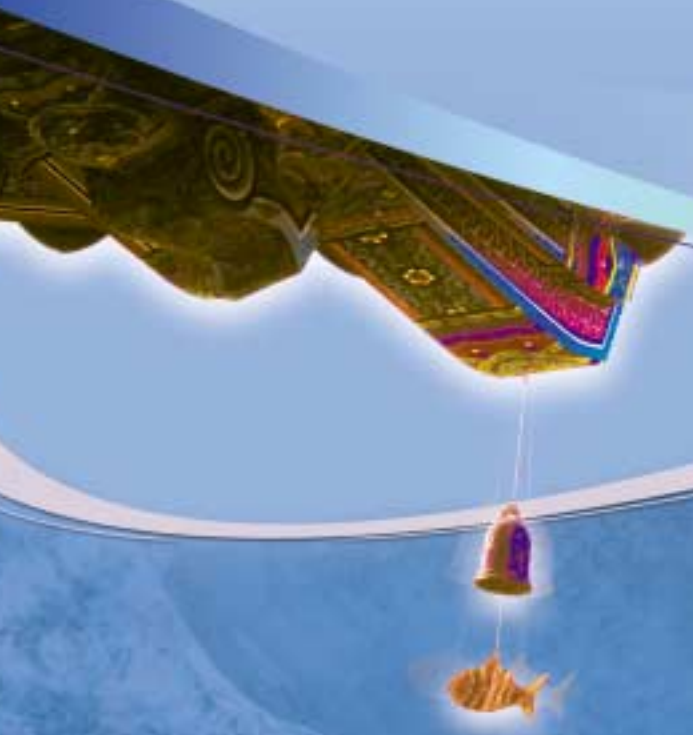
The 7th International Conference on Cellular Engineering

September 6-9, 2005
The Centennial Hall, Yonsei University
Seoul, Korea

from DNA to CLINICS

Program and Abstracts



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Poster Sessions

All posters are displayed throughout the conference session days (Set up on **Tuesday 6th Sep. between 10:00-13:00**; Remove on **Friday 9th Sep. by 13:00**)

■ Poster Session 1

10:40-11:20 WEDNESDAY 7 SEPTEMBER **EXHIBITION HALL, MUSEUM, 1F**

P01 Cell surface-localized ATP detection with immobilized firefly luciferase
Makiko Nakamura, Masayasu Mie, Hisakage Funabash1, Kimiko Yamamoto, Joji Ando, Eiry Kobatake (Japan)

P02 Intracellular Direct Detection of RNA Transcription by Fret Imaging
Tamaki Endoh, Hisakage Funabashi, Masayasu Mie, and Eiry Kobatake (Japan)

P03 Electrochemical Detection of Cell Response during Pathogen Infection
Jeong Woo Choi, Y Kim, JY Kim, W Lee, JS Kim, YK Jang (Korea)

P04 24-Well-Microplate with Sensors for Metabolic, Morphologic and Electrophysiologic Parameters of Living Cells or Tissue
Johann Ressler, J. Wiest, H. Grothe, M. Brischwein, T. Asmus, K. Wienand,B. Wolf (Germany)

P05 Response Of Cultured Cell Under High Pressure Environment
Tetsuya Miwa, Sumihiro Koyama, Tomoji Takayama, Satoshi Konishi, Junko Hayashi, Koki Horikoshi, Masuo Aizawa (Japan)

P06 NPC1 Gene Deficiency Leads to Lack of Neural Stem Cell Self-Renewal and Abnormal Differentiation through Activation of P38 MAP Kinase Signaling
Se-Ran Yang, Kyung-Sun Kang, Kyoung-Hee Byun, Sun-Jung Kim, Brian Hutchinson, Bong-Hee Lee, Makoto Michikawa, Yong-Soon Lee, (Korea)

P07 In Vivo Bone Formation by Human Marrow Stromal Cells in Biodegradable Scaffolds That Release Dexamethasone And Ascorbate-2-Phosphate
Hyongbum Kim, Inho Jo, Hwal Suh, Sangmee Ahn Jo, Jung Min Lee (Korea)

