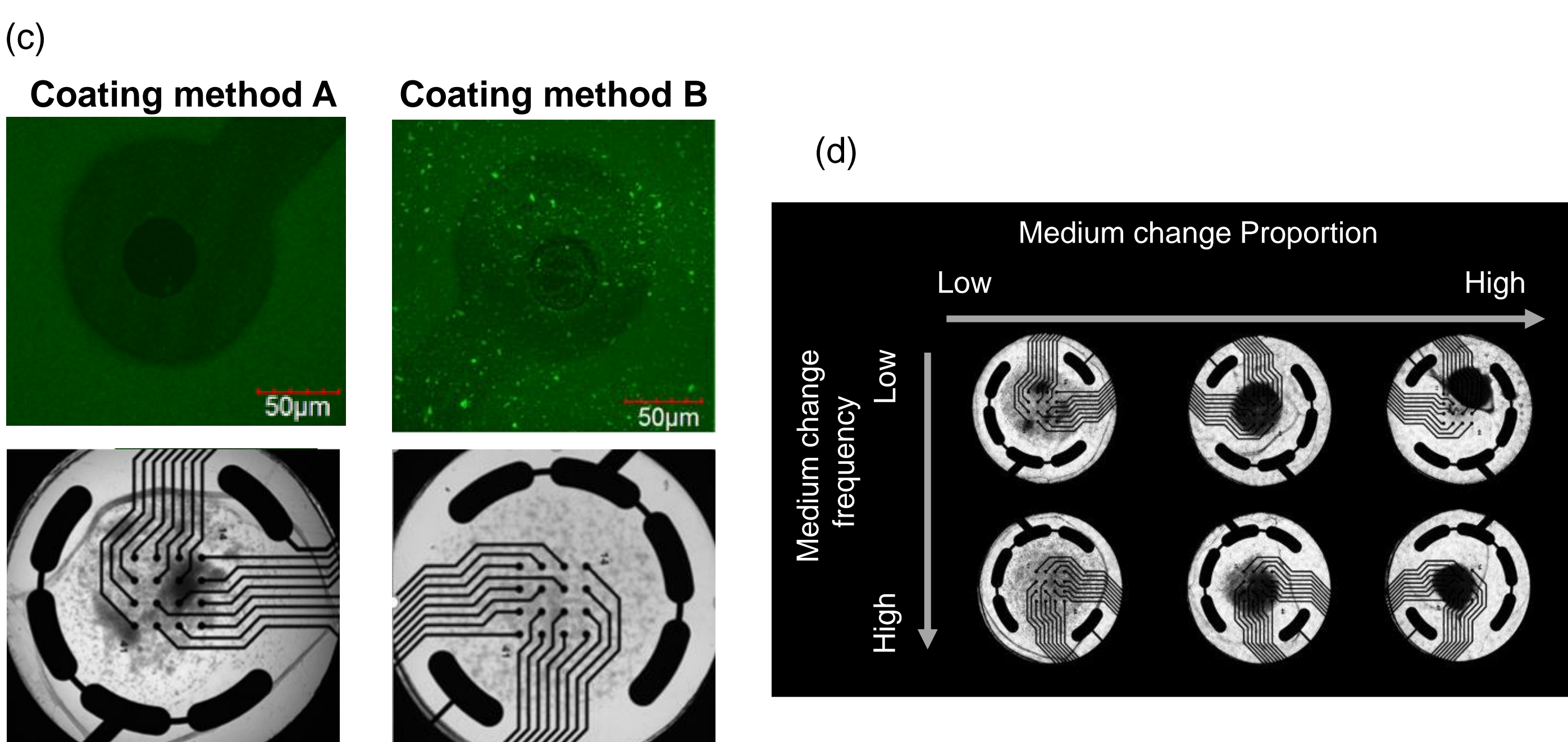
Quick Tissue Neurons show expected responses to neurotransmitters, receptor antagonists, channel blockers, antiepileptic drugs, and convulsant.

*Since the IC50 value of amoxicillin is unknown, the IC50 value for other GABA receptors of β-lactams is described.

2. Optimization of culture stability

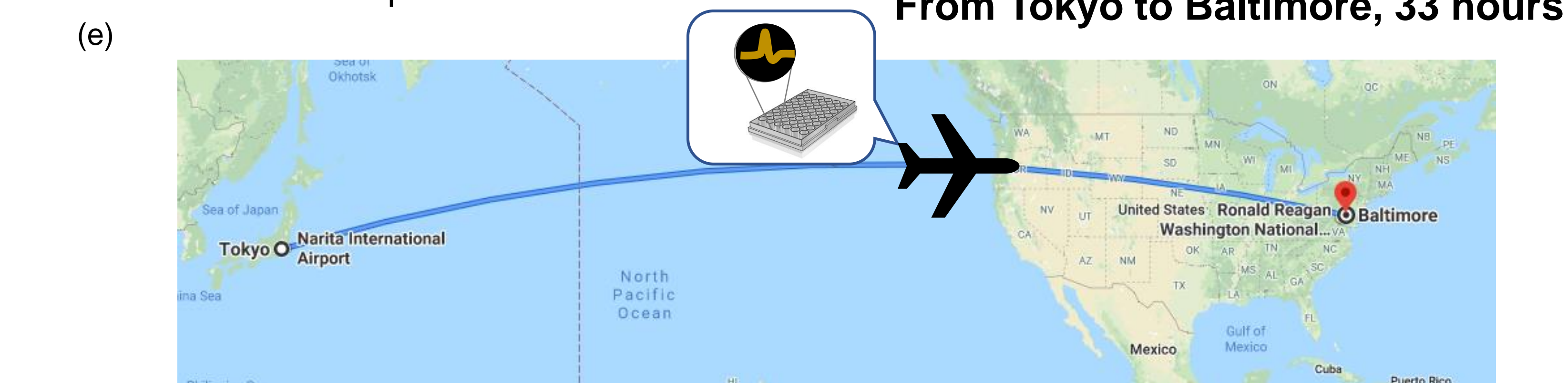
Shinnsuke Koshizuka, Momoko Shionoiri (RICOH).

Neurons: Quick-Neuron™ Excitatory, Elixirgen Scientific
 Medium: Brainphys based medium



3. Overseas transportation of live-cell MEAs

Neurons : Quick-Neuron™ Excitatory, Elixirgen Scientific
 Astrocytes : Human astrocytes, ThermoFisher
 Culture : DIV49-52
 Medium : Neurobasal plus based medium



(e) The course of the transportation. (f) The number of active electrodes of MEAs before and after the transportation. (g) Response to 4-Aminopyridine (4-AP), a potassium channel blocker, after the transportation.

Conclusions and future works

Conclusions

- ✓ Quick-tissue neurons exhibit excellent pharmacological responses and are suited to neurotoxicological assays.
- ✓ Optimized culture conditions enable transportation of live-cell MEAs overseas.

Future work

- ✓ We will evaluate the cells with a larger number of compound.