3D Spheroid Culture Workflow using iPSC-induced Human Neurons

Kaiping Xu[1], Zhong-Wei Du[1], Anju Dang[2], Ben Dungar[1], Kurt Laha[1]

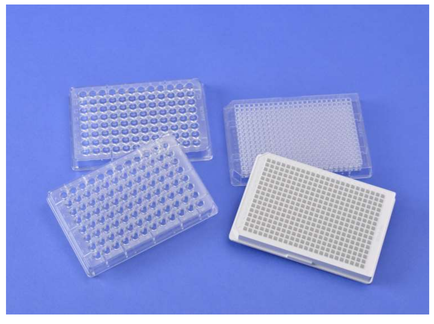
1. BrainXell, Inc., Madison, WI, USA; 2. S-BIO, Hudson, NH, USA

Introduction

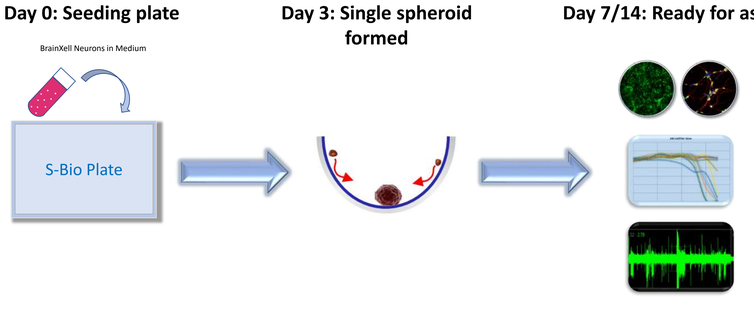
Human induced pluripotent stem cells (iPSC) derived neurons are now considered a more relevant in vitro model system for psychiatric and neurological diseases. They can be used for the development of physiological cell models, human disease models, and drug library screening. Three dimensional (3D) cultures are also being pursued as a more physiologically relevant system because they provide a microenvironment, cell-to-cell interactions, and biological processes that better represent in vivo conditions. The implementation of 3D spheroid culture plays an important role as an alternative approach for drug development and therapeutic applications in central neural system (CNS) disorders. To develop a neuronal 3D spheroid culture system, we tested iPSC-derived human motor neurons and cortical glutamatergic neurons using the S-BIO PrimeSurface Ultra Low Attachment Micoplates. After 2 hours, plated neurons started to settle down at the bottom of the well and form large clusters. On day 3, 3D spheroids could be seen clearly under phase contrast. On day 7, the spheroids were more condensed. The size of the 3D spheroids was proportional to the number of neurons seeded per well. In addition to the morphological assessments, a cytotoxicity assay and MEA assay were performed to demonstrate the suitable of 3D spheres as a platform for various applications. In both assays, the spheroids yielded expected results. The results presented here demonstrate the feasibility of generating uniform and reproducible spheroids using human neurons and the potential application for neurotoxicity studies.

S-Bio Plates for Spheroid Formation

S-BIO PrimeSurface® cultureware are ultra low attachment (ULA) dishes and plates that promote scaffold free, self assembly of spheroid formation. The plates are pre-coated with unique ultra hydrophilic polymer that enables spontaneous spheroid formation of uniform size. PrimeSurface 96 and 384 ULA plates have good optical clarity making them highly suitable for bright field imaging, fluorescent imaging and confocal microscopy.