P08 Bone Regeneration Using Adipose-Tissue Derived Stem Cells and Demineralized Bone Matrix
Young-Il Yang, Ji-Yeon Seo (Korea), Marga Massey, Jane Shelby (USA)

P09 Study of Composite Skin Equivalents Using Gelatin and Fibrin Sealant for Wound
Hye Na Kang, O.H Kwon, I. S. Shin , S. N. Kim, C. M. Hong, S. H. Yoo, J. H. Lim, Y. L. Kim, M. J. Ahn, W. H. Lee, H. K. Min, S. N. Park (Korea)

P10 Construction of Tissue-Engineered Artificial Tissue by Using Perfusion Culture System
Dong Lim Seol, S.J. Jeong, W.H. Jang, M.H. Cho, S.J. Lee, Y.I Yang (Korea)

P11 Peripheral Nerve Regeneration by a Novel Microporous PLGA/Pluronic Nerve Guide Conduit
Se Heang Oh, Jun Ho Kim, Sung Ho Ghil, Jin Hwan Yoon, Kyu Sang Song, Byeong Hwa Jeon, Il Woo Lee, and Jin Ho Lee (Korea)

P12 The Role of p38 MAP Kinase and c-Jun N-terminal Protein Kinase Signaling in the Differentiation and Apoptosis of Immortalized Neural Stem Cells
Se-Ran Yang, Kyung-Sun Kang, Sung-Dae Cho, Nam-Shik Ahn, Ji-Won Jung, Joon-Suk Park, Eun-Hye Jo, Jae-Woong Hwang, Sung-Hoon Kim, Bong-Hee Lee, Yong-Soon Lee (Korea)

ANALYSIS OF LOW-DENSITY NEURONAL NETWORKS ON MICRO-CONTACT PRINTED MICROELECTRODE ARRAYS

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Abstract: Hippocampal neuronal networks were cultured on poly-L-lysine stamped microelectrode array. The neurons plated with low density enabled us to monitor electrical activities in a single cell level and to observe the signal propagation through the network. We found that electrically functional synapses still existed even in the density as low as 200 cells/mm^2. The cells were evoked by electrical current and high-K$^+$. To identify what kinds of synapse are dominant in the cultured neurons, DNQX, AMPA antagonist were applied into the cultures. By scanning electron microscopy and immunocytostaining, the cultured neurons were visualized.

Introduction

Several methods have been developed to precisely pattern proteins which can affect the attachment and growth of cells in culture [1,2]. Especially stamping on planar microelectrode arrays (MEAs) enables us to pattern neurons at exact location close to the electrode sites. Using these technologies, investigators have produced neuronal networks on the planar microelectrode arrays, but failed to investigate the networks on a cell level because of the difficulties in culturing the small number of neurons. Our primary goal is to determine the fewest number of neurons that can be used to form long-lived cultures to permit continuous extracellular recording and stimulation. Secondly, we verify the physiological functionalities of cultured neuronal networks by various methods such as treatment of high-K$^+$ and antagonists. We also performed scanning electron microscopy(SEM) for visual inspection and immunocytostaining to identify dendrites, synapses, and nuclei in the patterned neurons.

Materials and Methods

The MEAs were fabricated by the semiconductor process reported previously [3]. The polydimethylsiloxane (PDMS) stamp was molded from the stamp master which was designed to match the patterns of MEAs. The fabricated PDMS stamp has the pattern of 2㎛-wide lines and 20㎛ circles at the crossing points. The procedure of stamp fabrication and stamping on the microelectrode array is shown in Figure 1. Flourescein isothiocyanate (FITC) labeled poly-L-lysine (PLL) was applied to the PDMS stamp for 1 hour after dissolved at 1 mg/ml in borate buffer solution and transferred to the surface of electrodes by custom-made alignment tool for 1 hour with the pressure of 50 gm/cm^2 [6].

