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Preliminary Study of Multichannel Flexible Neural Probes Coated with Hybrid Biodegradable Polymer

Yasuhiro Kato[1], Itsuro Saito[2], Takayuki Hoshino[3], Takafumi Suzuki[3], and Kunihiko Mabuchi[3]
[1]Dept. of Advanced Interdisciplinary Studies, Grad. School of Engineering, The University of Tokyo
[2]Division of Artificial Biomechanism, Dept. of Life Science,
Research Center for Advanced Science and Technology, The University of Tokyo
[3]Dept. of Information Physics and Computing,
Grad. School of Information Science and Technology, The University of Tokyo
Yasuhiro_Kato@ipc.i.u-tokyo.ac.jp

***Abstract*—Two types of intracortical multichannel flexible neural probes coated with a hybrid biodegradable polymer were designed and fabricated. The hybrid biodegradable polymer was made of polyethylene glycol and biodegradable polymeric microspheres containing nerve growth factor (NGF) and incorporated to improve the stiffness for flexible neural probe insertion, and promote regrowth of damaged neural tissues around the probe. The type-A neural probe has a groove structure designed to be seeded with a large amount of the hybrid biodegradable polymer. The type-B neural probe has a unique configuration like skeleton to minimize the volume of the flexible probe and buffer injurious micromotion between the probe and the tissues after implantation. In this preliminary study, the efficacy of the released NGF from the microspheres with the PC12 cells was examined. Further, neural probe implantation and neural signal recording with an acute experiment were studied.**

I. Introduction

Micro Electro Mechanical System (MEMS) technologies have been established to fabricate multichannel neural probes for interfacing with the nervous systems for the past decade. A wide variety of neural probes have been applied in Brain-Machine Interfaces, electrophysiological investigations, and medical treatments. However, no suitable probes are for chronic neural recording. One main reason is the death of neural tissues damaged by probe insertion and placement in the brain. Thus, we have been developing a new flexible neural probe to regrow damaged neural tissues for chronic recording, while incorporating a drug delivery system (DDS) based on MEMS technologies (Fig. 1) [1-4].

In this preliminary study, we designed and fabricated two types of flexible neural probes. Both of the neural probes were based on parylene C (poly-monochloro-paraxylylene), which was flexible and biocompatible material [5]. The type-A neural probe has a groove structure on the probe. This was designed to be seed with a large amount of the hybrid polymer, since the small wells were limited to fill and deliver the volume of bioactive components for the long-term. The type-B has a skeleton configuration to minimize the volume of the flexible probe. This design was aimed to provide a space with recovery for the neural tissues damaged and pressed by neural probe insertion, and flexibility to buffer injurious micromotion between the probe and the neural tissues after implantation, as shown in Fig. 2.

Biodegradable polymeric microspheres with bioactive components of nerve growth factor (NGF) and polyethylene glycol (PEG) were mixed, and seeded into the groove or coated on the skeleton-like probe. These were added with the aim of providing a route for the damaged tissue around the neural probe and improving the mechanical stiffness of the flexible neural probe for insertion. The DDS material was a biodegradable polymer of poly(lactic-co-glycolic acid) (PLGA), which was used to deliver the NGF for an optimal period. In this study, the efficacy of the two types of flexible neural probes and the biodegradable polymeric microspheres were examined.

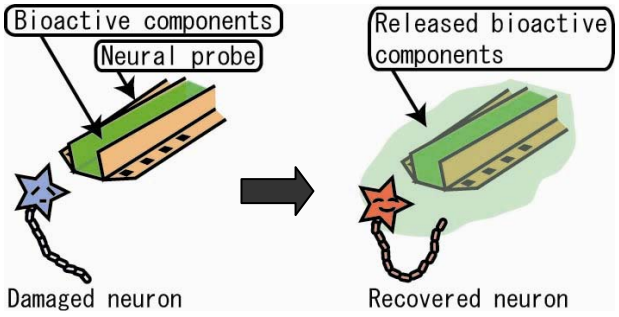

Fig. 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the groove of the type-A neural probe.

