Neuronal Network High Throughput Screening Device

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Neuro-degenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis (ALS) are ty pical brain intractable diseases, for which cause nor treatment method is unknown in spite of more than h undreds years of researches. I think that the most important reason for this situation is due to nonexiste nce of suitable excellentdevices for the cause analysis and drug development.
Synapse currents from the neuronal network were successfully observed by the incubation type planar patch clamp [1] for the first time at 2014 [2], To use this measurement for the high throughput screening based on planar patch clamp, it is required to develop the well-defined neuronal network, since the network characteristics must be well understood among the multi point of measurements. However, it is very clear that to form the well-defined network is almost impossible, since it is known that one neuron forms more than 1000 of synapses with surrounding neurons self-organizingly (randomly). To overcome this difficulty, We have proposed "partially controlled neuronal network", where single pyramidal cell locates inside of the targeted cell cage and the random network is formed around the cell cage. Here, the micro through hole for the channel current measurements is formed inside of the cell cage. We have formed the sensor chip with the cell cage patterns (Fig.1) using several instruments supported by "Nanotechnology platform of Monbukagakusho" installed at Nagoya University and Institute for Molecular Science. An example of the neuronal network formed by low density culture with setting a single cell on the micropore inside of the cell cage is shown in Fig. 2.
The planar patch clamp chip with neuronal network on its surface was set on the stage of the patch clamp device shown in Fig. 3. The stage is designed so that the position alignment between the micro-pore of the chip and the micro-fluidic circuit formed in the stage is automatically attained.
Using the device shown in Fig. 3, spontaneous synapse currents such as mEPSC and mIPSC have been successfully observed. The quality of the current recording was equivalent to that of pipette patch clamp reported.
As a future plan, we are going to construct the 96 well device which contribute to the high throughput screening applications by arranging 96 unit devices shown in Fig. 3.

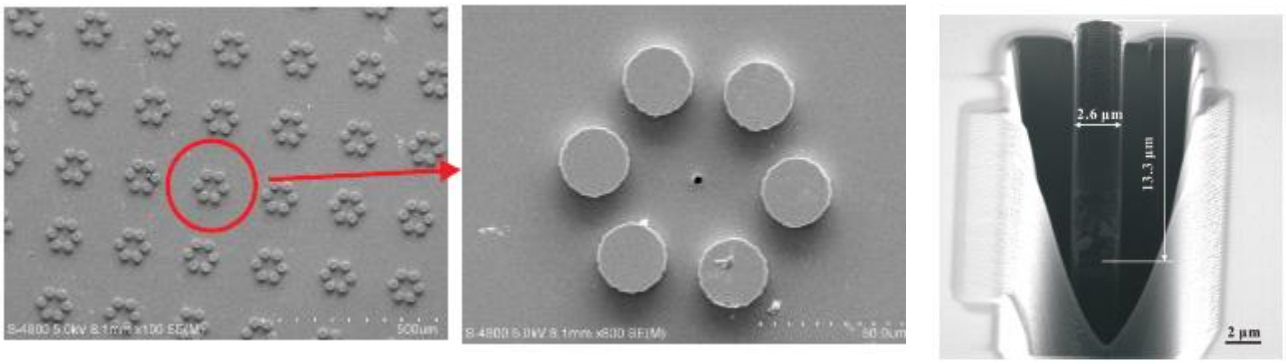

Fig. 1 The top side view of the cell cage pattern on the SOI substrate observed by SEM (left). Cross sectional view of the micropore observed by FIB (right).

