StemoniX® microBrain® 3D: A high-throughput model for drug discovery and toxicology

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INTRODUCTION

- StemoniX microBrain 3D is a high-throughput model designed to aid researchers with drug discovery and toxicology.
- Available in both 96 well and 384 well formats.
- The format of microBrain 3D better recapitulates the complex three dimensional structure found in the human cerebral cortex.
- Benefiting from human induced pluripotent stem cell (iPSC) technology, microBrain 3D offers significant benefits in lot-to-lot variation and ethical constraints compared to primary cell types.
- Harboring a co-culture of neurons and astrocytes, microBrain 3D utilizes the complex interactions between different cell types, and sub-types, to allow for an accurate representation of human cerebral cortical tissue.
- The microBrain 3D cortical spheroids are highly homogenous in size and composition. Derived from a single human donor, the iPSC-derived neural cells in the spheroid develop naturally during the course of differentiation.

METHODS

Spheroid Preparation

Neural Progenitor Cells (NPCs), derived from human iPSCs, were dissociated and seeded into Corning round-bottom 384 or 96 well cell culture plates. Under a proprietary differentiation protocol, the cells were matured and media changed regularly using StemoniX NeuralX® media.

Microscopy

Phase contrast images were obtained on a ZEISS CellDiscoverer 7.

Gene Expression

RNA was extracted using Ambion® PureLink™ RNA Mini Kit. cDNA was prepared using Invitrogen's SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR. Qiagen RT2-Profiler PCR Array system targeting Neurotransmitter Receptors was run on a BioRad CFX384™ Real Time System using SYBR Green detection to determine gene expression fold change for a panel of 84 genes.

Calcium Oscillation

Calcium oscillation studies were carried out on the Fluorescent Imaging Plate Reader (FLIPR) Tetra High-Throughput Cellular Screening System from Molecular Devices. Cells were media changed to phenol-free NeuralX media and incubated with FLIPR Calcium 6 Assay Kit by Molecular Devices for two hours prior to data acquisition. After incubation a panel of drugs were added which contained neuromodulatory compounds including GABAergic and Glutamatergic agonists and antagonists. Calcium oscillation data was analyzed using JMP.

Immunocytochemistry (ICC)

Spheroids were extracted from plates and washed with DPBS +/+. Immediately after washing spheroids were fixed using 4% Paraformaldehyde for 10 minutes. The spheroids were washed twice more using DPBS +/+ prior to permeabilization with 0.1% Triton™ X-100 in Odyssey Blocking Buffer for 15 minutes. Following permeabilization the spheroids were blocked for a further 30 minutes using Odyssey Blocking Buffer. Primary antibodies were prepared as per manufacturer specifications and incubated overnight at 4°C. Spheroids were washed in DPBS +/+ a total of three times before addition of secondary antibodies, prepared as per manufacturer specifications for one hour. DAPI was added and incubated at room temperature for 5 minutes. The spheroids were then washed a final three times in DPBS +/+ and then imaged using a ZEISS CellDiscoverer 7.

RESULTS

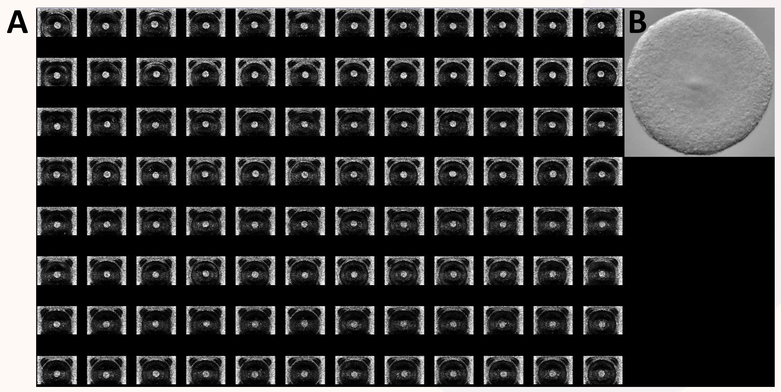

Figure 1: A) Representative image of the microBrain 3D 96 well product. Image has been inverted using the ZEISS CellDisoverer 7 to better visualize spheroids; B) Single spheroid taken at 5X using the ZEISS CellDiscoverer 7.

