Interconnected human brain spheroids for investigating synaptic plasticity of neuronal circuitry

Chiara Ausilio[123], Annachiara Scalzone[1], Paolo Antonio Netti[123]

[1] Center for Advanced Biomaterials for Healthcare (CABHC), Istituto Italiano di Tecnologia (IIT), 80125 Naples, Italy.
[2] Dipartimento di Chimica Materiali e Produzione Industriale (DICMaPI),Università di Napoli Federico II, 80125 Naples, Italy.
[3] Interdisciplinary Research Centre on Biomaterials (CRIB), Università di Napoli Federico II, 80125 Naples, Italy.

e-mail: chiara.ausilio@iit.it

Introduction and Approach

Three-dimensional (3D) brain models allow to replicate molecular aspects of learning and memory processes in *in vitro* platforms. Such systems, mimicking the human brain miroenvironment, may be considered as promising approach to develop effective and patient specific treatments for neurodegeneration.[1] Our research is focused on the implementation of interconnected brain spheroids-based platform to delve into the biological feedback loop of learning systems.

Materials and Methods

Neuronal spheroids were obtained from human iPSCs-derived neural stem cells (NSCs). They were differentiated and interconnected within a dedicated microfluidic device (Fig. 1A) 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 spheroids.[2] Once the connection is established, high density multi-electrode arrays (HD-MEAs) will further unveil the mechanisms governing learning processes within these interconnected systems. (Fig. 1B) Stimulus-response training will be used to investigate the communication between interconnected spheroids and, more importantly, the adaptation of neuronal networks in response to applied stimuli.

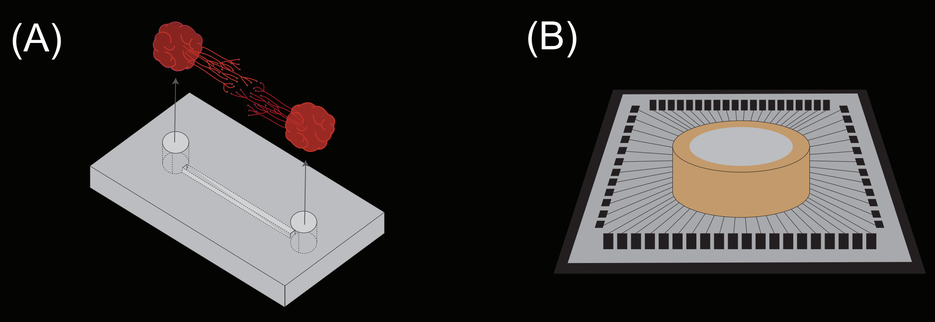

Figure 1. (A) Microfluidic device used to connect brain spheroids. (B) High density multi electrode array (HD-MEA) device used to monitor the electrical acitvity of interconnected brain spheroids.

Results

Spheroids differentiation

The NSCs differentiation protocol was validated by analysing the presence of neuronal markers (Fig. 2) , such as Nestin, specific for progenitor cells, and tubulin- βIII, specific for microtubules, after (i)1, (ii) 3,(iii) 4 and (iv) 5 weeks of culture.

