MEA Assay Using Human Glutamatergic Neurons (BX-0300 or BX-0350)

BACKGROUND

BrainXell, Inc. specializes in the differentiation of human iPSCs to a variety of neuronal cell types that provide researchers biologically relevant systems for CNS drug discovery and toxicity screening. Researchers can choose from a variety of highly pure, functionally specialized subtypes that exhibit biochemical and functional characteristics of human neurons. These neurons provide an ideal in vitro experimental system for a wide range of neurophysiological investigations.

Neuroscience researchers are turning to multi-electrode arrays (MEA) to evaluate neuronal activity of multiwell plate cell cultures. MEA systems measure extracellular voltage changes that occur as neurons fire action potentials. Such measurements reveal the firing patterns of individual neurons as well as the patterns of neuronal networks that exist in the cell culture. Such measurements are non-invasive and allow for repeated measurements to be made over the life-time of the cell culture. MEA can be used to study the physiological behavior of neurons, acute and long-term pharmacological responses, and disease phenotypes.

This application protocol describes the use of BrainXell Cortical Glutamatergic Neurons and Layer V Glutamatergic Neurons to perform consistent and reproducible MEA assays to investigate neuronal activity.

MATERIALS PROVIDED BY BRAINXELL

- One vial of 5 million cryopreserved human neurons (200 µL)
- 100 µL Neuron Seeding Supplement at 1000X
- 50 µL Neuron Day 4 Supplement at 1000X

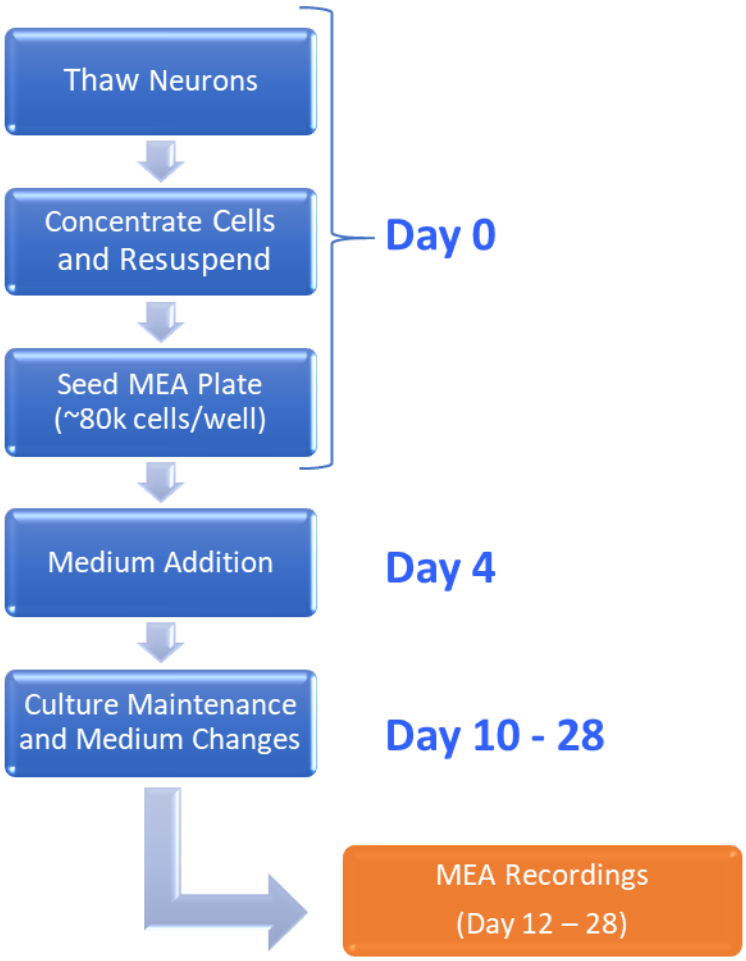
ADDITIONAL MATERIALS NEEDED

- DMEM/F12 Medium (Life Technologies #11330-032)
- Neurobasal Medium (Life Technologies #21103-049)
- B27 Supplement (Life Technologies #17504-044)
- N2 Supplement (Life Technologies #17502-048)
- GlutaMAX (Life Technologies #35050-061)
- Geltrex (Life Technologies #A1413201)
- BDNF (Peprotech #450-02)
- GDNF (Peprotech #450-10)
- TGF-β1 (Peprotech #100-21C)
- 24-Well CytoView MEA Plates (Axion Biosystems # M384-tMEA-24W)
- BrainPhys Medium (STEMCELL Technologies # 05790)

EQUIPMENT NEEDED

- Maestro Edge (Axion Biosystems)

WORK FLOW



CELL CULTURE PROCEDURE

Thawing and Seeding the Neurons

1. Gather the components for the Seeding Medium according to the recipe below. Note that BDNF, GDNF, and TGF-β1 are supplied as lyophilized powders. Follow the manufacturer's instructions for reconstitution. We recommend creating stock solutions of 10 µg/mL for BDNF, 10 µg/mL for GDNF, and 1 µg/mL for TGF-β1.
2. Thawing neurons and seeding of one 24-well plate requires approximately 20 mL of seeding medium. Working in a cell culture hood (biological safety cabinet), combine all components in a sterile 50 mL conical tube. Allow the medium to equilibrate to room temperature for 15 minutes. Do not warm the medium in a 37°C water bath.
3. Remove a cryovial from the liquid nitrogen and place in a 37°C water bath. To minimize contamination, avoid submerging the cap. Gently move the vial within the bath to increase the rate of thawing.
4. As soon as the last of the ice melts, which will take ~90 seconds, remove the vial from the water bath. Disinfect the vial by spraying it with 70% ethanol and transfer it to the cell culture hood.