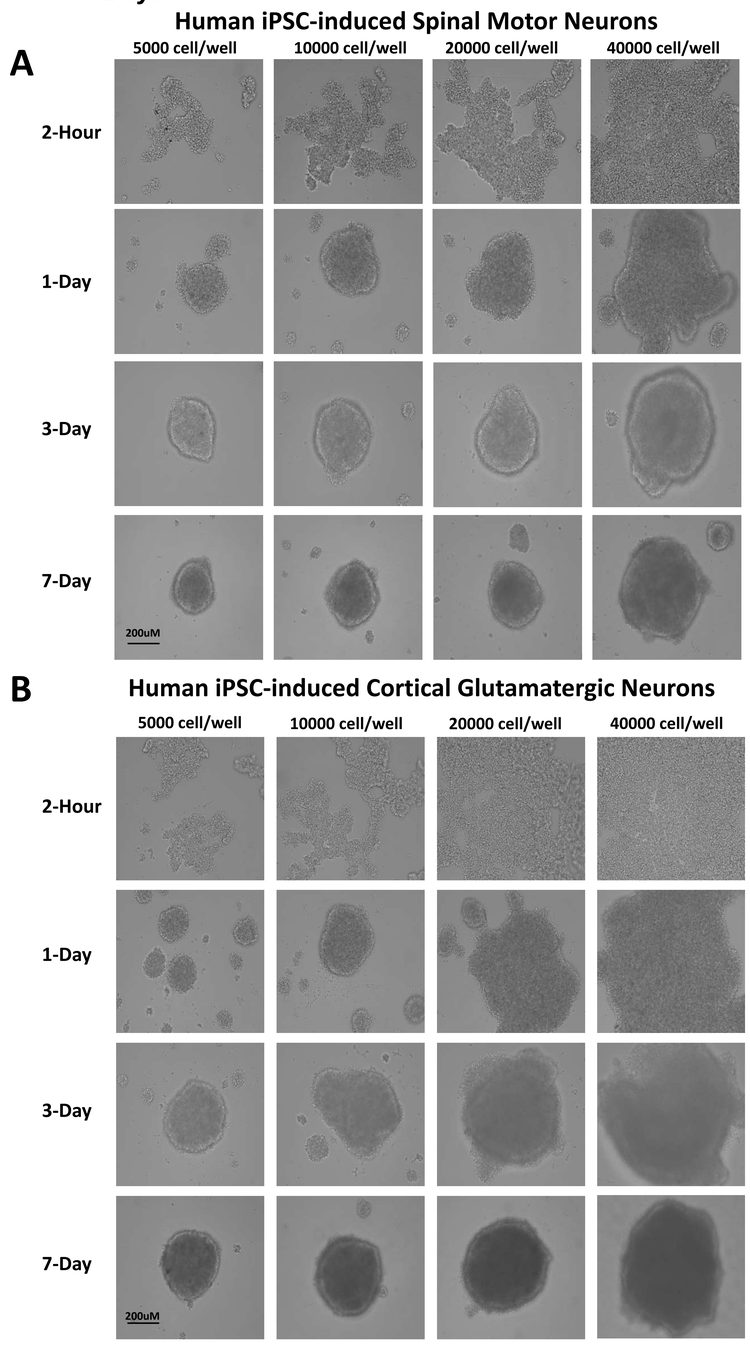


Spheroid Formation Workflow



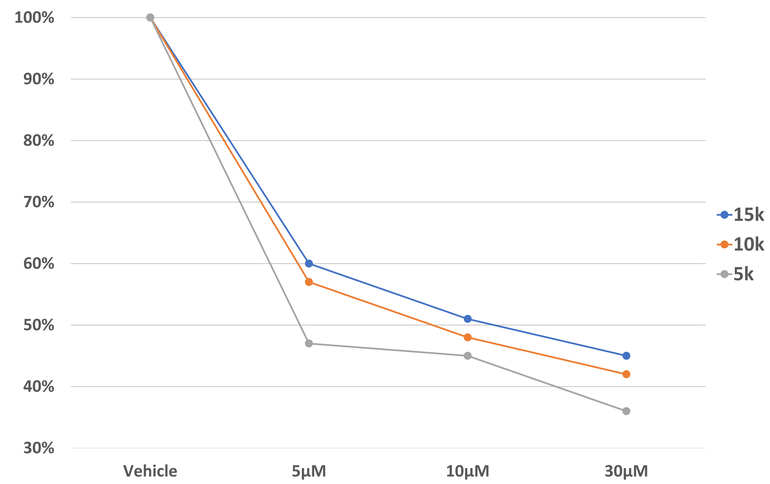
BrainXell Human iPSC-induced Neurons Cultured as 3D Spheroids

1. Human iPSC-induced Spinal Motor Neurons and Cortical Glutamatergic Neurons Form Uniform, Single 3D Spheroids within 3 Days



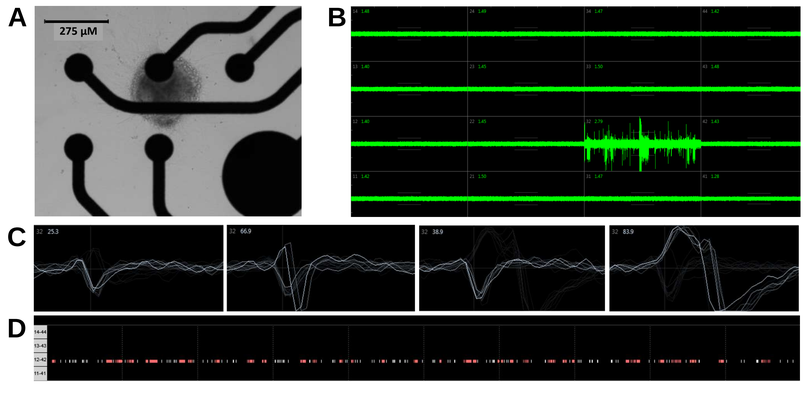
(A) BrainXell iPSC induced spinal motor neurons were seeded at 4 different densities on S-Bio PrimeSurface® 96-well plate. On Day 3, uniform and single 3D spheroids were formed. (B) BrainXell iPSC induced cortical glutamatergic neurons were seeded at 4 different densities on S-Bio PrimeSurface® 96-well plate. On Day 3, uniform and single 3D spheroids were also formed.

2. Cytotoxicity Assay with 3D Spheroids



BrainXell iPSC induced mixed cortical neurons seeded at three densities on an S-Bio PrimeSurface® 96-well plate (15k, 10, 5k). Day 14 treated for 72hr with Rotenone concentrations 1uM, 10uM and 30uM. Assayed with CellTiter-Glo® 2. All seeding densities showed similar and consistent responses to the toxin. The spheroid size correlated to toxin response.

3. MEA Assay with 3D Spheroid for Functional Assessment



(A) Phase contrast microscope image (10X) of individual spheroid transferred to MEA plate at 14DIV. (B) 3 Days after transfer electrical activity was recorded. Single spheroid covers a single electrode and showed prominent activity. (C) Qualitative examination of individual waveforms indicates activity of several neurons was recorded by a single electrode. (D) Raster plot of activity. Firing rate was 11 Hz and burst frequency was 0.7 Hz.

Conclusions

PrimeSurface® ultra low attachment plates with a u-shaped bottom provide a suitable culture system for iPSC-derived human neurons to be cultured as uniform 3D spheroids. The process is consistent, robust and amenable to a wide range of assays.