RESULTS

RNA Expression of Neurotransmitter Receptors

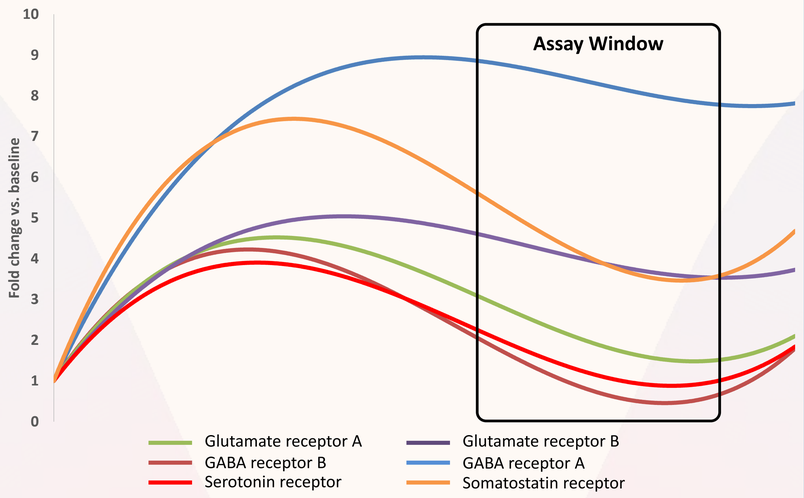

Figure 2: RNA expression of neurotransmitter receptors over time recorded through qPCR. Data was adjusted to Day 0 NPCs as baseline and is represented as fold-change versus baseline over time.

Immunocytochemistry

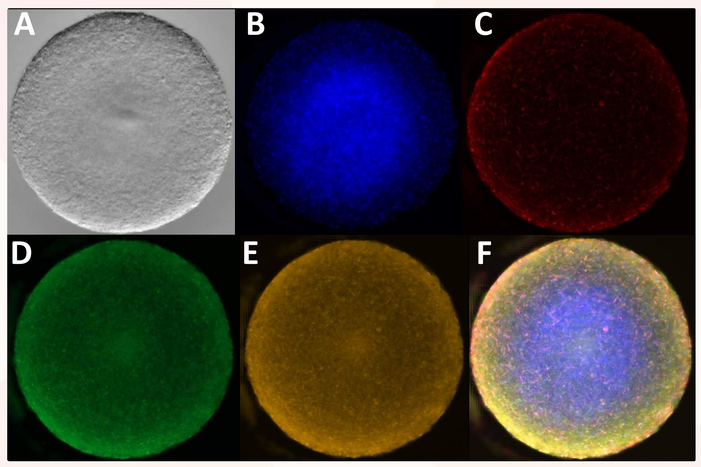

Figure 3: Immunocytochemistry of a 3D 384 spheroid taken at 5X. A) Phase contrast; B) DAPI; C) Glial Fibrillary Acidic Protein (GFAP); D) Synapsin I ; E) Microtubule-associated Protein 2 (MAP2); F) Merge.

Plate Variability

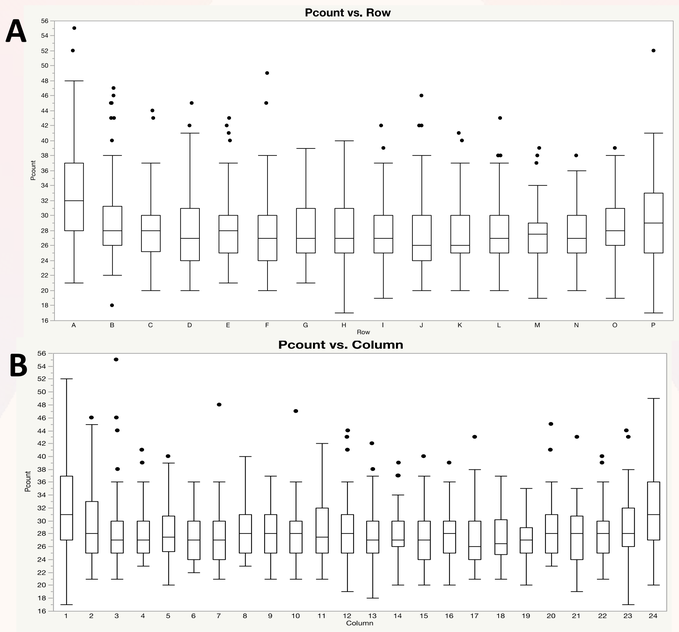

Figure 4: A) Peak count versus row at baseline read; B) Peak count versus column at baseline read. Both figures data captured on the FLIPR Tetra and analyzed in JMP.

