ボッシュプロセスによるプレナーパッチクランプチップの製作及びチップを利用した神経細胞ネットワーク結成

Fabrication of planar patch clamp chip by Bosch process and neuron network formation on this chip

○王志宏[1,2]，宇野秀隆[1,2]，中尾聡[3]，高田紀子[3]，青山正樹[3]，鈴井光一[3]
中原康[4]，杉浦広峻[4]，長谷川貴之[4]，新井史人[4]，山本英明[5]、宇理須恒雄[1,2]
名大・グリーンモビリティ[1]，JST・CREST[2]，分子研[3]，名大・工学研究科[4]，東北大学[5]
Zhi-Hong Wang[1,2], Hidetaka Uno[1,2], Satoru Nakao[3], Noriko Takada[3], Masaki Aoyama[3], Mitsukazu Suzui[3],
Yasushi Nakahara[4], Hirotoshi Sugiura[4], Takayuki Hasegawa[4], Fumihito Arai[4], Hideaki Yamamoto[5], Tsuneo Urisu[1,2]
[1]Nagoya Univ. Green Mobility, [2]JST・CREST, [3]IMS, [4]Nagoya Univ. School of Engineering, [5]Tohoku Univ.
E-mail: zhwang@gvm.nagoya-u.ac.jp

Neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) are intractable diseases for which neither cause nor reliable treatment method is established. The reason why these diseases are so difficult is; it is not easy to sample neurons from the brain during the lifetime of the patients, and there has never been suitable method for the cause analysis and developing treatment method. The measurement of ion-channel current, which contains the information with the release of the neuro-transmitter molecules at the synapse is considered to be the most useful method for analyzing the neuron-network function. It is expected that the incubation-type planar patch clamp finds its useful application in the high throughput screening device of neuron-network. Concerning this application, we have developed the cell cage substrate as shown in Fig.1 using Bosh process [1]. Bosch process is widely used for fabrication of high aspect ratio microelectromechanical system (MEMS) which need a highly anisotropic etch process to create almost vertical structure in silicon wafers. It consists of a series of time multiplexed etching and passivation processes using SF_6 and C_4F_8 gases. With the optimized condition which control the flow rate of SF_6 , C_4F_8 , O_2 etc. and several parameters such as applied bias voltage and bias power, it can etch the silicon wafer almost vertically with smoothed sidewall.

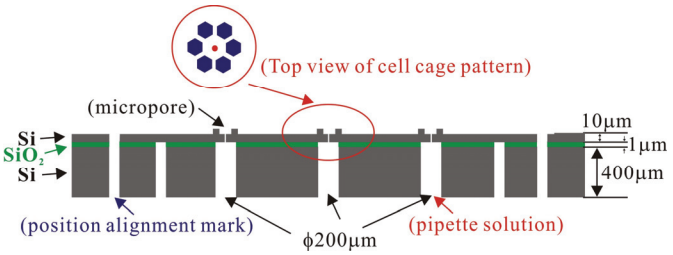

Fig. 1 Cross section of the incubation-type planar patch clamp chip, and the top view of the cell cage pattern.

We have successfully fabricated the Si chip with ~2 μm diameter micropore using SOI substrate. The penetration of micropore has been confirmed with the backside SEM investigations. At 950 °C with O_2 flow rate 1 L/min and 95 °C water vapor, the 0.2 μm thick oxide layer was formed on the chip surface by 2 hours thermal oxidations. After plasma treatment at 400 mTorr of O_2 pressure for 5 min, poly-l-lysine (PLL, 0.01%) was coated and left for 2 days in the incubator, and then low density cultures based on Banker method [2] of dissociated neurons from embryonic day 18 (E18) rat embryos rats were carried out. Fig. 2 depicts the immunofluorescence image of a single neuron cultured for 5 days. In this case the neuron was brought into the cell cage using Enpendorf single cell manipulator. After 5 days incubation, the single neuron has appropriately polarized and developed the extensive axon and dendrites in the cell cage as shown in Fig. 2. Present results show that new high quality and mass productive fabrication way of the planar patch clamp chip can be opened by the Bosch process for the first time. We have succeeded in the single neuron culture in the area surrounded by cell cage according to the Banker method.

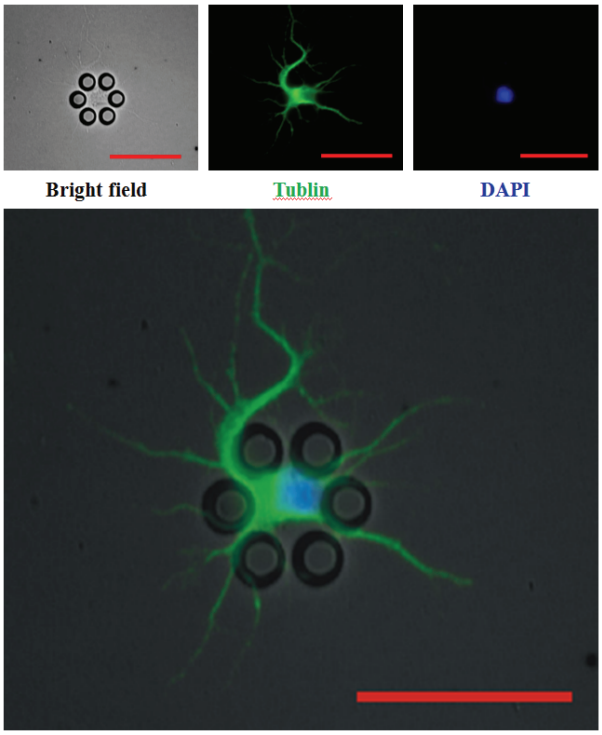

Fig. 2 Immunofluorescence image of neuron after 5 days culture. Scale bar: 50 μm.

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[2] S. Kaech and G. Banker, Nature protocols, 1 (2006) 2406