ENGINEERING HUMAN BRAIN ORGANOIDS FOR INVESTIGATING 3D NEURONAL ANATOMY AND CIRCUITRY.

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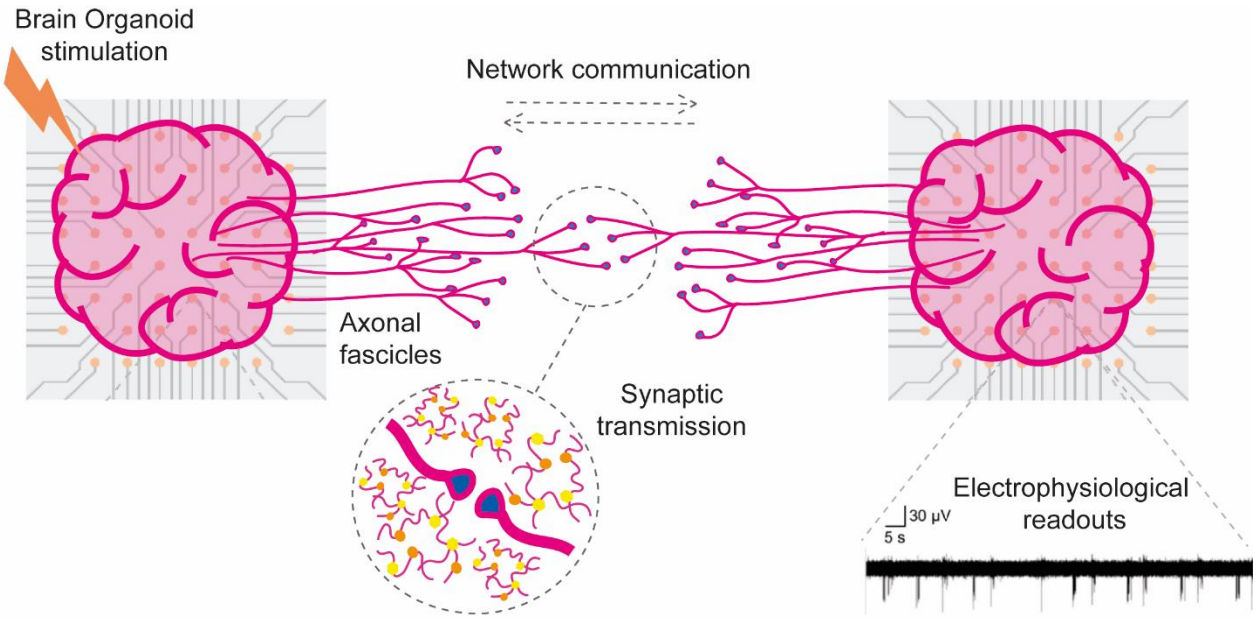

The brain is the most sophisticated and fascinating organ in the human body. Its intricate structure includes around 86 billion of electrically excitable neurons, supported by glial cells, working together to process and transport information controlling the various function of the body.[1] Neurons communicate through the synapses in a highly dynamic way: the electrical signal propagating along the axon of a presynaptic neuron (action potential) is transduced into a chemical signal, which is sensed by the post-synaptic cell and transduced once again in a new electrical signal (postsynaptic potential). [REF] Importantly, it is widely known that the neuronal extracellular matrix components are actively involved in various fundamental processes providing physical and biochemical cues guiding cell proliferation, differentiation and migration and mediating the formation of the intricate neuronal network. [2]

In the last decades, several engineering approaches based on 2D culture systems have been implemented to investigate neuronal behavior, trying to mimic the ECM biochemical and biophysical cues.[3] For instance, previous works have demonstrated how positively or negatively charged surfaces can electrostatically interact with neuronal cells as well as extracellular matrix (ECM) components-coated surfaces can mediate their attachment and growth. [4], [5]However, these strategies still hardly resemble the extreme complexity of the native brain environment, in which a plethora of signals are continuously and dynamically presented to the cells.

In this scenario, inspired by the complexity of the human brain, several methods have been introduced to surpass the shortcoming of 2D *in vitro* models, robustly replicating the brain microphysiology. Here, induced pluripotent stem cells (iPSCs) have found wide application in neurobiology, mediating the development of 3D systems such as spheroids, organoids, micro-tissue engineered neural networks (microTENNS) mimicking the human brain *in vitro*.[6], [7]

Such new and complex platforms may allow to study the molecular and cellular mechanisms characterizing the intricate neuronal network in the brain, paving the way towards the development of novel therapeutics. Notably, the brain functionality is based on the interconnectivity between cortical areas by axons extending from one region to another. Indeed, recent studies have replicated this connection by fusing or connecting organoids mimicking the axonal connection between multiple regions of the brain. [8], [9]In this context, the aim of this PhD project is focused on the engineering of a brain organoids-based platform to investigate neuronal cells interaction and connectivity. Briefly, brain organoids will be obtained starting from human iPSCs-derived neural stem cells (NSCs) (iXCells Biotechnologies, USA) which will be differentiated into mature neurons within 4 to 6 weeks. [10] The iPSCs-derived NSCs differentiation protocol will be optimized and validated by analysing the presence of neuronal markers, such as microtubule associated protein-2 (MAP2) and tubulin- βIII. The differentiated neuronal organoids will be placed into a dedicated microfluidic device made of two chambers connected via a microchannel whose function is to guide the spontaneous formation of axon fascicles which extend reciprocally between the two organoids. Once optimised the connection between the two organoids, the synaptic transmission alongside the axon fascicles will be evaluated. Thus, one organoid will be electrically stimulated, and the response of the other will be measured by means of multi electrode array (MEA) systems.[11] Importantly, considering the role of the glia during neural development [1], the later step will include the incorporation of glial cells to further replicate the native brain microenvironment. These cells will create a sort of matrix which support and mediate the connection between NSCs-derived organoids. We expect that such 3D systems in which connected brain organoids can actively communicate, may pave the way towards the implementation of *in vitro* platforms to deeply investigate and characterize neuronal behavior while exploiting the advantages of recreating a highly physiological microenvironment.

References

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