Exploring human Stem Cells to mimic neural microenvironment

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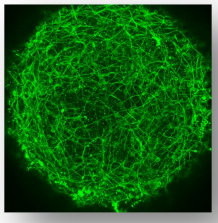
SUMMARY :

The demand for robust and predictable human in vitro models that can bridge the gap between preclinical and clinical stages of drug discovery, and contribute to increase the understanding of human diseases, is steadily increasing. Neural fate and functionality are highly regulated processes that integrate a wide range of external cues, such as nutritional status, growth factors, mechanical stress, cell-cell and cell-extracellular matrix (ECM) interactions. Indeed, brain microenvironment plays an important role in neurodevelopment, function and degeneration.

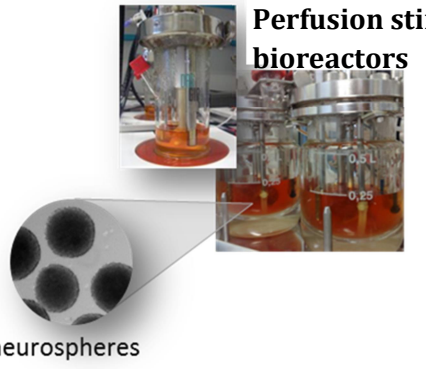
We employ perfusion stirred-tank bioreactors for 3D neural differentiation of an array of human stem cell sources. This strategy induces neural progenitor cell aggregation and subsequent differentiation into complex tissue-like structures with reproducible ratios of neurons, astrocytes and oligodendrocytes. The generated neurons elicit spontaneous calcium transients and stimuli-induced neurotransmitter release. Under whole-cell current-and-voltage clamp, recordings showed polarized neurons and voltage-dependent ionic currents. Differentiated glial cells presented astrocytic functions, such as glutamate clearance and glutamine synthesis. Moreover, gene expression of synaptic and ion transport machinery, as well as accumulation of neural proteoglycans suggests that this 3D differentiation strategy better mimics neural tissue microenvironment than other differentiation methods currently available.

Applications of these models as tools for preclinical assessment and in disease modelling will be discussed.

ILLUSTRATION :



differentiated neurosphere: beta-III tubulin (green)



Perfusion stirred-tank bioreactors

neurospheres

KEYWORDS :

3D cell models human stem cells stirred-tank bioreactors neural differentiation extracellular matrix

REFERENCES

Gualda EJ et al (2014) “Imaging of human differentiated 3D neural aggregates using Light Sheet Fluorescence Microscopy”, *Frontiers in Cellular Neuroscience,* 8, 221.

Simão D et al (2016) “Evaluation of helper-dependent canine adenovirus vectors in a 3D human CNS model”, *Gene Therapy* 23, 86-94.

Simão D et al (2016) “Functional metabolic interactions of human neuron-astrocyte 3D in vitro networks” *Scientific Reports* 6, 33285.

Simão D et al (2016) “Perfusion Stirred-tank Bioreactors for 3D differentiation of Human Neural Stem Cells” Bioreactors for Stem Cells Biology: Methods and Protocols - Methods Mol Biol. 1502, 129-42 , Springer Science.