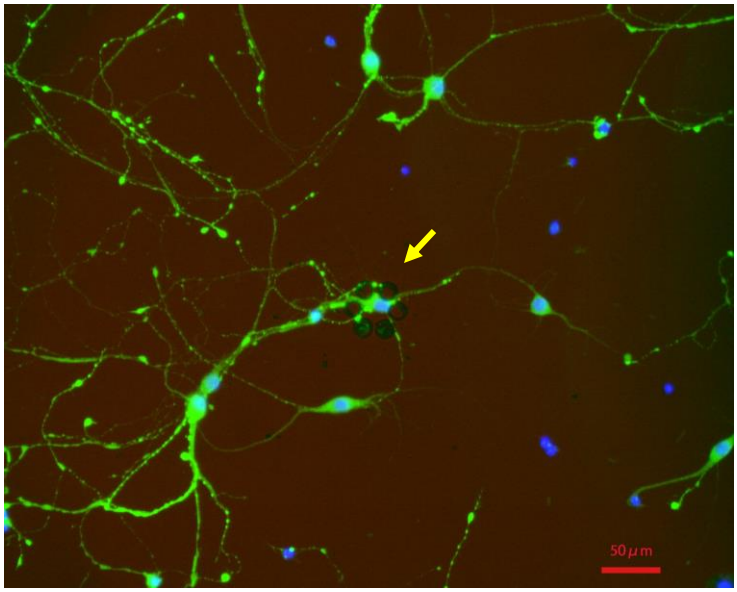

Fig.2 Rat hippocampi neuronal network formed by low density culture. A single cell is set on the micro-pore in the cell cage (arrow). 11 days culture. Green: synapsin, Blue: DAPI. Objective lens, x10.

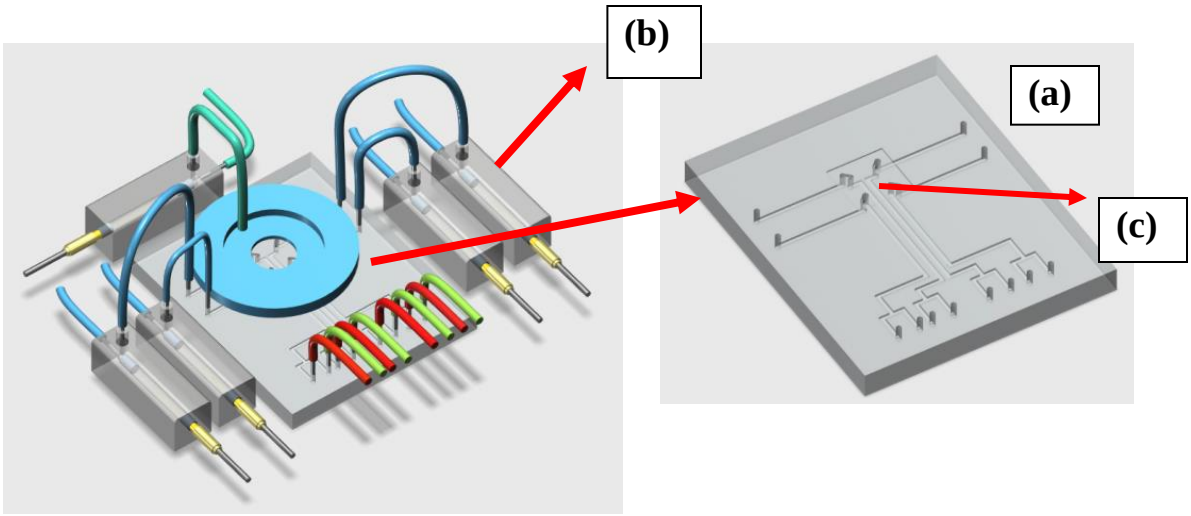

Fig. 3 Incubation type planar patch clamp device. (a): stage, (b) salt bridge type stable electrode. (c) dimple for setting the chip.

[1] Tsuneo Urisu, et al, "Incubation type Si-based planer ion channel biosensor", *Analytical and Bioanalytical Chemistry*, **391** (2008) 2703-2709.
[2] Hidetaka Uno, et al, "Improvement of performances in incubation-type planar patch clamp biosensor by using salt bridge electrode and plastic (PMMA) substrates" Sensors & Actuators B: Chemical, **193** (2014) 660-668.