Nano-Bio Science

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Abstract

This paper briefly introduces the nano-bio science research and related research being carried out in our research group. The research is based on the fusion of neuroscience, bio-molecular science, and nanotechnology. This interdisciplinary research is extremely promising for creating a new field of science and new knowledge. Nano-bio science could be a key to understanding mysterious biological phenomena and developing new technologies that will lead to a novel, innovative social life in the future.

1. Introduction

It is well known that the memories and learning in our brain are induced by the plasticity of the neural connections at the synapses. Although the mechanisms have long been discussed, they are still uncertain. National projects such as the "Decade of the Brain" in the US and our Brain project promoted the understanding of brain functions and mechanisms. Recent developments in nanotechnology have made it possible to analyze biological phenomena that used to be difficult to analyze, and have produced new tools and technologies for their analysis. Biotechnology has advanced greatly. The combination of nanotechnology and biotechnology, known as nano-bio or bio-nano technology could be a key to understanding biological functions such as plasticity, and developing applications.

DNA (deoxyribo-nucleic acid)/protein chips and DNA tweezers/motors are good examples for understanding these technologies. Lithographical techniques are quite important in this case. Optical tweezers are also quite useful for detecting single molecules. Myosin movement along an actin filament has been visualized by measuring evanescent fields [1], [2] (**Fig. 1**). Applications of these technologies have developed not only in the field of detection, but also in various fields including genomic study. These technologies have also greatly advanced gene-therapy, drug-delivery, and tissue engineering. Considering clinical usage, there are some interesting reports of trials conducted with blind patients using CCD (charge coupled device) cameras in lieu of their eyes. As the patients needed to have long electrodes inserted in their brains, we must try to reduce the painfulness of the treatment. Nano-bio technology should solve this problem.

Here I report our approach to this field, especially the nano-bio technology based on the fusion of neu-

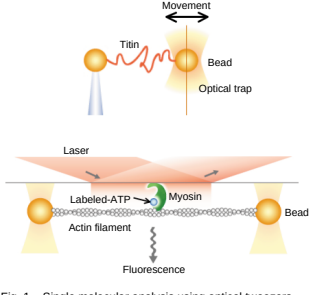

Fig. 1. Single molecular analysis using optical tweezers.
(a) Titin (b) myosin movement along the actin filament.

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roscience and nanotechnology, to develop an understanding of the brain plasticity mechanism and the nano-bio device architectures. First, I describe the neuroscience approach using a multi-electrode array and sensors to analyze brain functions. The array can be an interface between neurons and electrical instruments, allowing detection and stimulation. The information processing mechanism in our brain systems will also be discussed. Then, I discuss the use of DNA/peptides and/or synthesized polymer as a wire for nano-devices. Nano-gap electrodes with an electrode gap of only a few nanometers have been developed to detect the electro-conductive characteristic of the wires.

2. Approach to nano-bio science

We are conducting this research under two main projects (**Fig. 2**): one focusing on bio-molecular science, especially, bio-molecular wires including DNA, and the other aiming to understand the signal transduction mechanism including synaptic efficacy such as learning and memories. In the bio-molecular science project, we are trying to develop a novel method of analyzing electrical properties such as conductance. A nano-gap electrode is one of the novel methods that we developed recently. It is fabricated by electroplating. We manipulate the molecule (e.g., DNA or a synthesized polymer) onto this electrode with an atomic force microscope (AFM) with or without chemical end-terminal modification.

The aim of this research is to create an architecture for nano-bio devices to unify the principle of neural information processing in the brain with bio-molecular circuitry (**Fig. 3**). Future goals could include a bio-computer based on a novel signal processing system and a health-support device that would send information interactively to keep our condition healthy. The

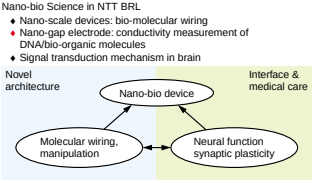


Fig. 2. Nano-bio science in NTT Basic Research Laboratories.

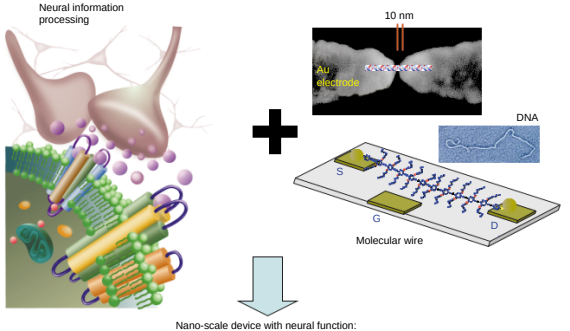


Fig. 3. Nano-bio device architecture.

