# Optimized Conditions for MEA Assay with Human iPSC-Derived Spinal Motor Neurons, Glutamatergic Neurons and Mixed Cortical Neurons

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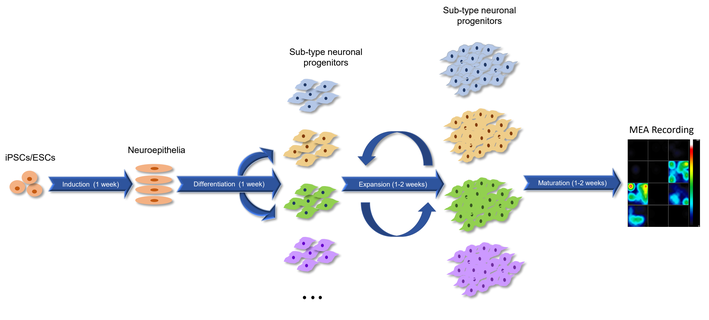
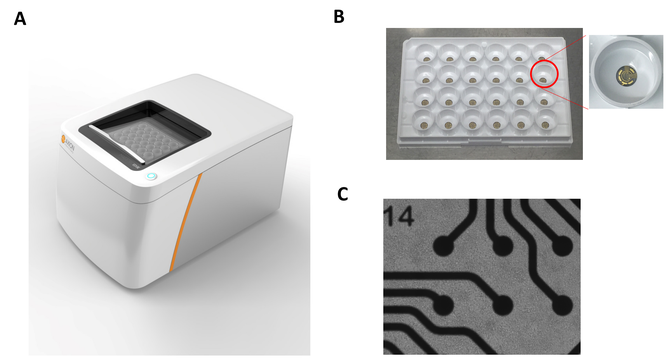
## Introduction

Human induced pluripotent stem cells (hiPSC) derived neurons are now considered a more relevant model system for neurological and psychiatric diseases in vitro. They can be used as a platform for neurological disease modeling, drug discovery and toxicity screening. Neural electrical activity is one of the essential parameters for assessing the functionality of the nervous system. Micro-electrode array (MEA) systems provide a non-invasive and label-free means to assess electrophysiological activity from thousands of neurons in the same plate over time. More and more researchers are using micro-electrode array (MEA) with hiPSC-derived neurons to characterize neuronal phenotypes and perform drug screening. But achieving stable and consistent MEA recordings within the shortest possible culture time remains a challenge. In order to generate a robust protocol for MEA recording on hiPSC-derived neurons, we evaluated several conditions, which could affect culture performance (1. neuron seeding density; 2. seeding medium; 3. astrocyte co-culture). These conditions were evaluated with BrainXell's hiPSC-derived spinal motor neurons, cortical glutamatergic neurons and mixed cortical neurons. Our data demonstrate that different neuron types have different optimal seeding densities that can generate the most consistent and robust neuronal activity. Inclusion of BrainPhys neuronal medium as the cultures mature also contributes to consistent, synchronized signals, and astrocyte co-culture accelerates the network maturation. With our current protocol, cortical glutamatergic neurons started to show consistent synchronized signal as early as day 12 after seeding, and spinal motor neurons and mixed cortical neurons started to show on day 18. The synchronized network activity lasted for at least two weeks. The presented data demonstrate the suitable application of hiPSC-derived neurons coupled with MEA technology as a noninvasive human neuronal test system that can be used for drug discovery and toxicity screening.