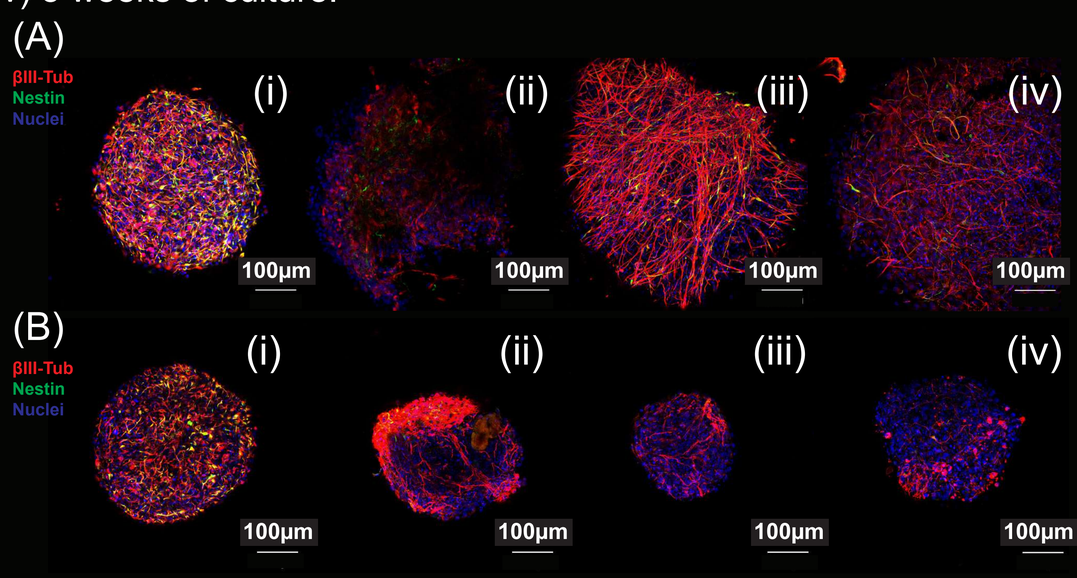

Figure 2. Fluorescence micrographs of NSCs-derived spheroids. Cells were staneid against neuronal markers (A) before and (B) after the differetiation process. The images were acquired at four different time points: (i) 1,(ii) 3,(iii) 4 and (iv) 5 weeks.

Ca^{2+} wave propagation

The electrical activity of neurons constituting the spheroids was investigated by means of Ca^{2+} imaging after 4 weeks of differentiation (Fig. 3A). The dynamics of the fluorescence intensity profiles was also evaluated (Fig. 3B).

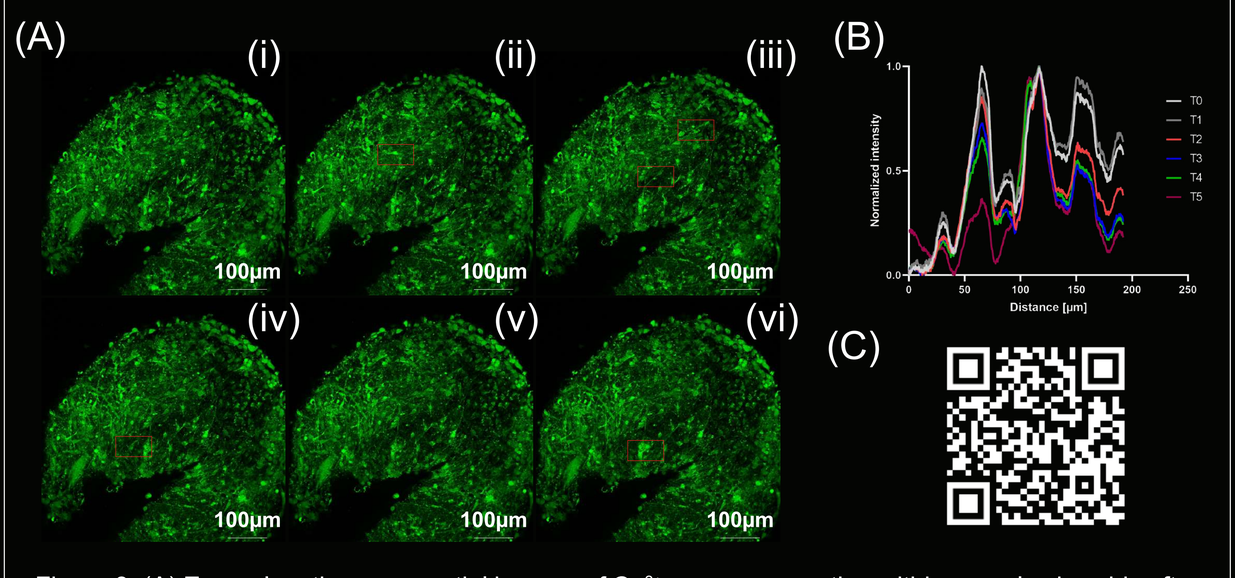

Figure 3. (A) Exemplary time-sequential images of Ca^{2+} wave propagation within neural spheroids after 4 weeks of differentiation. Cells were labelled with Fluo-4 AM. Each frame was acquired after 0.10s (i-iii). Red squares indicate the propagation of the Ca^{2+} wave. (B) Fluroescence intensity profiles were calculated for each frame. (C) Scanning QR-code for the video of the calcium wave prorpagation.

