BIOHYBRID NEURAL PROBE: A NEURAL PROBE HAVING CULTURED NEURONS BETWEEN AN ELECTRODE AND TISSUE

Keisuke Okita1, Midori Kato-Negishi1, Hiroaki Onoe1,2, Riho Gojo1,2, Tetsuhiko Teshima1 and Shoji Takeuchi1,2

1. Institute of Industrial Science, The University of Tokyo, JAPAN
2. Takeuchi Biohybrid Innovation Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology (JST), JAPAN

ABSTRACT

We propose a neural probe combined with a neuronal spheroid (neurospheroid) cultured on electrodes for deep brain stimulation. Since neurons of the spheroid extend their axons deep into the brain tissue, the neural probe can electrically stimulate deep brain area via the neurons without inserting an invasive probe. Here we first fabricated an electrode and then successfully placed a neurospheroid at the electrodes tip, named a “biohybrid neural probe”. Furthermore, we tried to make the fabricated probe contact with cultured cortical cells on glass. We believe that our biohybrid neural probe has a potential to provide minimally invasive stimulation to the brain tissue.

KEYWORDS: Neurospheroid, Spheroid, Neural Probe, Electrode, Deep Brain Stimulation

INTRODUCTION

Recently interest in neural interfaces has increased because these interfaces can be used to link the nervous system, mainly the brain tissue, to an electrical system [1]. Since neural interfaces have an implantable neural probe, by linking to an electrical system, we can stimulate the brain tissue electrically and record its electrical activity through the probe. Especially the electrical stimulation using these interfaces is widely used in a neurosurgical treatment such as deep brain stimulation (DBS). DBS provides therapeutic benefit for several neuropathologies, including Parkinson’s disease (PD), epilepsy, chronic pain, and depression [2-5]. Main concerns in conventional neural probes are damages to the brain tissue when they are inserted into the brain tissue and reductions in signal-to-noise ratio because of the unstable probe position in the tissue [6]. To overcome these problems, various flexible probes that can deform in the inserted organs have been reported to date [7,8]. Although they can attain implantation with less damage than different kinds of probes till then, most of these probes have rigid needles to insert and injure the brain tissue.

In this paper, we propose a probe without needles: this probe has a microchamber on its tip and a single neurospheroid cultured on the microchamber for interfacing between electrodes and target tissue (Fig. 1). We here present a fabrication of electrodes with a cultured neurospheroid on the electrode tip. Using this probe, we demonstrated the contact between the probe and cultured cortical cells while keeping the neuronal activity of the neurospheroid. As we previously studied, when neurospheroids are transplanted onto the host brain tissue, they can interconnect to the host brain tissue via neuronal processes and formed neuronal network [9]. Accordingly, putting the probe having a neurospheroid onto the brain tissue, the probe has potential to stimulate the brain tissue through the neurospheroid.

Figure 1. Conceptual design of Biohybrid Neural Probe. Biohybrid Neural Probe has a neurospheroid on its tip. The spheroid is contacted on and synchronized with the tissue. We can stimulate the tissue through the spheroid fixed on the tip.
FABRICATION OF THE ELECTRODE

Figure 2 shows the fabrication process of two electrodes (i.e., stimulation and reference electrodes). First we deposited gold and chromium layers on glass substrate (Fig. 2.(A)). The thickness of the gold layer is 300 nm. S1818 photoresist was spincoated on the surface and patterned. Then the gold and chromium layers were etched with a gold etchant and HY solution to form a pair of electrodes (Fig. 2.(B)). We then spincoated the 100-μm-thick SU-8 photoresist on the surface. The resist was exposed to UV and developed using SU-8 developer to form an insulating layer for the electrodes and to make a microchamber at the tip (Fig. 2.(C)). Finally we coated polyethyleneimine (PEI) on the surface of the fabricated electrodes for neurospheroid attachment (Fig. 2.(D)).

Figure 3 shows the bright field photograph of the fabricated electrodes (Fig. 3(A)) and the scanning electron microscope image of the microchamber at the electrode tip (Fig. 3(B)).

FORMATION OF NEUROSPERHIOIDS

To form uniform-sized cortical neurospheroids, the cerebral cortices of Wister rats (embryonic days 17-19) were dissected and cultured. The cortices were dissociated with papain and then the resultant cell suspension was plated at a density of 4 × 10^5 cells/ml on a polydimethylsiloxane (PDMS) microchamber array. Then neurospheroids were formed in the PDMS microchamber array (Fig. 4(A,B)). The diameter of each microchamber was 100 μm. After 3 days culture, a formed neurospheroid was taken from a PDMS microchamber and put into a microchamber of the electrode tip.

ANALYSIS OF THE NEURONAL ACTIVITY OF A NEUROSHEROID

After 10 days culture, we investigated the neuronal activity of a neurospheroid. In a PDMS microchamber, we examined [Ca^{2+}]i fluxes of a neurospheroid by Ca^{2+} imaging system. The neurospheroid was loaded with the Fluo-4/AM solution at 37°C for 60 minutes. After washing out excess dye, we observed many cortical neurons in the neurospheroid exhibited spontaneous synchronized [Ca^{2+}]i oscillations (Fig. 5(A,B)). This result indicates that our preparation of neurospheroids allows us to maintain the neuronal activity of neurons in the neurospheroid.

FABRICATION OF BIOHYBRID NEURAL PROBE AND CONTACT WITH CORTICAL CELLS

Finally, we demonstrated contacting the neurospheroid of the probe with cultured cortical cells on a glass plate. After the neurospheroid was transferred into a microchamber of the electrode and cultured for 5 days. Figure 6(A) shows the cultured neurospheroid on the electrode. Furthermore, we succeed in contacting the electrode having a cultured neurospheroid onto cortical cells on a glass plate coated with PEI. This result indicates that the neurospheroid robustly adheres to the electrode tip and we can easily handle this biohybrid neural probe.
CONCLUSION

We proposed a neural probe having a neurospheroid on the tip, biohybrid neural probe, and demonstrated a contact between the probe and cultured cortical cells via the neurospheroid. Since the neurospheroid may extend its axons when it is put onto the brain tissue, we believe that our biohybrid neural probe would enable us to stimulate electrically deep area of the brain tissue without invasion.

REFERENCES

CONTACT
*Keisuke Okita, Institute of Industrial Science, The University of Tokyo, 4-6-1, komaba Meguro-ku, Tokyo, JAPAN, Tel: +81-3-5452-6650; Fax: +81-3-5452-6649, E-mail: okita@iis.u-tokyo.ac.jp

Figure 5. Ca^{2+} imaging of neurospheroid. (A) (i,ii) Pseudocolor image of fluo-4 labeled neurospheroid for calcium imaging. (B) Changes in the fluorescent intensity of the four spots (neurons) in the neurospheroid.

Figure 6. Placement the electrode with neurospheroid on cortical cells. (A) Image of a neurospheroid on the electrode tip. (B) Fluorescent image of the electrode putting onto the cortical cells cultured on a glass plate. Cortical cells and neurospheroid were stained with Cell Tracker Red and Green respectively.