## BrainXell Core Technology




## Axion Maestro Edge System and Cytoview Plate




(A) Axion Maestro Edge System
(B) 24-well Cytoview Plate
(C) Bright field image of hiPSC-derived spinal motor neurons on Day 12 after seeding

## Result 1: BrainPhys Medium Supports Neuronal Network Activity

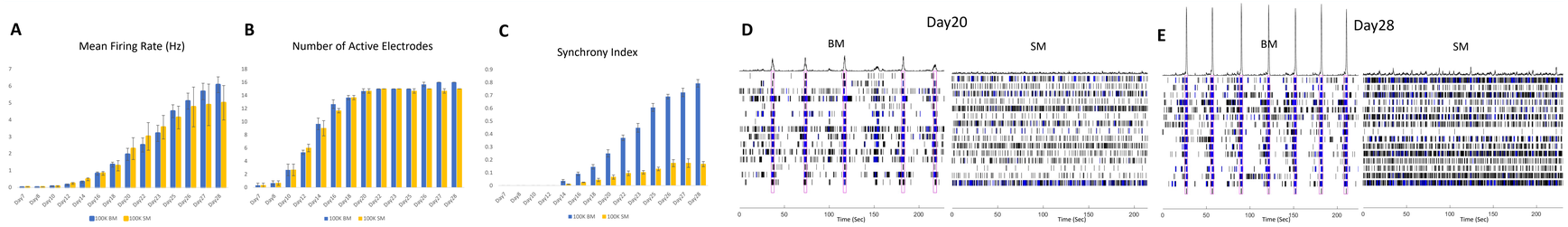



Figure 1: Compared to seeding medium, BrainPhys medium cultured hiPSC-derived spinal motor neurons have more activity and greater synchronization.
(A). Mean firing rate is higher in BrainPhys medium cultured spinal motor neurons. (B). Both seeding medium and BrainPhys medium cultured motor neurons can reach >80% active electrodes on Day20 and support a high active electrode rate through Day28. (C). BrainPhys medium cultured neurons have a much higher synchrony index compared to seeding medium cultured neurons. (D). Example raster plot on Day20. (E). Example raster plot on Day28. SM: Seeding Medium (DMEM/F12:Neurobasal 1:1), BM: BrainPhys Medium

## Result 2: Optimal Neuron Seeding Density Supports Stable Neuronal Electrical Activity

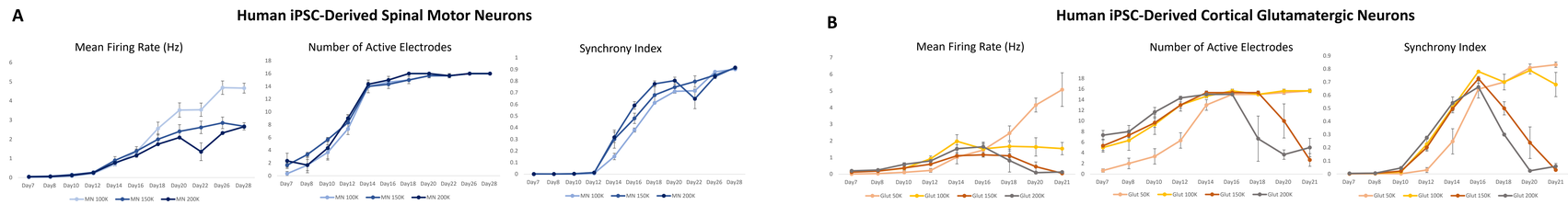



Figure 2: Neuron seeding density titration on hiPSC-derived spinal motor neurons and cortical glutamatergic neurons.
(A) Motor neuron seeding density titration included 100K cells/well, 150K cells/well and 200K cells/well. Evaluation of mean firing rate, number of active electrodes and synchrony index data together indicated an optimal seeding density of 100K cells/well for motor neurons.
(B) Glutamatergic neuron seeding density titration included 50K cells/well, 100Kcells/well, 150K cells/well and 200K cell/well. Evaluation of mean firing rate, number of active electrodes and synchrony index data together indicated an optimal seeding density of 100K cells/well for glutamatergic neurons.