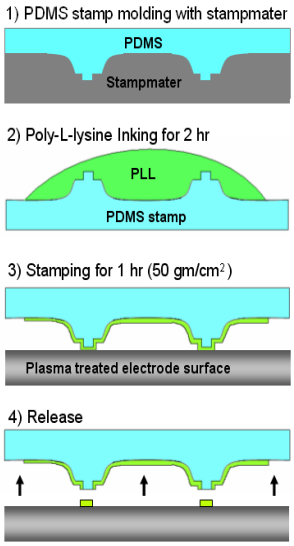

Figure 1: Fabrication of PDMS stamp and stamping process

Hippocampal neurons were extracted as previously described [4]. Dissociated cells were seeded at the densities of 100, 200, and 400 cells/cm^2 on the PLL-patterned MEAs in order to find out how sparse a culture can still have electrical connectivity. The culture was performed in minimal essential medium(MEM) supplemented with 10% horse serum and 0.1% pyruvic acid. After several hours, the medium was replaced with serum-free neurobasal media(Gibco) supplemented with B27 (Gibco) and 0.5mM L-glutamine. Cultures were maintaned at 37℃, 5% CO_2.

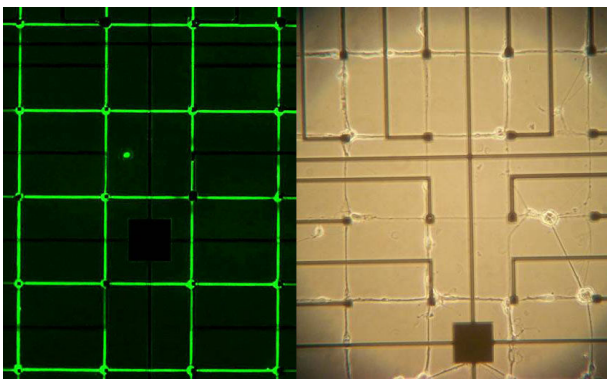

Figure 2: PLL stamped pattern on microelectrode array(left) and cultured hippocampal neurons on the electrodes at DIV 7 (right)

Half of the media were replaced with fresh media twice a week.

For electrophysiological experiments, the culturing media was replaced with recording media (HEPES). MEAs were mounted into the custom-made connector, which was placed on a resistively heating stage. Ag/AgCl reference electrode was placed into the recording media. Electrical activities from the recording sites were amplified with the gain of 10,000 and filtered (0.3~5 kHz, 40 dB/decade) by A-M systems differential AC amplifier model 1700. The signals were sampled at the frequency of 20 kHz and digitized by data acquisition board (NI 6024E, National Instruments). During recording the amplitude of background noise were maintaned below 10μVrms. Electrical stimulation was applied through one of electrode sites by isolated stimulator (ISO-Flex, AMPI, Israel). The stimulation current pulse width was fixed with 50 μs, and was applied through a electrode site for stimulation.

High K^+ HEPES Hank's buffer solution was made to see how the spontaneous activity changes. The concentration of K^+ was adjusted to 10 mM by adding potassium chloride. In order not to give cells excessive osmopressure, the osmolarity was also adjusted to around 285 mM/kg. The osmolority was measured by Vapor Pressure Osmometer 5520 (VAPRO^{TM} , Wescor). 6,7-dinitroquinoxaline-2,3-dione(DNQX), glutamate antagonist was also applied to the culture with the concentration of 1µM and 2µM. After the application, the media exchanged for fresh media without DNQX to see if the activity of neurons recovers or not.