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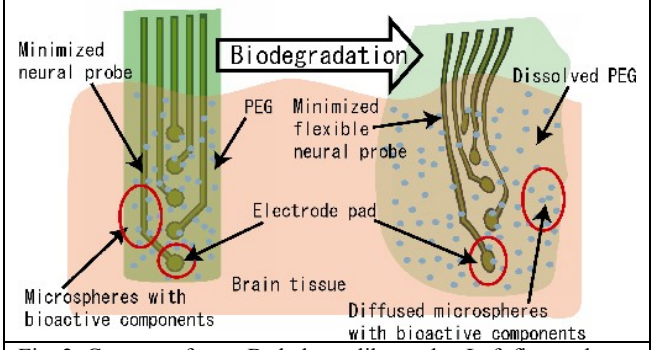

Fig. 2. Concept of type-B skeleton-like probe. Left figure shows implanted skeleton-like neural probe coated with hybrid biodegradable polymer of microspheres and PEG. Right figure shows that each minimized probe is flexible to brain tissue and the hybrid polymer is dissolved to promote recovery of damaged tissues around the neural probe.

II. Methods

A. Neural Probe Fabrication

Both of the type-A neural probe and the type-B neural probes were constructed using a surface micromachining technique with parylene C and gold layers on a silicon wafer. The fabrication processes of the neural probes were as follows, respectively.

1) Type-A neural probe: A 10-µm layer of parylene C was deposited on a silicon wafer by a parylene coater (LABCOTER2 PDS2010, Specialty Coating Systems). A 200-nm gold layer was then deposited with a vacuum evaporator and patterned to delineate the individual recording pads, connecting pads, and wire traces. Next, a thick photoresist (AZP4903, Clariant Japan) was spin coated onto the surface to a thickness of 30 µm. A 10-µm layer of parylene C was again deposited. A 300-nm aluminum layer was then deposited and patterned on the parylene C layer as a mask to define the outline of the neural probe. The parylene C layer was patterned by O_2 reactive ion etching (RIE). After wet-etching the aluminum layer by a mixed acid aluminum etchant (838-38012, Wako Pure Chemical Industries, Ltd), the neural probe was peeled off the silicon substrate with tweezers. A cross-sectional schematic diagram of the manufacturing process is shown in Fig. 2.

2) Type-B neural probe: The method to fabricate the skeleton-like probe was basically the same as the type-A neural probe. First, a 300-nm aluminum layer was deposited on a silicon wafer as a sacrificial layer to release a thin final device from the wafer. Then, a 5-µm layer of parylene C and a 200-nm gold layer were deposited. An additional 5-µm layer of parylene C was deposited. A 300-nm aluminum layer was deposited and patterned on the parylene C layer as a mask of the configuration for the skeleton-like neural probe. The parylene C layer was patterned by O_2 RIE. After dissolving the aluminum layers by a mixed acid aluminum etchant, the neural probe was easily released from the silicon substrate.

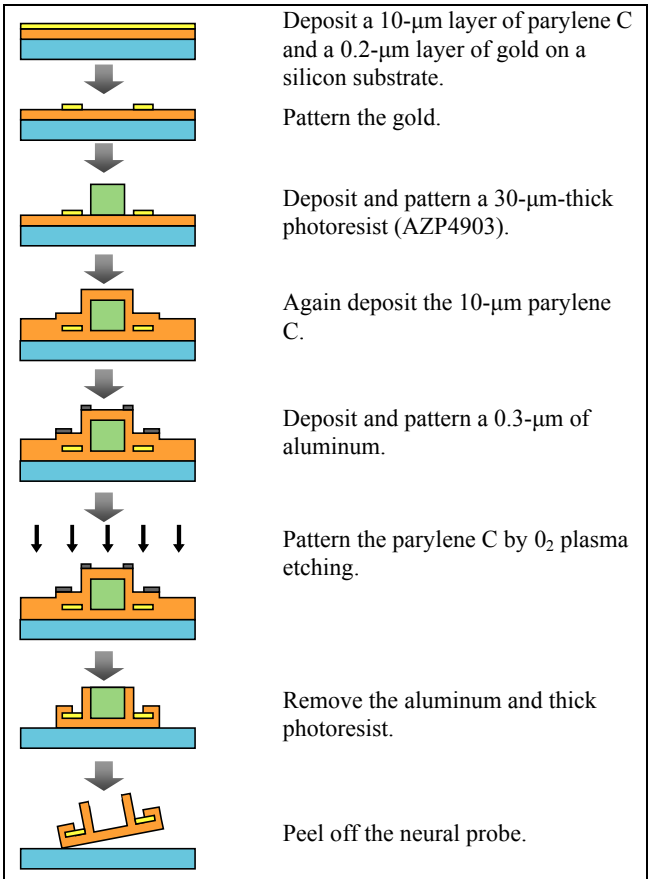

Fig. 3. Schematic of type-A neural probe fabrication. ■: Silicon, ■: Parylene C, ■: Gold, ■: Thick photoresist (AZP4903), ■: Aluminum

