Application Note

Human iPSC-derived excitatory neurons and astrocytes differentiated by Quick-Tissue™ technology for functional drug screening on MEAs

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Introduction

Human induced pluripotent stem cells (hiPSCs) hold much promise in providing unique opportunities to advance understanding and treatment of human disease. hiPSCs can be expanded in culture indefinitely and differentiated into a variety of cell types, thereby providing a valuable and reproducible supply of trillions of cells for extended research. Such hiPSCs can be established from healthy or diseased individuals to study normal physiology as well as specific diseases and contribute towards drug discovery and new therapies. We have developed a rapid differentiation method, namely Quick-Tissue™ technology, resulting in the conversion of hiPSCs to highly homogenous neurons in 7 to 10 days[1] and functional astrocytes in 1 month. Based on the expression of markers, these neurons and astrocytes display high levels of purity and low batch-to-batch variation and have been consistently produced from dozens of different hiPSC lines, including lines derived from patients and lines with known mutations. We find that these hiPSC-derived neurons and astrocytes exhibit expected responses to known drugs when they are tested using the micro- (or multi-) electrode array (MEA) system. These hiPSC-derived cells thus have great potential to revolutionize high-throughput phenotypic and toxicology screening through establishment of 2D or 3D models (e.g., spheroids, organoids, and organ-on-a-chip) of entirely human origin, thereby accelerating the advancement of regenerative medicine, tissue engineering and personalized therapeutic strategies.

Results and Discussion

Excitatory Neurons

Quick-Tissue™ technology, Elixirgen Scientific's proprietary transcription factor-based technology, allows rapid and reproducible differentiation of hiPSCs into neurons without sacrificing the purity of the cells. We have characterized our neurons using immunofluorescent staining for marker expression and MEA with human primary astrocytes[2]. Our Quick-Neuron™ Excitatory - Human iPSC-derived Neurons exhibit typical morphology with neurite outgrowth and express a variety of neuronal markers, such as the pan-neuronal marker tubulin beta 3 class III (TUBB3) and the glutamatergic neuron marker vesicular glutamate transporter 1 (vGLUT1/SLC17A7) by day 10 of differentiation (or by day 7 post-thaw: Figure 1A). Excitatory neurons can be maintained in culture for at least 7 weeks while exhibiting stable firing activity and are suitable for a variety of applications such as neurotoxicity assays.

Astrocytes

Quick-Tissue™ technology also allows rapid and reproducible differentiation of hiPSCs into astrocytes. We have characterized our astrocytes using immunofluorescent staining for marker expression, glutamate uptake assay, and MEA with iPSC-derived Excitatory Neurons. Our Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes exhibit typical morphology including the characteristic spiky star-shape, and express a variety of astrocyte markers, such as the Aldehyde Dehydrogenase 1 Family Member 1L (ALDH1L1), Glial Fibrillary Acidic Protein (GFAP) and S100 Calcium Binding Protein B (S100B) by day 49 of differentiation (or by day 7 post-thaw: Figure 1B). At day 56 of differentiation Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes show a level of glutamate uptake comparable to that of human primary astrocytes (Figure 1C). These astrocytes slowly proliferate and can be maintained in mono-culture in our recommended medium conditions for several weeks or can be maintained in co-culture with excitatory neurons in a neuronal culture medium for weeks for the formation of a mature neural network.

MEA-drug responses

Having established that Elixirgen Scientific's Quick-Neuron™ Excitatory - Human iPSC-derived Neurons and Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes display physiologically relevant markers and expected functionality, we next explored their utility in MEAs. MEAs offer a powerful, scalable, and highly sensitive platform to investigate the formation and the maturation of neural networks by hiPSC-derived neurons and astrocytes in a high-throughput manner and were previously demonstrated with hiPSC-derived neurons and human primary astrocytes[2]. Unlike

traditional Ca^{2+} oscillation assays, MEAs offer the unique advantage of allowing the evaluation of dose-dependent responses of neurons to specific drugs in the same well.