RESULTS

Size Distribution

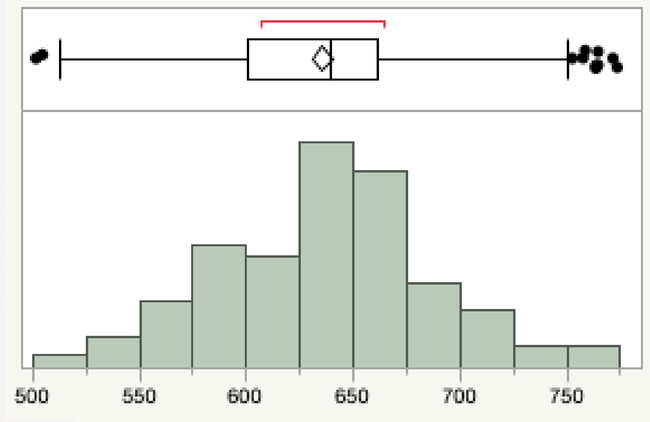

Figure 5: Representative distribution of spheroid size across a 384 well plate. Data acquired using the ZEISS CellDiscoverer 7 and analyzed in JMP.

FLIPR

microBrain 3D response after 30 minutes drug addition across assay window

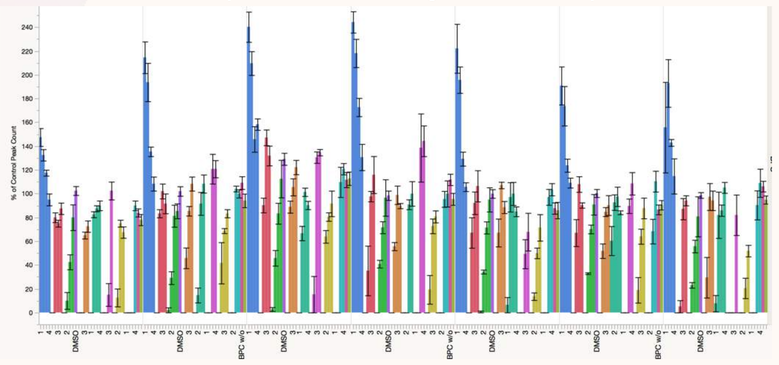

Figure 6: Panel of calcium oscillation modulatory compounds evaluated across product assay window and normalized. Data analyzed using JMP.

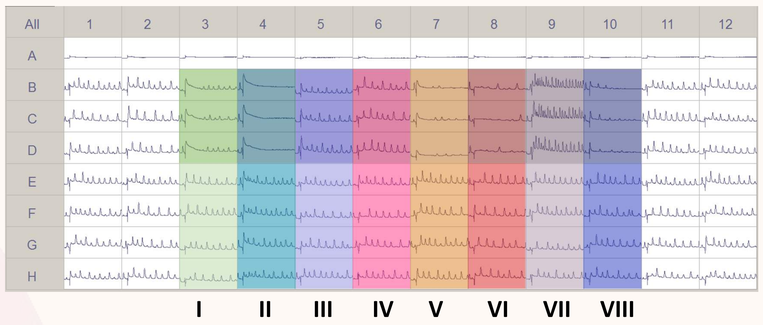

Figure 7: FLIPR trace after 30 minutes of incubation with I) $GABA_A$ receptor agonist; II) Glutamate receptor agonist; III) Glutamate receptor agonist; IV) GABA receptor agonist; V) GABA receptor agonist; VI) AMPA/kainate antagonist; VII) K+ channel blocker; VIII) Glutamate receptor antagonist. Rows B to D contain higher concentration of respective compounds than rows E to H.

CONCLUSIONS

- microBrain 3D provides a physiologically relevant representation of the human cerebral cortex for drug discovery and toxicology.
- microBrain 3D expresses a broad range of neurotransmitter receptors as well as cell markers indicating the presence of mature neurons and astrocytes typically found in the human cortex.
- Plate variability analyses confer a highly consistent product well-to-well and lot-to-lot containing spheroids of stable size.
- microBrain 3D demonstrates a broad spectrum of functionality in response to neurotransmitter modulatory compounds