Hydrogel-based Microfluidic Chip for Site-specific Chemical Treatment of 3D Neuronal "Opto-nets"

Sanjeev Kumar Mahto, Anat Marom, Josué Sznitman, and Shy Shoham

Recent advancement in hydrogel scaffolds has created tremendous opportunities to manipulate and understand cellular behavior in more realistic physiological conditions [1]. Particularly, neurons encapsulated in hydrogel matrices have emerged as promising *in vitro* models of neuronal tissue that closely mimic the realistic and complex three-dimensional (3D) brain's cytoarchitecture [2]. In addition, hydrogel-based sophisticated *in vitro* model systems are envisaged to be powerful screening platforms for toxicity testing and drug discovery applications [3]. However, the selective chemical treatment of the micro-environments of such artificial neuronal tissues remains a major challenge. Here, we present the design, fabrication and application of a novel microfluidic neuronal device for site-specific chemical treatment of 3D brain-like neuronal networks. The device consists of a bottom well-like base for growing the neuronal culture directly in a transparent 3D hydrogel matrix (Matrigel, BD Biosciences), separated by a microporous high density polyethylene terephthalate (PET) membrane (3 µm pore size) at the mid-section from a patterned arrays of microchannels on top (Fig. 1a), and allowing for a localized exposure or site-specific treatment of the neuronal population to drugs/toxicants (Fig. 1b). The microfluidic platform was characterized and validated for optimal 3D culturing of dissociated cortical neurons that we harvested from embryonic day 18 (E18) Sprague-Dawley rats. Viral agents carrying the genetically encoded calcium indicator GCaMP6 were used to transfect the neurons embedded in a hydrogel matrix in order to optically monitor the activity patterns in the network (Fig. 1c). Neuronal networks were imaged in 3D using a fluorescence microscope equipped with a piezoelectric actuator capable of moving the objective lens to multiple focal planes. To test the efficacy of the device, several drugs were locally introduced through one of the top channels resulting in spatially-localized alteration in the activity patterns of the 3D neuronal network. Altogether, hybrid microfluidic-hydrogel platforms that combine bio-realistic growth conditions and optical access hold potential for high-throughput toxicity testing, drug discovery and point-of-care applications.

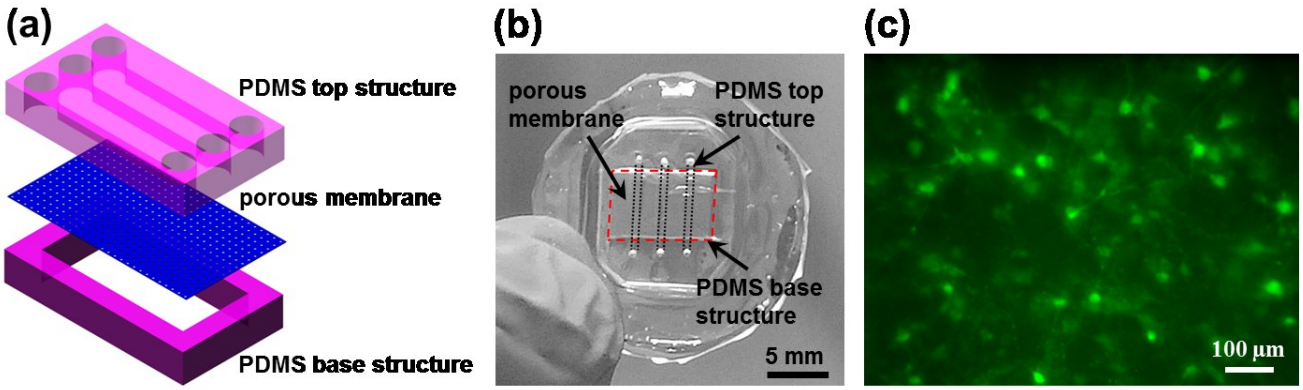

Figure 1. (a) Schematic illustration of the fabrication process of a microfluidic neuronal device that is composed of three basic components. (b) Structure of an assembled microfluidic device. (c) Fluorescent image of neurons encapsulated in hydrogel scaffolds and transfected with a genetically encoded calcium indicator, GCaMP6 (transfection at 1 DIV followed by incubation for 13 DIV).

REFERENCES

[1] O. Sarig-Nadir, N. Livnat, R. Zajdman, S. Shoham, and D. Seliktar, "Laser photoablation of guidance microchannels into hydrogels directs cell growth in 3-D," *Biophys. J.*, vol. 96, pp. 4743-4752, June 2009.

[2] Y. Fan, F. Xu, G. Huang, T. J. Lu, and W. Xing, "Single neuron capture and axonal development in three-dimensional microscale hydrogels," *Lab Chip*, vol. 12, pp. 4724-4731, Nov. 2012.

[3] S.-F. Lan, and B. Starly, "Alginate based 3D hydrogel as an *in vitro* culture model platform for the toxicity screening of new chemical entities," *Toxicol. Appl. Pharmacol.*, vol. 256, pp. 62-72, Oct. 2011.

This work was supported in part by the ERC Starting Grant #211055.

S. K. Mahto, A. Marom, J. Sznitman and S. Shoham are with the Technion-Israel Institute of Technology, Haifa 32000, Israel (corresponding author to provide phone: +972-4829-4125; fax: +972-4829-4599; e-mail: sshoham@bm.technion.ac.il).