Human Neural Microtissues Derived from Induced Pluripotent Stem Cells for Toxicity Testing

S. DELAURA1, D. A. FLURI2, R. MARCHAN3, W. MORITZ2, *E. M. JONES1, J. G. HENGSTLER2, J. M. KELM2;
1Cellular Dynamics Intl., Madison, WI; 2InSphero AG, Schlieren, Switzerland; 3Leibniz Res. Ctr. for Working Envrn. and Human Factors, Dortmund, Germany

Abstract

In vivo animal models are primarily employed to assess for neurotoxic effects of chemicals and potential candidate compounds in drug development. In vitro testing is mainly limited to HTS compatible low complexity 2D cultures using cell lines and more sophisticated low throughput explant rodent cultures. Cell sourcing for primary human brain material is inherently difficult and ethically controversial. Recently developed strategies to generate embryonic stem cell- and iPSC-derived pluripotent neuronal and glial cell types offer an invaluable source to create a new generation of in vitro test systems for neurotoxicity, drug discovery, and disease modeling. We report here the generation of a scaffold-free 3D microissue (MT) model derived from human iPSC-derived cell types. The spheroidal aggregates are produced in high throughput compatible hanging drop plates. Owing to the standardized production process, microtissues are highly size consistent and culture handling, such as maintenance, compound dosing or downstream analytics are compatible with robotics. The human brain microtissues consist of iPSC-derived astrocytes in co-culture with iPSC-derived neurons. Microtissue constructs exhibit stable three-dimensional architecture over time periods longer than four weeks and display positive staining for the neuronal markers β-III-tubulin, synaptophysin and the astrocyte marker GFAP. These microissue models provide three-dimensional biological complexity with high reproducibility. When combined with high throughput automation, this in vitro screening-friendly system closely recapitulates in vivo human biology enabling new choices for phenotypic screening.

iCell® Neurons Characterization

Schematic of cortical neuron differentiation process

Identity and purity assessment of iCell Neurons by flow cytometry and high content imaging (HCl). HCl of βIII-Tubulin and Nestin double stain are shown. Flow cytometry for purity characterization is performed by using expression of βIII-Tubulin (pos) and nestin (neg) (98.3%) post-thaw.

Hanging Drop Technology

(A) Schematic representation of the GravityPLUS™ plate. (B) Overview depicting the production of microtissues in hanging drops in the GravityPLUS™ plate and subsequent transfer to the tissue receiver and assay plate (GravityTRAP™).

IPSC-derived Cells Form Stable Microtissues

(A) Overview scan images of human brain MTs in GravityTRAP plate after transfer. (B) Stability of tissue diameters over a four week time course for different initial seeding densities. (C) Immunohistochemical analysis of human brain MT at day 5. Hematoxylin and Eosin (H&E) staining (left), astrocyte specific GFAP staining (middle) and neuron specific βIII-Tubulin staining (right) are shown. (D) Confocal micrograph of whole mount human brain MT stained for βIII-Tubulin (white), GFAP (red) and DAPI (blue).

Conclusions and Outlook

Future strategies to assess neurotoxicity in vitro will largely depend on improved predictivity of the models and on the capacity to increase throughput of such systems. Reported here, neuronal MT provide three-dimensional biological complexity in comparison to conventional monolayer cultures. In combination with a standardized automation-compatible and highly reproducible production process, this adds another tool to the portfolio of in vitro testing systems. Further development of brain MT models will mainly focus on the implementation of functional assays and the adoption of commonly used 2D endpoints to the 3D system.

This work was supported by: E.U. 7th Framework program, FET Open, Project # 266067