3F(eature)s model: modularity, heterogeneity and three-dimensionality to design *in vitro* neuronal model

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Abstract

In this work, we present a novel experimental platform to build *in vitro* interconnected (*i.e.*, modular) heterogeneous (*e.g.*, cortical-hippocampal) and three-dimensional (3D) neuronal cultures plated on Micro-Electrode Arrays (MEAs) to extracellularly record the electrophysiological activity continuously.

Introduction

The human brain is the most complex organ of our body. It gives rise to all our thoughts, actions, memories, feelings, and experiences. However, because of such high complexity, understanding human physiology as well as pathogenesis is not straightforward. Set-ups for *in vivo* studies are often very complicated, time consuming, and with low reproducibility. For this reason, there is the need to develop new *in vitro* systems capable of mimicking as much as possible the human brain. In addition, a successful model would minimize animal use for drug screening applications, deliver a highly reproducible system, and significantly lower costs in light of the current demand for pharmacological development.[1] In this work, we go beyond state of art by presenting an innovative experimental platform to realize interconnected (*i.e.*, modular) heterogeneous (*i.e.*, cortical-hippocampal) 3D neuronal cultures plated on MEAs whose electrodes are arranged in a four-cluster displacement (Figure 1b).

The possibility to have a 3F(eature)s *in vitro* model will pave the way to design realistic brain-on-a-chip models to be exploited for both basic science as well as clinical applications.[2] In this work, we show the effect of the 3Fs in cortical-hippocampal *in vitro* assemblies.

Materials and Methods

We used a polymeric device to recreate modular heterogeneous cultures.[3] It consists of two compartments separated by parallel microchannels that prevent the movement of cell bodies between them. The dimensions of the device are reported in Figure 1a. To create a biocompatible scaffold where neuronal networks can grow in a 3D fashion, we used pre-sterilized and precoated glass microbeads (Ø = 40 µm, Distrilab-Duke Scientific, Thermo Fisher). About 4-6 layers were stacked, reaching a height of about 220 µm. The microbeads were placed onto a Transwell® porous membrane (Costar Sigma) where they self-assembled forming a uniform layer. Then, dissociated cells were seeded on the monolayer of microbeads. After 8 hours, the suspension of neurons and microbeads was moved from the Transwell® membrane and deposited over the 2D neuronal network previously plated on the MEA (Figure 1c). The 2D neuronal network was directly plated on the active area of the MEA to ensure good communication between the substrate-embedded electrodes and the 3D assembly.

To quantify the effects of the modular and 3D connectivity, as well as heterogeneity, we compared the 3D cortical-hippocampal (Cx-Hp_{3D}) configuration with 2D homogeneous cortical-cortical (Cx-Cx_{2D}), hippocampal-hippocampal (Hp-Hp_{2D}) and heterogeneous cortical-hippocampal (Cx-Hp_{2D}) controls and with 3D homogeneous (Cx-Cx_{3D} and Hp-Hp_{3D}) ones. We recorded 20 minutes of spontaneous activity from *n*= 5 MEAs after 21 days *in vitro* (DIVs) for each configuration.

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Results

We firstly assessed the electrophysiological activity of Cx-Cx and Hp-Hp networks. We observed a higher value of mean bursting rate (number of bursts per minute, MBR) in hippocampal networks than in cortical ones ($p = 0.009$), as of the percentage of random spiking activity (spike outside the bursts, %RS, $p = 0.009$). On the contrary, cortical bursting activity displayed a higher number of spikes inside the bursts (SxB, $p = 0.009$). Such results are reported in Table 1. The differences between cortical