Elixirgen Scientific's Quick-Neuron™ Excitatory - Human iPSC-derived Neurons and Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes were cocultured on MEA plates for 43 days post-thaw and their spontaneous firing activity was monitored. Among the wells prepared, these neurons and astrocytes were uniformly plated and did not show any sign of cell growth or proliferation, or peeling (Figure 2A). We found that they showed stable firing and frequent strong network bursts over the duration of recording (Figure 2B), indicating that these cells formed mature neuronal networks and that the network formation is reproducible across the wells (Table 1). Next, they were exposed to known compounds at 3 increasing concentrations sequentially. The compounds used are categorized as receptor agonists, antagonists, or ion channel blockers. Analysis of spontaneous firing activity (i.e., spikes) indicated that these neurons displayed expected responses to each of the compounds examined (Table 2). Acetaminophen served as a negative control and induced only moderate changes in the spike count at concentrations of 1-100 µM. Interestingly, with the histamine receptor agonist histamine, the number of spikes increased at low dosages of 0.1-1 µM but did not change beyond the range of the negative control at the higher dose of 10 µM. When a kainate receptor agonist, kainic acid, was applied at low dosages of 0.1-1 µM, the number of spikes showed moderate changes comparable to those found in the negative controls, but decreased at 10 µM. Glutamate had a similar effect - causing suppression at a higher dosage. Additionally, when GABA and dopamine were applied to the cultures at sufficient concentrations, the number of spikes decreased, indicating the presence of inhibitory synapses among the network of these neurons. An AMPA/Kainate receptor antagonist CNQX and an NMDA receptor antagonist D-AP5 suppressed neuronal activity at 0.5-50 µM and 1-100 µM, respectively, whereas the administration of a GABA receptor antagonist picrotoxin resulted in only small changes in the firing frequency at 0.1-10 µM as were found in the negative controls. Antiepileptic drug Carbamazepine, a sodium channel blocker, serves as a suppressor of neuronal activity and, as expected, the number of spikes decreased when administered at 10 and 100 µM. When a potassium channel blocker 4-Aminopyridine (4-AP) was added at 3 µM or greater, the number of spikes increased. Collectively these data confirm the expression of various receptors (e.g., NMDA, AMPA, and Kainate) in Quick-Tissue™ iPSC-derived neurons (co-cultured with Quick-Tissue™ iPSC-derived astrocytes) that are functional and demonstrate their feasibility in MEA-based drug-screening.

