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Fine-tuning a brain spheroid model as a screening platform for neuroprotective therapies against ischemic stroke

Aguilar Román, Javier, Romero Bernal, Marina, González Díaz, Ángela, Del Río Mercado, Carmen, Montaner Villalonga, Joan.

Institute of Biomedicine of Seville (IBiS), Hospital Universitario Virgen del Rocío, CSIC, Universidad de Sevilla, 41013 Seville, Spain.

Department of Neurology, Hospital Universitario Virgen Macarena, 41009 Seville, Spain

Tutor académico: Rojas González, Ana Isabel

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ABSTRACT

Motivation: Stroke is the second cause of death and a leading cause of disability worldwide. Ischemic stroke represents more than 80% of all events and it is due to the presence of a clot blocking the blood flow. European legislation has already assumed the goal of no longer needing to use animals for scientific research in the future. To improve current in vitro models, a two-stage 3D brain spheroid model was previously described to recreate the blood-brain barrier and serve as a tool to assess ischemic damage and neuroinflammation. Our objective is to fine tune this organoid model, so we can use it as a screening platform of different neuroprotective therapies against ischemic stroke.

Methods: Six different human brain cells were cultured following manufacturer´s recommendations. In order to be able to visualize each cell type in the 3D model, immunocytochemistry protocols were stablished. Cells were fixed and immunolabeled using a suitable primary antibody for each type of cell followed by the appropriate Alexa 568 secondary antibody. Negative controls were also prepared following the same protocol without the addition of the primary antibody. As a fist approach for spheroid formation, astrocytes, microglia, oligodendrocytes and cortical neurons were co-cultured in round bottom 96 well plates building the spheroid core. After 48h microvascular cells and pericytes were added to the culture creating the surface area. Finally, spheroids images were taken employing bright field microscopy and diameter was measured.

Results: Fluorescent images confirmed that the immunolabeling protocols for each cell type were set up appropriately. Red fluorescence staining indicated cell´s detection by their specific markers. Fluorescent signal was absent in negative controls, confirming the specific binding. Finally, two-stage spheroids (4.000 cells) were formed. After 48, one round-shaped spheroid per well was observed. Spheroid diameter was 313.9± 22 µm after 48 h and 690.9±64 µm after 4 days.

Conclusions: We have fine-tuned the immunocytochemical technique to identify each cell line that will be used to analyse brain spheroids. The first trial for spheroid formation showed homogenous shape and size. Next step would be testing smaller spheroids (2.000 cells) and verifying that each cell type is correctly located in the 3D structure. If results are satisfactory these spheroids could be used as a tool to test potential neuroprotective therapies.

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