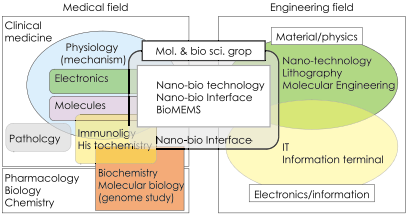


Fig. 4. Research field of Molecular and Bio- Science Research Group.

latter could be implanted in tissue or the brain. Or it could be a medical device to cure a patient's disease. Parkinson's disease and vision support for the blind are very attractive targets.

Our field covers medicine such as physiology, immunology, and clinical medicine, and engineering including nanotechnology, molecular engineering, and information technology (IT) (**Fig. 4**). We are in an interdisciplinary field bridging these two research fields.

3. Neuro-engineering

In order to understand the signal processing mechanism in the brain, we need to consider a simplified brain, which is easier to analyze than a real (complex) brain under normal conditions. Dissociated culture is a good example of such simplification. The number of inputs to the neurons can be control depending on the development and culture day. As shown in **Fig. 5**, Wistar rats were anesthetized with ethyl ether before dissection. We removed the hippocampus from a newborn rat (postnatal two-day-old, P2) and cortex*1 from embryo (embryonic day 18, E18). After dissociation with enzymes (trypsin or papain), cells were plated onto a multi-electrode array and culture substrates. The array and substrates were pre-treated with laminin and poly-D-lysine for two days before plating. Dissociated cells were cultured on them for 1-2 weeks in a medium containing L-glutamine, glutamate, gentamycin, and B27 supplement in neurobasal medium (Gibco) for serum-free conditions and for 2-3 weeks in a medium containing DMEM (Dulbecco's minimum essential media), 5% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum, penicillin and streptomycin, insulin, and 10% glucose under 5% CO_2 at 37°C for conditions using serum.

Neurons grow randomly and make connections among themselves, so it is necessary to control the neural outgrowth to avoid complexity. Photolithography has been used to make an ordered pattern on the substrates. Multi-electrode arrays are also fabricated from an indium tin oxide (ITO) membrane by conventional lithography,

An example of guided neural outgrowth on substrate patterns is shown in **Fig. 6**. It is critical for neurons to grow on the surface. They mainly consisted of cell bodies (about 20 μm across) and neurites. At the tip of each neurite, there was a growth cone. These growth cones were about 10 μm in size. They had needle-like very thin filopodia, which acting as sensors/anchors to decide where growth should occur. These filopodia were less than 0.1 μm long. They cannot survive without being attached to the surface. Therefore, surface adhesion is very important and the filopodia, the growth cone tips, play an important role in neural growth/survival. Close correlation between adhesion to the substrates and neural outgrowth has been reported [3]-[8]. Therefore, surfaces are usually treated with extracellular matrices, such as poly-lysine and laminin before the neurons are plated. However, neurons can survive even if the substrates do not have coatings. We found that the surface three-dimensional structure and the surface conditions such as electro-properties were very important for neurons

*1 The cortex is a part of the brain, located near the surface. It has several layers and regions. For example, the visual, auditory, and motor region are in the cortex. It is believed to play an important role in long-term memory.

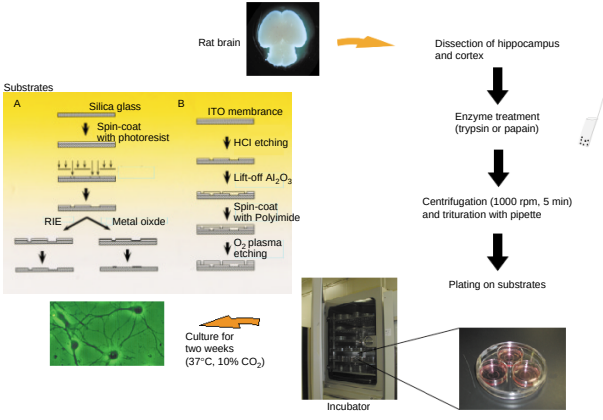
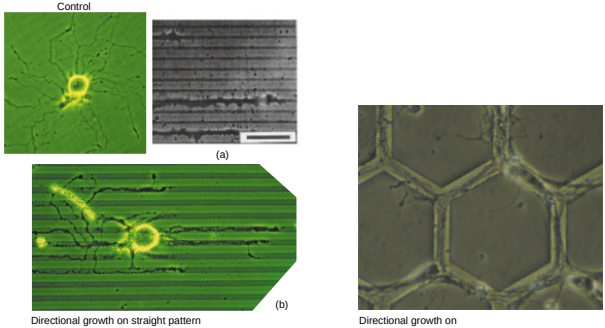


Fig. 5. Primary cell culture method.