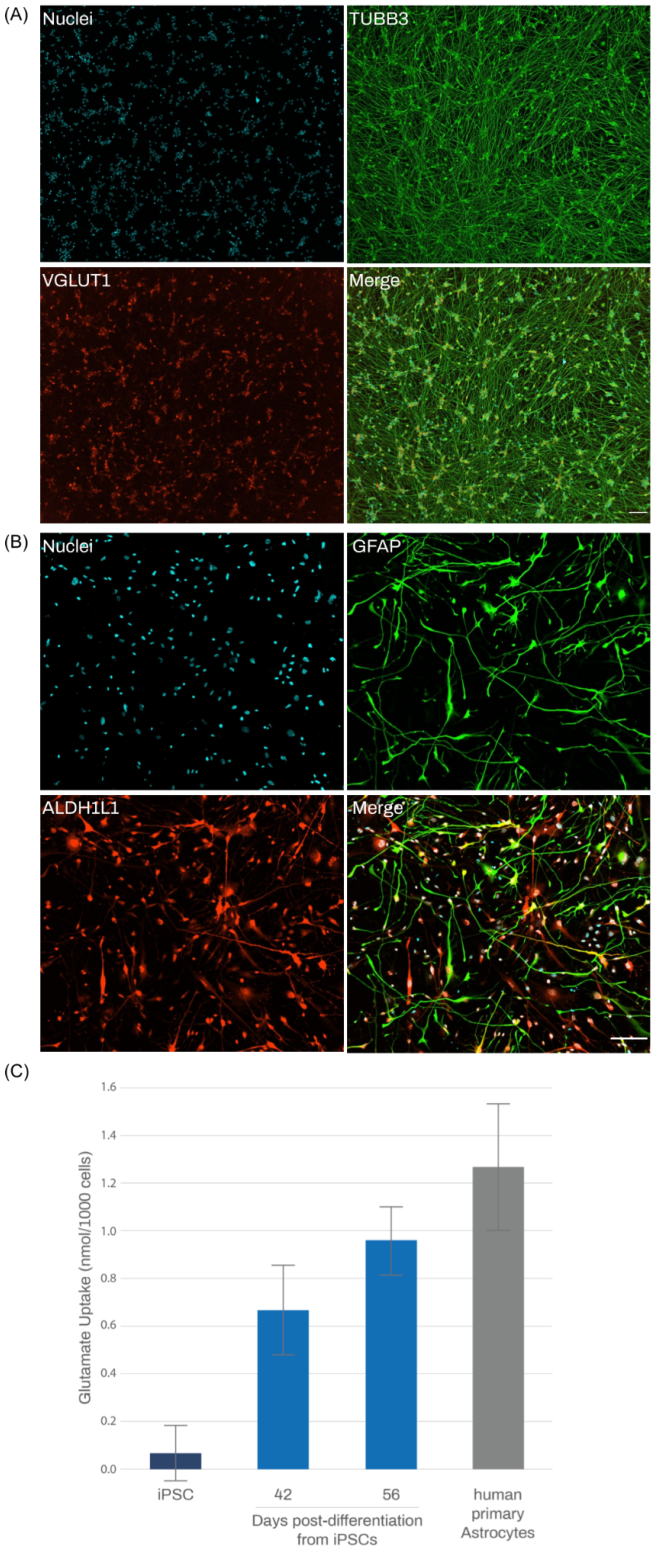

Figure 1: Characterization of Quick-Tissue™ Excitatory Neurons and Astrocytes. (A & B) Fluorescence images of iPSC-derived neurons at Day 10 (A) and iPSC-derived astrocytes at Day 49 (B). Scale bars = 100 µm. Nuclei were stained with Hoechst 33342. (C) Assessment of glutamate uptake over 60 minutes in iPSCs, iPSC-derived astrocytes 42 and 56 days after differentiation, and human primary astrocytes.

Conclusion

Animal models have not fully been able to predict drug responses in humans. Attrition of drugs due to neurotoxicity and problems in the central nervous system is a serious and expensive issue for pharmaceutical companies. hiPSC-derived neurons and astrocytes possess great potential to establish human cell-based in vitro platforms to screen out toxic small compounds, and may serve as better alternatives for the study of drug efficacy and toxicity[3-6] without batch-to-batch variance and ethical issues. Furthermore, in vitro models developed using patient-derived hiPSCs with known mutations associated with specific diseases may allow for prediction of patients' response to treatments and therapies. The results presented here demonstrate that Elixirgen Scientific's hiPSC-derived Quick-Neuron™ Excitatory Neurons are physiologically relevant, have characteristic gene expression profiles, and are amenable to toxicity screening, pharmacogenomics, drug discovery, disease modeling, and regenerative medicine. When they are combined with Elixirgen Scientific's hiPSC-derived Quick-Glia™ Astrocytes, assays like MEAs will become more physiologically relevant to humans without ethical and technical challenges in obtaining primary cells and can be personalized, particularly when the same hiPSC line is used to differentiate the two cell types