Brain spheroids connection

Brain spheroids were differentiated into a microfluidic device (Fig. 4A). Cells were labbeled using Calcein-AM dye, cell-permeant dye determing cell viability. Fig. 4B shows a single neuronal spheroid within the chamber of the microfluidic device. The images display how cell growth and neurite sprouting appear to be guided by the presence of the microchannel.

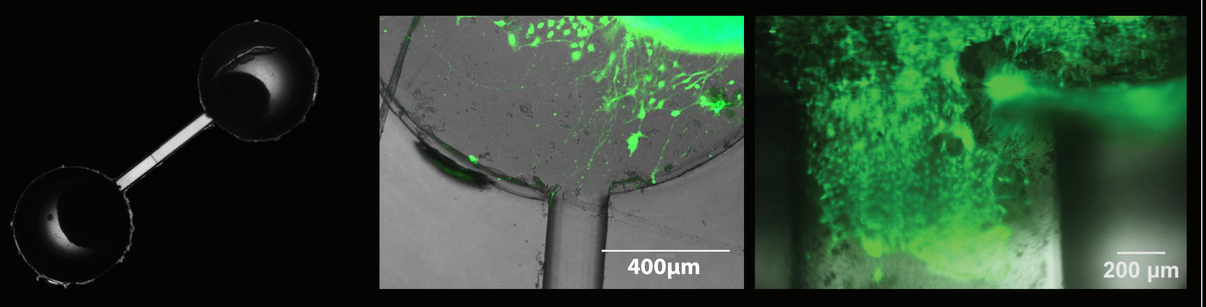

Figure 4. (A) Images of two differentiated spheroids cultured into the microfluidic device. (B) Fluorescence image of brain spheroids cutlutred into the mirofluidic chamber (calcein-AM in green) during the differentiation process.

Brain spheroids on HD-MEA

Brain spheroids were cultured on HD-MEAs to monitor their electrical activity after the differentiation (Fig. 5A). Cell viability was monitored by using Neurofluor membrane-permeable fluorescent probe that selectively labels neurons in live cultures (Fig. 5B).

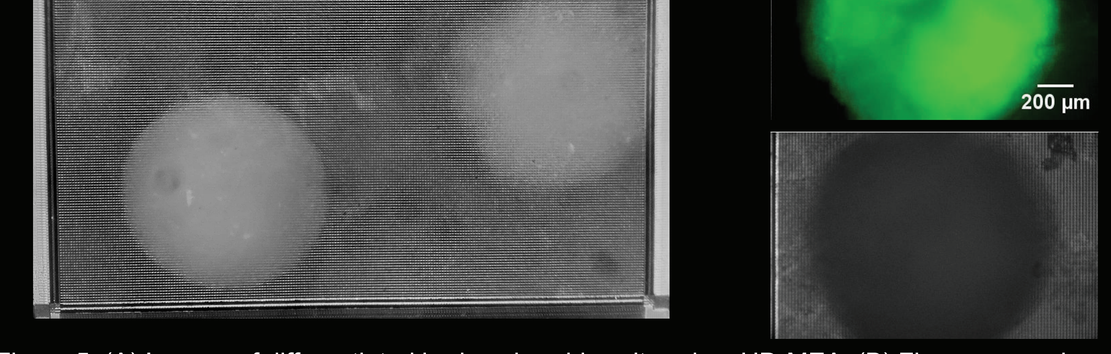

Figure 5. (A) Images of differentiated brain spheroids cultured on HD-MEA. (B) Fluorescence image of living differentiated spheroids cultured on electrical devices (Nuerofluor in green) after two weeks of cutlure.

Conclusions and future perspectives

Interconnected brain spheroids may be used to recapitulate the complexity of the native brain microenvironment, paving the way towards the implementation of 3D *in vitro* platforms, providing realiable models to deeply investigate and characterize learning and memory process, while exploiting the advantages of recreating a highly physiological microenvironment.

References
1. L. Smirnova et al., «Organoid intelligence (OI): the new frontier in biocomputing and intelligence-in-a-dish», Front. Sci., vol. 1, p. 1017235, feb. 2023, doi: 10.3389/fsci.2023.1017235.
2. T. Kirihara et al., «A Human Induced Pluripotent Stem Cell-Derived Tissue Model of a Cerebral Tract Connecting Two Cortical Regions», iScience, vol. 14, pp. 301–311, apr. 2019, doi: 10.1016/j.isci.2019.03.012.