After the series of electrophysiological experiments, the neurons were fixed with 4% paraformaldehyde at 37℃ for 15 minutes, made permeable with 1% triton in HEPES Hank's buffer for 15 minutes at room temperature (RT), and incubated in 6% bovine serum albumin for 30 minutes at RT to block nonspecific

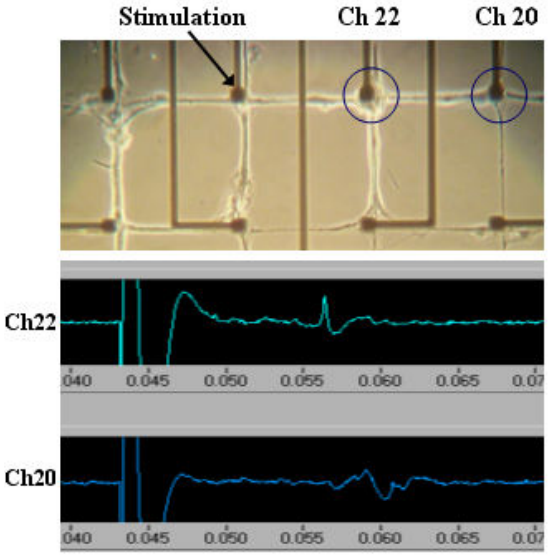

Figure 3: Electrically evoked responses by current stimulation with the pulsewidth of 50μs, and the amplitude of 200μA

bindings. Samples were exposed to a mixture of rabbit polyclonal anti-MAP2 (1:500, Sigma) and mouse

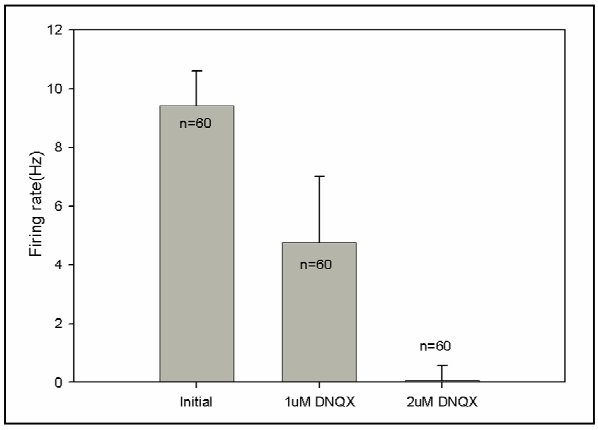

Figure 4: DNQX dose-dependent spontaneous activities

monoclonal anti-synaptophysin (1:200, Sigma) for 1 hour at 37℃. To identify these primary antibodies, Texas Red goat anti-rabbit (1:200, Molecular Probes) and Alexa 488 goat anti-mouse (1:200, Molecular Probes) were used as secondary antibodies. MAP-2 is a maker for the identification of the neuronal cell bodies and dendrites, and synaptophysin is one of the presynaptic proteins which show the locations of the synapses. After the immunostaining, cells were applied with DAPI for staining nuclei. The samples were observed with an Olympus BX41WI epifluorescence upright microscope and the images were captured by an optronics magnafire CCD camera (Olympus, Melville, NY).

For scanning electron microscopy, the samples were dehydrated with ethyl alcohol and critical point dried [9]. The dehydrated samples were observed using SEM after coated with gold.

Results

The cells were well patterned on the poly-L-lysine as shown at Figure 2. Almost neurons were attatched exactly on the poly-L-lysine stamped surface. When cells were plated at 200 cells/cm^2, spontaneous activity could be recorded as early as 7 days in culture. By 21 days in culture, levels of spontaneous activity were

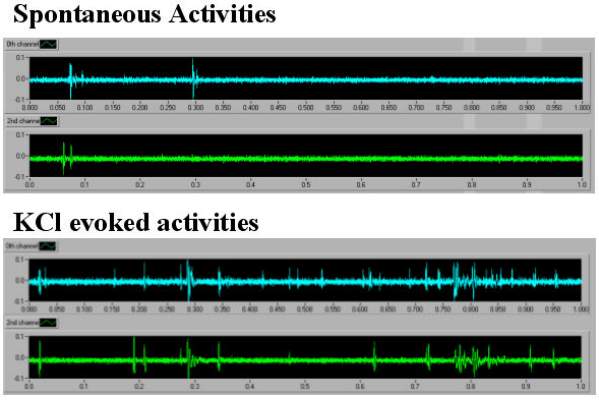

Figure 5: Firing rate increasement by high K^+ treatment

constant. In Figure 3, electrical stimulation (200 μA

current amplitude, 50 μspulse width) evoked neuronal activities. Signal propagation through the network was also observed.

