Detection of network burst activity in a MEMS-based 3D neuronal cell culture system

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This work presents the analysis of network burst (NB) activity in a microfluidic brain-on-chip (BoC). To measure long-term 3-dimensional (3D) neuronal network dynamics, it is crucial to provide appropriate compound exchange from fresh medium in a flow induced stress-free environment, which has been implemented by the microbioreactor design originally described by Schurink and Luttge[1]. Figure 1 illustrates the device schematics (a) and final assembly (b). Cells inside Matrigel™ were seeded into the culture chamber. Medium was loaded into microfluidic channel, encircling the culture chamber, to provide nutrients to cells through the PES tube and for waste removal. As a preliminary result, previously, we reported that human induced pluripotent stem cells (hiPSCs)-derived cortical neurons can be cultured up to 21 days-*in vitro* (DIV) by using this system. It was shown that spontaneous spikes, having 100µV amplitudes, occurred at 7DIV. Moreover, burst activity, consisting of higher amplitude (400µV) spikes, was observed at 14 and 21DIV[2]. Here, our extended electrophysiological analysis, including time points of 8, 26, 27, 29, 33, and 37 days, revealed significant change in bursting patterns as of day 14, when such a culture is performed in our MEMS-based 3D culture system using Matrigel™ as a scaffold. Figure 2 demonstrates spike (a), burst (b), and NB rates (c) obtained over 37DIV and fixed cells (d) at day 18 by presenting neurons in 3D. A collection of activities in these electrophysiological recordings provided strong clues for the maintenance of hiPSC-derived cortical neuron cultures in 3D.

3D cell culture models fill the gap between animal and 2D cell culture studies and harness the potential to reduce the extensive use of animals in (bio)medical studies. Despite all these benefits, quantitative data collection and interpretation of 3D cell cultures has been challenging by traditional microcopy techniques[3]. The introduction of MEA for electrophysiological recordings of dynamic behavior of hiPSC-derived cortical neurons in 3D cultures are the foundation for further advances in microfluidic BoC technology, including drug testing, in a label free and non-invasive manner.

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[3] F. Pampaloni, E. G. Reynaud, and E. H. K. Stelzer, Nature Reviews Molecular Cell Biology, **8**, (2007), pp. 839–845.

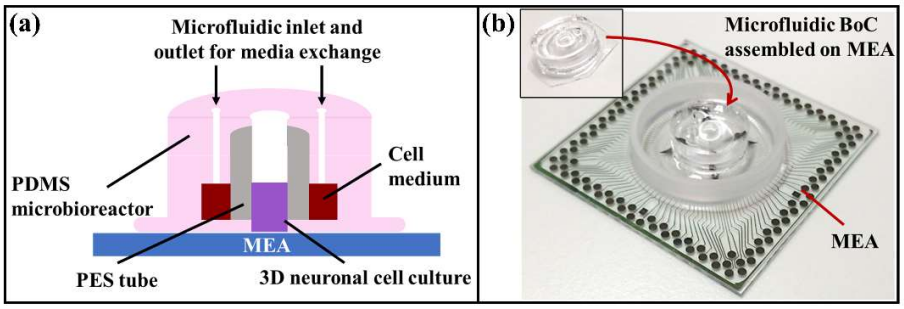

Figure 1: (a) Schematic of microbioreactor and (b) assembled MEMS-based 3D cell culture system, fabricated by using soft lithography of polydimethylsiloxane (PDMS) in a mechanically machined poly (methyl methacrylate) (PMMA) mold and porous polyether sulfone (PES) cylindrical membranes. Measurements were carried out via 2100MEA system (Multi Channel Systems GmbH, Germany) by using 120 electrodes MEA.

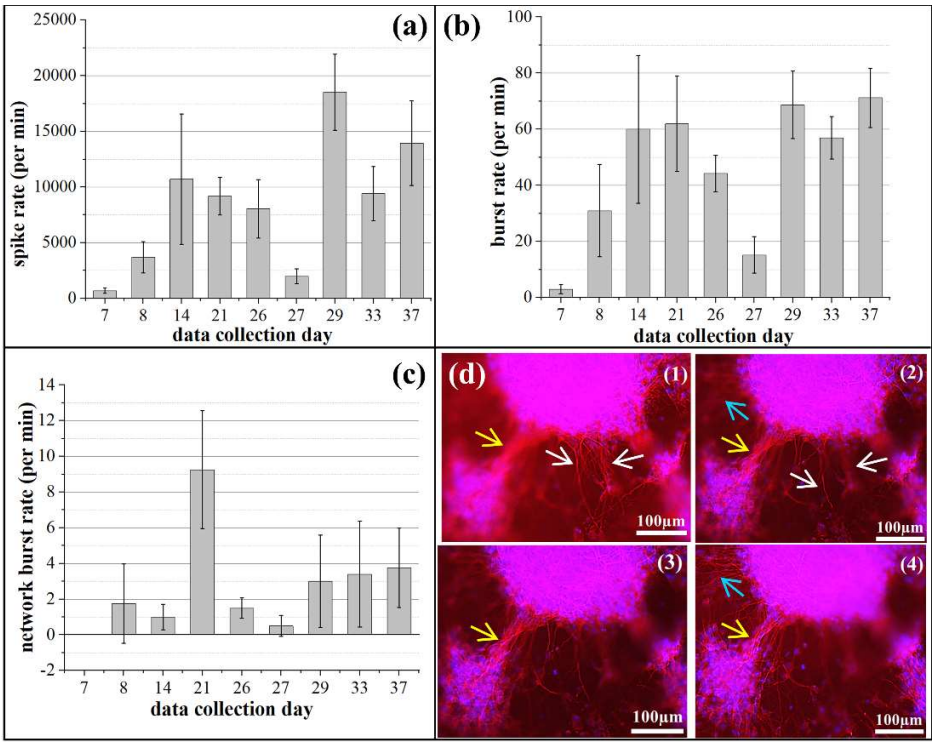

Figure 2: Electrophysiological recordings of hiPSC-derived cortical neurons in 3D, presenting the quantity of spikes (a), bursting rate (b), and NB rate (c). As early as 8DIV, network between neurons were observed. Data is presented as mean ± standard deviation. In overall, all means are significantly different at p=0.05 level (n=1, One-way ANOVA test, 4-5 measurement at each day). (d) Fixed cell images showing MAP2 (red) and DAPI (blue) stained neurons in 3D. Yellow, white, and cyan arrows indicate appearing and disappearing parts of neurons obtained by z-stack setting from bottom (1) to top (4) of 3D culture.