## Result 3: Astrocyte Co-culture Accelerates Neuronal Network Maturation

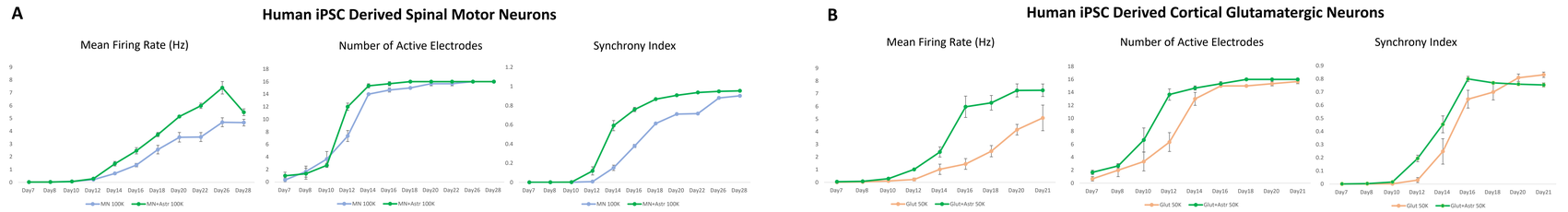



Figure 3: Astrocyte/neuron co-culture and neurons only  culture comparison for hiPSC-derived spinal motor neurons and cortical glutamatergic neurons.
(A) hiPSC-derived spinal astrocytes co-cultured with iPSC-derived spinal motor neurons increases the mean firing rate and accelerates the occurrence of a synchronized signal. Neuron to astrocyte ratio is 10:1. Motor neuron seeding density is 100K cells/well.
(B) hiPSC-derived cortical astrocytes co-cultured with iPSC-derived cortical glutamatergic neurons increases the mean firing rate and accelerates the occurrence of a synchronized signal. Neuron to astrocyte ratio is 10:1. Cortical glutamatergic  neuron seeding density is  50K cells/well.

## Result 4: Optimized Conditions Generate Consistent and Robust MEA Recording

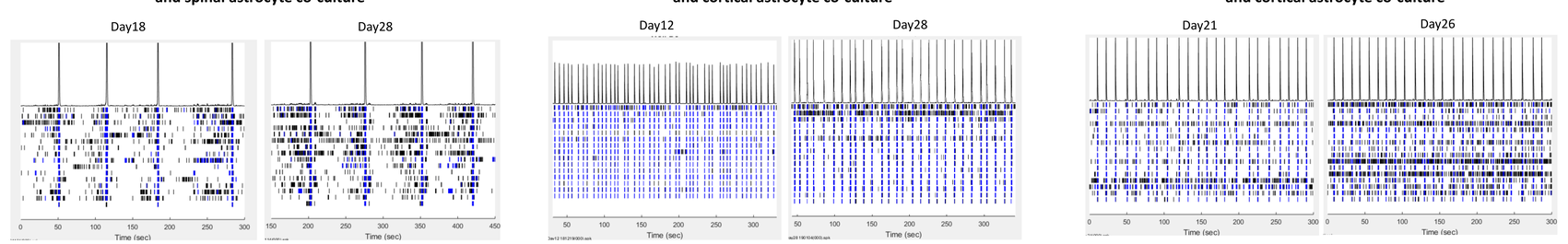



Figure 4: Example raster plots using optimized culture protocol for hiPSC-derived spinal motor neurons, cortical glutamatergic neurons and mixed cortical neurons.
Two time points were selected for each sub-type of neurons, highlighting the period of time in which each culture exhibited a stable synchronized signal. Mixed Cortical neurons have 80% of cortical glutamatergic neurons and 20% cortical GABAergic neurons

## Conclusions

1. Three types of BrainXell's hiPSC-derived neurons were tested on Axion Maestro Edge System. All three types could generate consistent and robust MEA recording within 2-3 weeks after plating.
2. All data demonstrate the suitable application of hiPSC-derived neurons coupled with MEA technology as a noninvasive human neuronal test system that can be used for drug discovery and toxicity screening.