Directional growth on straight pattern (a) 1 μm wide (dark pattern) (b) 10 μm wide (dark pattern)

Directional growth on hexagonal pattern (10 μm wide)

Fig. 6. Guided neural outgrowth on substrate patterns.

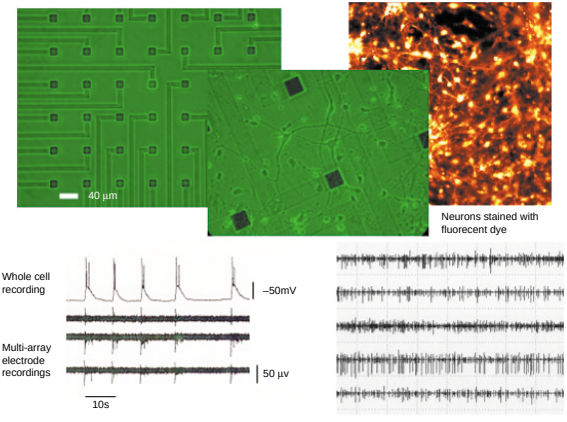


Fig. 7. Neural activity measurement using MEAS.

growth [6]-[8]. When the neurons are plated on the patterned surface, they might survive on the substrates according to this mechanism. Neurons recognize and trace the pattern fabricated on the substrates with nano-level accuracy. Results show that neurons grew along the pattern.

4. Neural activity measurement

Measurement of electrical properties is a primary method to understand neural activity. A glass micropipette was commonly used for a long time until Sakmann and Neher reported a patch clamp technique in 1976 [9], [10]. The patch clamp and whole cell recording are very useful and popular these days. Not only the membrane potential, but also single-channel activity can be measured. However, as this method breaks the cells, it is unsuitable for longer measurements, such as observing developmental changes. To enable this, we developed a multi-electrode array system (MEAS). Although this method is based on extra-cellular recording, we can measure neural activity without breaking the cells and can measure daily and monthly changes in the same sample. Furthermore, there are 64 recording electrodes, which can be selected for stimulation. Therefore, we can use MEAS to determine the distribution of electrical activity and its spatial changes and propagation. Each electrode is square with a side 10-40 μm long and arrayed with an interval of 50-200 μm. They are fabricated from ITO by conventional lithography and modified with platinum black.

As described above, MEAS is suitable for longer measurements such as observing development. Changes in synaptic activity were measured versus the number of days of culturing after plating. No significant spontaneous activity among the electrodes was observed during the first week. Around three weeks of culturing, remarkable synaptic activity was detected. The signals from different electrodes indicated synchronized periodic responses [11]-[13] (**Fig. 7**), indicating that synaptically active neural networks had been established. Tetanic stimulation modified this network activity. Changes in the latency of

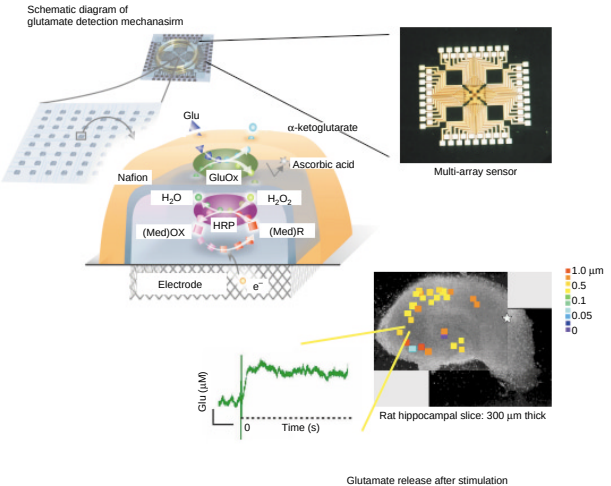


Fig. 8. Multi-site glutamate sensor based on MEAS.

evoked responses were induced. Repetitive stimulation seemed to optimize the evoked response. The results indicate that MEAS lets us discuss the synaptic plasticity*2 mechanism and it could be the tool for drug evaluation.

Glutamate, which is known as an excitatory neurotransmitter, is a good candidate as a key substance in synaptic plasticity. To understand the synaptic plasticity mechanism, we are required to measure the glutamate release/concentration during synaptic events. Therefore, we fabricated a multi-site glutamate sensor based on MEAS. Glutamate oxidase and horseradish peroxidase in Os-polymer were layered on the electrode. This multi-layered sensor array allows us to detect glutamate release from a synapse and to determine the glutamate distribution. We found that the distribution depending on the stimulation, i.e., which active region among those in the hippocampus*3, could easily be visualized [14]-[19] (**Fig. 8**).