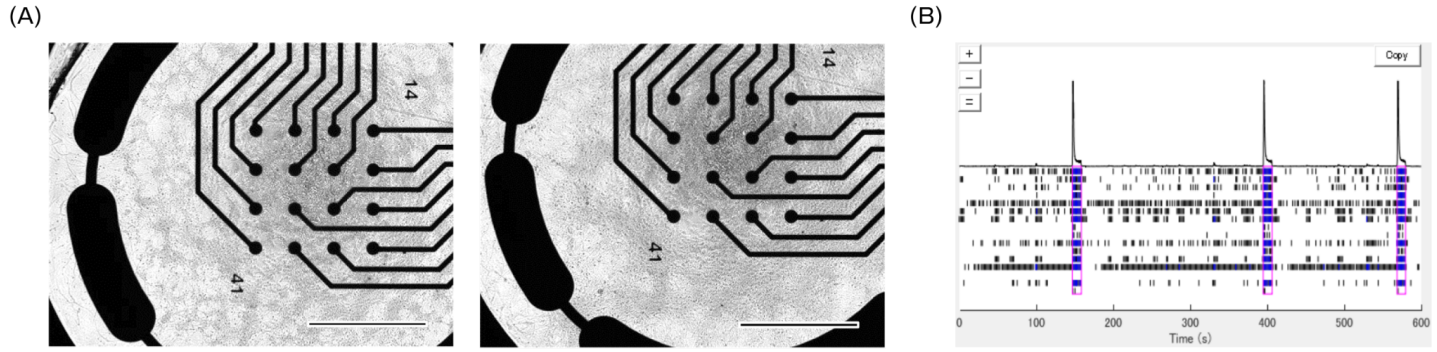

Figure 2: Quick-Neuron™ Excitatory - Human iPSC-derived Neurons cocultured with Quick-Glia™ Astrocyte - Human iPSC-derived Astrocytes on a CytoView MEA plate. (A) A representative well on the MEA plate shows cells plated homogeneously as seen on days 22 (left) and 43 (right). Scale bars = 1 mm. (B) A raster plot of the MEA recording for the representative well shown in A demonstrates that during a 10-minute recording, there are three strong synchronized network bursts (pink rectangles) detected without compound treatment.

Table 1. Well-to-well consistency in parameters during 10-minute recording on day 43

		Measured parameters			Coefficient of variance		
	Number of wells (n)	Number of Spikes (±SD)	Number of Active Electrodes (±SD)	Number of Network Bursts (±SD)	Number of Spikes	Number of Active Electrodes	Number of Network Bursts
Plate 1	48	17,899 ± 5,177	14.7 ± 1.06	2.5 ± 0.71	0.29	0.071	0.28
Plate 2	47	17,570 ± 4,260	14.9 ± 0.94	2.6 ± 0.57	0.24	0.062	0.21

Table 2. Summary of drug responses measured by the MEA system.

Mechanism	Receptor/Channel	Drug	Conc. Range	Relative # of spikes*		
Vehicle control	-	DMSO	0.2, 0.3, 0.4%	0.4	0.3	0.4
Negative control	-	Acetaminophen	1, 10, 100 µM	0.7	0.9	0.7
Agonist	Glutamate	Glutamate	0.3, 3, 30 µM	0.3	-0.7	-1.7
	GABA	GABA	0.1, 1, 10 µM	-0.7	-7.5	-12.1
	Dopamine	Dopamine	0.1, 1, 10 µM	-2.5	-2.5	-2.1
	Histamine	Histamine	0.1, 1, 10 µM	1.5	1.7	-0.8
	Kainate	Kainic acid	0.1, 1, 10 µM	0.6	0.3	-3.0
Antagonist	AMPA/Kainate	CNQX	0.5, 5, 50 µM	-3.0	-3.0	-3.0
	GABA(A)	Picrotoxin	0.1, 1, 10 µM	0.4	0.6	1.0
	NMDA	D-AP5	1, 10, 100 µM	-2.3	-3.0	-3.0
Ion Channel Blocker	Sodium	Carbamazepine	1, 10, 100 µM	0.0	-2.0	-3.0
	Potassium	4-AP	0.3, 3, 30 µM	0.8	2.3	2.6

* The number of spikes relative to that of a baseline (before DMSO administration) is shown as a value in a log2 scale. The gradient of red indicates increase in the number of spikes, whereas the gradient of blue indicates decrease in the number of spikes.