5. Slowly add 800 µL of seeding medium to the vial at a rate of ~1 drop/s using a 1 mL pipette tip. This process should take about 30 seconds per vial.
6. Gently transfer the contents (1mL total) from the vial to new sterile 15 mL conical tube.
7. To collect any residual cells, gently add another 1 mL of seeding medium to the vial and then transfer to the conical tube.
8. Slowly add an additional 3 mL of seeding medium to the conical tube using a 5 mL serological pipette. Gently swirl the conical tube while adding the medium. This process should take about 1 minute.
9. Remove 20 µL from the cell suspension and count the number of viable cells per mL with a hemocytometer using the trypan blue exclusion method to identify dead/viable cells. Perform counting while tube is being centrifuged during step 10.
10. The cells must be concentrated to achieve a neuron concentration of 6,700 viable neurons/µl. Centrifuge the neurons at 1000 RPM (160xg) for 3 minutes and then carefully remove the liquid above the cell pellet down to ~200 µl. Gently resuspended the pellet in the remaining media. Gauge total volume by gently drawing up into 200µl micropipette. Then add necessary volume to achieve a concentration of 6,700 neurons/µl as determined by the cell count performed in step 9. For example, if the tube has 5 Million viable neurons, the total resuspension volume will be ~745 ul.
11. Mix completely and then plate 12 µL in the center of each well onto a PDL-coated 24-well MEA plate one well at a time. This seeds ~80,000 neurons over the electrode array.
12. Transfer the plate to a humidified incubator at 37°C with 5% CO_2.
13. While incubating, prepare Geltrex for use from a 15 mg/mL stock. Add cold DMEM/F12 directly to an aliquot of frozen Geltrex to yield a 1:10 dilution. For example, if aliquots of Geltrex have a volume of 100 µL, add 900 µL of cold DEME/F12. Immediately place this mixture at 4°C to allow the Geltrex to thaw and dissolve. Add Geltrex to the remaining seeding medium to achieve a final concentration of 15 µg/mL (e.g. 150 µL of prepared Geltrex added to 15 mL of seeding medium).
14. After 30 minutes incubation remove plate from incubator and place in hood. Gently add 190 µl to each well, filling the bottom of each well while avoiding direct washing of the cells. Gently add an additional 300 µl to each well to bring the total well volume to 500 µl.
15. Transfer the plate to a humidified incubator at 37°C with 5% CO_2. Note: the day the cells are plated is designated as Day 0.

Day 4 Medium Addition

1. On Day 4 (96 hours after seeding), prepare fresh medium following the Day 4 recipe described below. This medium includes a mixture of BrainPhys, DMEM, and Neurobasal Medium to allow the culture to gradually transition to BrainPhys medium. The medium also contains the Day 4 supplement, which aids neuronal maturation.
1. Gently add 500 µL/well to the entire plate bringing the total volume in each well to 1 mL.

Day 10 and Onward Medium Changes

1. Change half the medium (500 µL/well) every 5-7 days starting on Day 10 (ex: Day 10, 16, and 22) or more frequently if you notice a change in the pH indicator of the media. The medium for these changes will consist of BrainPhys and the recommended supplements (see recipe below).

Media Compositions

		Component	Stock Conc.	Final Conc.	Volume
Seeding Medium	1	DMEM/F12 Medium	1X	0.5X	9.6 mL
	2	Neurobasal Medium	1X	0.5X	9.6 mL
	3	B27 Supplement	50X	1X	400 µL
	4	N2 Supplement	100X	1X	200 µL
	5	GlutaMAX	200 mM	0.5 mM	50 µL
	6	BDNF	10 µg/mL	10 ng/mL	20 µL
	7	GDNF	10 µg/mL	10 ng/mL	20 µL
	8	TGF-β1	1 µg/mL	1 ng/mL	20 µL
	9	Seeding Supplement	1000X	1X	20 µL
	*Geltrex is added to cell suspension immediately prior to plating				

		Component	Stock Conc.	Final Conc.	Volume
Day 4 Medium	1	DMEM/F12 Medium	1X	0.25X	4.8 mL
	2	Neurobasal Medium	1X	0.25X	4.8 mL
	3	BrainPhys Medium	1X	0.5X	9.6 mL
	4	B27 Supplement	50X	1X	400 µL
	5	N2 Supplement	100X	1X	200 µL
	6	GlutaMAX	200 mM	0.5 mM	50 µL
	7	BDNF	10 µg/mL	10 ng/mL	20 µL
	8	GDNF	10 µg/mL	10 ng/mL	20 µL
	9	TGF-β1	1 µg/mL	1 ng/mL	20 µL
	10	Day 4 Supplement	1000X	1X	20 µL

		Component	Stock Conc.	Final Conc.	Volume
Day 10 Medium and Onwards	1	BrainPhys	1X	0.5X	19.3 mL
	2	B27 Supplement	50X	1X	400 µL
	3	N2 Supplement	100X	1X	200 µL
	4	GlutaMAX	200 mM	0.5 mM	50 µL
	5	BDNF	10 µg/mL	10 ng/mL	20 µL
	6	GDNF	10 µg/mL	10 ng/mL	20 µL
	7	TGF-β1	1 µg/mL	1 ng/mL	20 µL

MEA ASSAY

Once the cell culture has reached DIV 12, prominent neural activity can be observed. For each recording, transfer the plate from the incubator to the Maestro Edge. Allow 15 minutes for the instrument and plate to come to equilibration (37°C, 5% CO_2) and then record for 15 minutes or desired length of time. After recording, return the plate to the incubator.