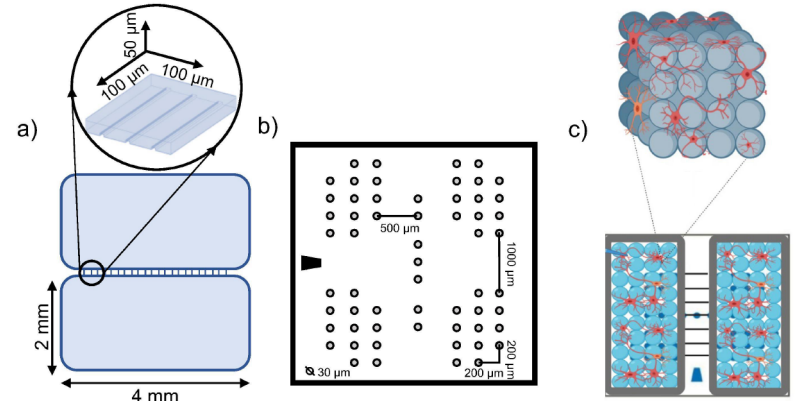

Figure 1. Sketch of the *in vitro* model: a) PDMS mask details; b) MEA-4Q active area configuration; c) 3D cell cultures made up of glass microbeads.

Table 1.

BURSTING PARAMETERS FOR THE DIFFERENT EXPERIMENTAL CONFIGURATIONS. DATA ARE AVERAGED OVER THE REALIZATIONS NUMBER OF EACH DATASET.

	Cx-Cx$_{2D}$	Cx-Cx$_{3D}$	Hp-Hp$_{2D}$	Hp-Hp$_{3D}$
MBR	8.6 ± 3.2	8.0 ± 2.2	19.9 ± 4.0	23.5 ± 13.1
BD	200.3 ± 66.8	343.9 ± 112.0	114.1 ± 41.6	139.2 ± 41.9
SxB	24.2 ± 4.2	67.1 ± 22.0	9.8 ± 1.5	15.5 ± 3.9
%RS	14.7 ± 6.3	5.4 ± 3.9	36.1 ± 9.2	21.0 ± 6.4

and hippocampal activity observed in the 2D configuration are also kept in the 3D configuration, indicating an intrinsic natural firing pattern of each neuronal family.[4]

Then, we identified the role of three-dimensionality (Cx-Cx$_{3D}$) and the hippocampal input (Cx-Hp$_{2D}$) on the cortical networks separately. The only presence of the 3D configuration strongly increased the cortical ensemble's spiking rate (+118%). On the other hand, hippocampal neurons induced a pronounced increase in the spiking (+184%) and bursting (+178%) activity in the cortex, suggesting the role of the hippocampal neurons as master. Moreover, hippocampal neurons modulated the network burst duration (NBD), independently from the spatial organization (in 2D −55%, and in 3D −56%). These findings were supported by the dynamics of the hippocampal activity, which displayed a shorter and faster bursting activity than the cortical one.

Considering the network bursting activity is mediated by the choral activity of the excitatory and inhibitory pathways of the network,[5] we investigated the different phases (*i.e.*, rise and decay) of the network bursts. In particular, we tried to understand how the simultaneous presence of the 3D topology and the hippocampal input modulated the population events shape. We found the neuron recruitment (rise phases, strongly regulated by the massive enrollment of the excitatory network) is fast ($\tau < 1$ ms), independently from the network organization. More interesting was the decay phase modulated by the GABAergic population. We observed a faster decay behavior in the heterogeneous cultures that could imply an inhibitory nature of the connections from the hippocampal assembly to the cortical one, which can shorten the duration of the network burst.

Discussion and Conclusions

Modularity, three-dimensionality, and heterogeneity are the 3Fs that an *in vitro* model should simultaneously embed to recreate *in vivo*-like dynamics and provide a valid experimental model for neuropharmacological applications. In perspective, such an experimental platform (that can be expanded to accomplish more than two interconnected populations) will allow understanding how brain cells are connected and interact and how neurological disorders impair these connections or destroy small/medium neuronal assemblies. The possibility of having an *in vitro* test-bench, where different neuronal populations are functionally connected, can also help in predicting how cells are responsive to drugs and in determining which neuronal families are more reactive to such drugs.

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