Materials and methods

Cell Culture

Elixirgen Scientific's Quick-Neuron™ Excitatory - Human iPSC-derived Neurons (Catalog No. EX-SeV-CW50065) and Quick-Glia™ Astrocytes - Human iPSC-derived Astrocytes (Catalog No. AS-SeV-CW50065) were thawed and maintained following the instructions in the user guides (https://www.elixirgensci.com/resources/).

Immunofluorescence Staining

Elixirgen Scientific's Quick-Neuron™ Excitatory - Human iPSC-derived Neurons and Quick-Glia™ Astrocytes - Human iPSC-derived Astrocytes were maintained for 7 days post-thaw under medium conditions instructed by the user guides prior to immunofluorescence staining.

Glutamate uptake assay

Human primary astrocytes (ScienCell Research Laboratories, Catalog number: 1800) and Quick-Glia - Astrocyte SeV kit cultures, harvested at either 28 days or 42 days post SeV infection, were plated at 7,800 cells/cm^2 in a Geltrex-coated 96-well plate (Corning, Catalog number: 353072) and grown in ScienCell Astrocyte medium (ScienCell Research Laboratories, without FBS, Catalog number: 1801) for 14 days. As a negative control, human iPSCs were plated in StemFit Basic04 medium (Ajinomoto, Catalog number: ASB04-C) two days before assay measurement using the plating density stated above. 30 minutes prior to assay execution, culture medium was replaced with Hanks' Balanced Salt Solution (HBSS) without phenol red (Fisher Scientific, Catalog number: 14025092). Subsequently, cells were exposed to 100 µM L-glutamate (Tocris, Catalog number: 0218) in HBSS for 60 minutes. Immediately after the assay, solution was collected and cells were dislodged using a 1:1 mixture of TrypLE Select Enzyme (Fisher Scientific, Catalog number: 12563029) and 0.02% EDTA (Lonza, Catalog number: 17-711E). The numbers of live cells were determined using a NucleoCounter NC-200 (Chemometec, Catalog number: 900-0200) cell counter. Glutamate concentration was measured by a bioluminescence-based Glutamate-Glo assay kit (Promega, Catalog number: J7021) and a SPECTRAFluor Plus microplate reader (Tecan). To determine the amount of glutamate cleared, all values were first background signal-subtracted using a sample treated with HBSS only and concentrations were thereafter acquired from the glutamate titration curve according to manufacturer's instructions.

MEA

Quick-Neuron™ Excitatory Neurons were plated at 0.8 x 10^5 cells/well of a CytoView 48-well MEA plate with 16 electrodes per recording area in each well (1.21 mm^2; Axion Biosystems), after mixing with Quick-Glia™ Astrocytes at 0.2 x 10^5 cells/well and 0.2 µg/well laminin similarly to our Application Protocol of the Excitatory Neurons and human primary astrocytes except that astrocytes were thawed using Medium N. Cultures were maintained at 37°C, 5% CO_2 for 49 days with 50% of the medium changed twice a week. Observation of the cultures and recording were done once a week. Cells in each well were exposed to a diluent of drugs (i.e., 0.1 % DMSO) first and then drugs in 3 increasing doses sequentially with intervals of 10-min recording followed by 10-min resting under 37°C, 5% CO_2. Data were collected immediately after administration of drugs using MaestroPro (Axion Biosystems) at a sampling rate of 12.5 kHz per electrode. After a series of recordings was completed, treated cells were washed with medium and incubated overnight. Finally, spontaneous spike firing activity was measured again to confirm that cells were not damaged by the treatment. Drug response was evaluated by measuring spike counts. Data were extracted by AxISNavigator 2.0.4 and analysis was performed using Neural Metric Tool.

References

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