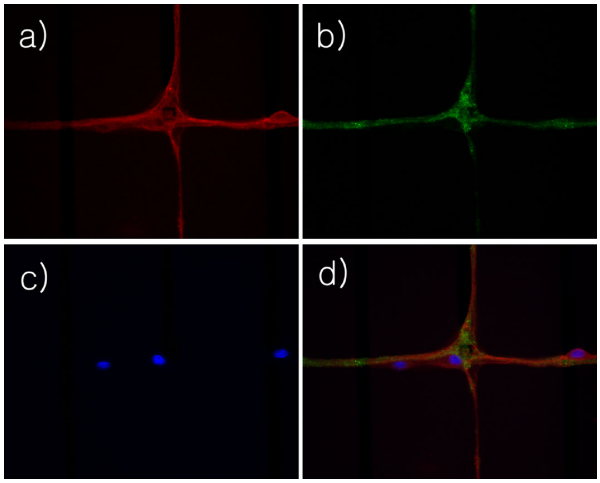

Figure 6: Fluorescence micrographs (a) dendrites and cell bodies by MAP-2 staining, (b) presynaptic proteins by synaptophysin staining, (c) nuclei by DAPI staining and (d) merged image

When high-K^+ HEPES Hank's buffer solution was applied, the activities increased dramatically as shown in Figure 5. The treatment of DNQX, glutamate antagonist, inhibited the spontaneous activities. 1µM DNQX blocked about half of activities, and all activites were ceased when 2µM DNQX applied. After washing out, the neurons started to fire again at the same rate as the initial state.

Figure 6 shows that a single cell body is located in the vicinity of electrode sites. The synapses between neurons are distributed along the neurites bundles, not some specific location.

The SEM image obtained from dehydrated neurons was like Figure 7. The electrode site had a little protrusion due to platinum electrodeposition. But it didn't prevent the neurites from growing through the surface of sites.

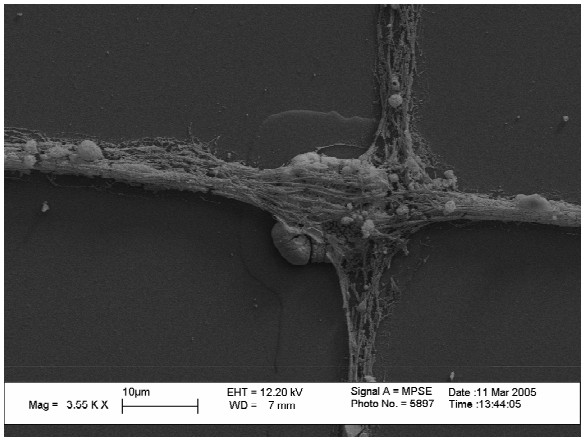

Figure 7: SEM image of patterned neurons near the electrode site

Discussion and Conclusion

Neurons were successfully cultured in low density for long term survival during more than 1 month. The cultured neuronal network was shown to have electrically functional synapses in spite of the low number of cells. The electrical activity of networks could be modulated by chemical treatments such as high-K^+ or neurotransmitter antagonists. The distributions of pre- and post-synaptic proteins were described using immunocytochemistry and their distribution mapped relative to cell bodies and patterns on the substrates. Results demonstrate the effectiveness and robustness of these cultures for studying network function. We believe this study is fundamental and also important for understanding functional circuits of neuronal networks and developing cell-based biosensors.

Acknowledgment

This work was supported by the International Collaboration Program, NBS-ERC(Nano Bioelectronics and Systems Research Center)/KOSEF(Korea Science and Engineering Foundation) and NIH, NS-044287, NSF, ECS-9876771.

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