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Microfluidic Chip For *In Vitro* Neuronal Cell Culture under Electrical Stimulation

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Extended Abstract

Microfluidic technologies have increasingly been used in neurosciences research to extend in vitro experimental capabilities[1], [2]. Hence, the main goal of this research work is developing an electrically stimulating microfluidic platform that allows performing axon outgrowth monitoring assays for high throughput screening by means of roll-to-roll (R2R) based manufacturing process.

The chip consists of two layers. The bottom layer is a screen-printed electrode layer, and the top layer consists of a polymer foil with macrochannels and microchannels microstructured design. This top layer has two separated channels in which cells are seeded and allow the monitoring of axons outgrowth through the designed microchannels using fluorescence microscopy. This chip can be electrically stimulated by a novel platform for multichannel electrical stimulation of cells that has also been developed. This electrical stimulus generator has been named Stim4Cell and operates in two modes: constant or pulsed current and voltage-controlled stimulation. Output current values range from 0.001 to 10 mA, supporting pulse frequencies from 10 Hz to 400 Hz with minimal pulse width of 50µs. The seize of the device is 14 cm x 8,5cm x 3 cm. Besides, an Android application that enables tuning of protocols for cell stimulation has been also developed for the Stim4Cell stimulator. Using this application, the current amplitude of the pulse can be individually set for each of the stimulating chambers, while pulse width and stimulation frequency are set for all pads. The device allows simple interface to the multi array electrodes designed for the well plate via the ribbon cable. It is designed to operate in the incubator under specific CO_2 and temperature conditions.

The stimulating platform was tested on NE-4C neuronal cells. Neuronal cells respond to electrical stimulation in two ways: by increasing their cell size and by increasing the cell number in culture. Following an analysis of the literature [3], [4], stimulation protocols were established with a frequency of 100 Hz and a pulse width ranging from 300 to 350 µs. Stimulation was conducted for 3 hours per day. NE4C cells were seeded and 24 h later, electrical stimulation was applied using Stim4Cell stimulator device. A 3-day protocol, providing a stimulus during 3h of 100Hz, and 300µs pulse width

showed that the cells elongated in a voltage-dependent manner. Controlling both the amperage and the stimulus duration, the platform can elicit a robust response in the cells, indicating that the pioneered chips can be used as a tool for monitoring in vitro neuronal models.

References

[1] H. U. Lee, A. Blasiak, D. R. Agrawal, D. T. B. Loong, N. V. Thakor, A. H. All, J. S. Ho, and I. H. Yang, "Subcellular electrical stimulation of neurons enhances the myelination of axons by oligodendrocytes.," *PLoS One*, vol. 12, no. 7, p. e0179642, 2017, doi: 10.1371/journal.pone.0179642.

[2] F.A Shaik, S. Ihida, Y. Ikeuchi, A. Tixier-Mita and H. Toshiyoshi. "TFT sensor array for real-time cellular characterization, stimulation, impedance measurement and optical imaging of in-vitro neural cells.," *Biosens. Bioelectron.*, vol. 169, p. 112546, Dec. 2020, doi: 10.1016/j.bios.2020.112546.

[3] A. T. Nguyen, S. Mattiassi, M. Loeblein, E. Chin, D. Ma, P. Coquet, V. Viasnoff, E. H. T. Teo, E. L. Goh and E. K. F. Yim, "Human Rett-derived neuronal progenitor cells in 3D graphene scaffold as an in vitro platform to study the effect of electrical stimulation on neuronal differentiation," *Biomed. Mater.*, vol. 13, no. 3, 2018, doi: 10.1088/1748-605X/aaaf2b.

[4] J. S. Jang, C. I. Choi, J. Yi, K. Butters, I. Kim, A. Bhagwate, J. Jen and S. Y. Chang. "High frequency electrical stimulation promotes expression of extracellular matrix proteins from human astrocytes," *Mol. Biol. Rep.*, vol. 46, no. 4, pp. 4369–4375, 2019, doi: 10.1007/s11033-019-04890-9.