Laser-trapping Raman spectroscopy is useful for analyzing glutamate content in synaptic vesicles. Drug induced changes in the glutamate spectrum were observed by holding a single synaptosome (5-600 nm in size) with a laser beam. The results indicate that synaptosomal glutamate movement could be analyzed with this technique [20].

*2 Synaptic plasticity: The change in connectivity of pre- and post- neurons depending on the input. It includes synaptic efficacy, such as learning and memory.

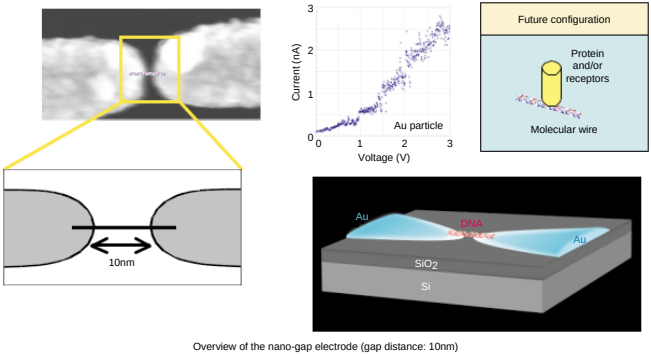
*3 The hippocampus is a part of the brain. The name comes from the fact that its shape looks like a sea horse. It is believed to play an important role in short-term memory.

5. Nano-gap electrode and conductivity measurement of a bio-molecule

A nano-gap electrode has been developed to investigate electrical properties at the single molecule level [21]. This project aims to understand whether a single molecule could be used as a conductive wire. This is extremely important for considering whether molecular devices (with protein, in future) such as nano-bio devices could be possible based on the single molecule level. Experiments were carried out with the electro-plated nano-gap electrode. Its primary electrode was fabricated from gold based on a Si substrate by electron-beam lithography. The gap between electrodes was 100 nm. The size was carefully reduced by electro-plating while controlling the current applied to the electrode. Then just before the current rapidly increased, the current injection was stopped to avoid contact. Thus, we achieved a 8-10 nm gap electrode. DNA or another bio-molecule was placed between the gap to measure its electrical conductivity (**Fig. 9**). The measured current increased discontinuously in a step-wise manner depending on the applied voltage, so we were able to discuss the transport mechanism.

6. Conclusion

In this paper, I briefly introduced nano-bio science and related research being carried out in our research group. As the idea is based on the fusion of neuroscience and bio-molecular science with nanotechonology, this interdisciplinary research field is extremely important for creating a new science field and new knowledge, which used to be very difficult to clarify. Because this is bio-related research, our goals are not limited to the telecommunication field, but also involve possible extension to the medical field of patient support. Patients might have nano-bio devices implanted to enable their health including brain condition to be monitored. Some might have implants to acquire vision or to cure diseases or disabilities (**Fig. 10**). In either case, if we collect this health-related information in a personal data bank, we could receive an alarm whenever our body condition becomes bad. It might be possible to control pain and disease in the near future. I believe that nano-bio science could be a key to creating and developing new technologies that lead to a novel, innovative social life in the future.



Overview of the nano-gap electrode (gap distance: 10nm)

Fig. 9. Electrical conductivity measurement in single molecule using nano-gap electrode.

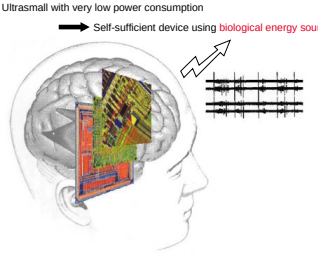


Fig. 10. Future applications.

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He received the B.E., M.E., and Ph.D. degrees in instrumentation engineering from Keio University, Yokohama in 1980, 1982, and 1986, respectively. In 1987, he joined NTT Basic Research Laboratories and worked in the bio-computer project. His research interests range from neuroscience including signal transduction, neuro-imaging, neurochemistry to surface science and molecular science. He is currently focusing on nano-bio science and nano-bio device fabrication. He studied cell physiology and developed a novel measurement system using magnetic resonance spectroscopy from 1980-1986. From 1986 to 1987, he studied neurophysiology at the Laboratory of Physiology, Faculty of Medicine, the University of Tokyo as a guest researcher. For the year 1990-1991, he was a visiting researcher at the National Institute of Health and studied GABA physiology at the Laboratory of Neurophysiology, NINDS. From 1997-1998, he was a guest researcher at the National Institute for Minamata Disease. He received Research Awards from NTT BRL in 1993, 1998, 2003 and a Distinguished Award in 1994. He received the Poster Award at Electrophoresis'99 and, the SLOWMAG Award at the Gordon Research Conferences in 2002. He is a member of Society for Neuroscience, International Brain Research Organization, Japan Neuroscience Society, the Japan Society of Applied Physics, and the Japanese Pharmacological Society.