B. Neural Probe Implantation

For preliminary *in vivo* testing, all animal experiments were performed in accordance with the institutional guidelines of the Animal Experiments Committee of the University of Tokyo. In the acute experiments for the probe insertion and neural recording, Wistar rats were conducted. Anesthesia was induced by isoflurane gas. The body temperature of the rat was maintained and the heart rate was monitored. The anesthetized rat was then fixed to a stereotaxic instrument, and the skin on the head was incised to expose the surface of the skull. After the dura matter was carefully removed, a neural probe with the hybrid biodegradable polymers was slowly inserted into the cerebral cortex of the rat and used to record the neural signals. The neural signals were amplified from 10000 to 20000 times and filtered from 500 Hz to 3 kHz. The signals were recorded and stored on a computer via an AD converter at a sampling rate of 10 kHz.

C. Preparation of Hybrid Biodegradable Polymer

NGF with ovalbumin (OVA) was encapsulated in the biodegradable polymeric microspheres of PLGA by a modified solvent evaporation method [6]. First, 0.2g of OVA and 30 µg of NGF in 4 ml of a chloroform solution with 1 g of PLGA were dispersed by a polytron homogenizer for 5 min at 10000 rpm. The formed emulsion was stirred in 300 ml of a

0.1% PVA solution for 3 h at room temperature. The microspheres were centrifuged, washed repeatedly with distilled water, freeze dried for 48 h, and then stored at -20°C prior to use. Before coating the neural probe for implantation, the hybrid biodegradable polymer was made by mixing the biodegradable microspheres and PEG.

In an experiment on the NGF release from the biodegradable polymeric microspheres, the 0.2 g of the microspheres were immersed in 10 ml of a phosphate-buffered saline solution (PBS) and incubated at 37°C. At each time point (24, 48, 72, 168, and 336 h), the suspension of the microspheres was centrifuged (10 min, 1000 rpm). Then, 5 ml of the supernatant was collected and replaced with 5 ml of fresh PBS. The microspheres were again suspended by vortexing for 5 min. The total amount of NGF released from the microspheres was measured using an Enzyme-Linked Immuno Sorbent Assay (ELISA).

The bioactivity of the NGF released from the microspheres was assessed with the PC12 cells (Riken Cell Bank). The PC12 cells were incubated on a collagen dish under serum-free conditions. The control material consisted of the PC12 cells and the microspheres without NGF.

III. Results and Discussions

A. Neural Probe

The type-A and type-B flexible neural probes were designed and fabricated as shown in Fig. 4.

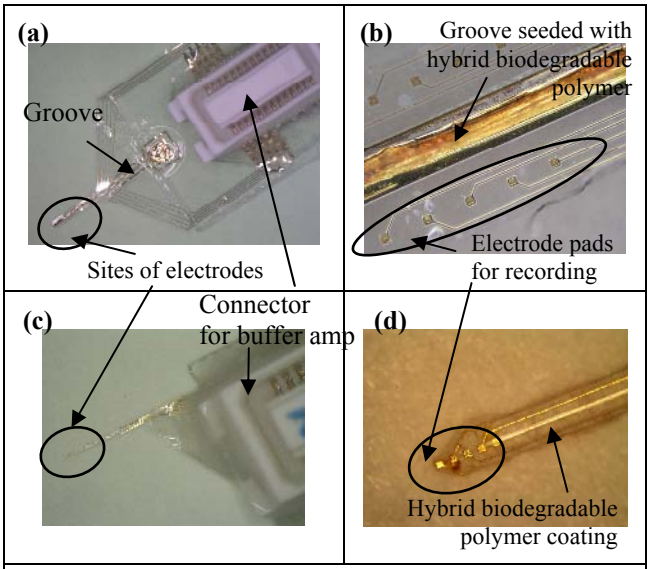

Fig. 4. Photos of neural probe. (a) is the type-A neural probe. (b) is magnified view of the electrode sites in tip of the neural probe. The hybrid biodegradable polymeric microspheres were seeded in the groove. (c) is the type-B neural probe. (d) is magnified picture of the electrode sites and each skeleton-lie probe coated with the hybrid biodegradable polymer.

The type-A neural probe has a single probe with ten electrode sites of 20×20 μm^2, a probe length of 3.7 mm, a probe width of 554 μm, a groove width of 120 μm, and a thickness of 20 μm. The impedance of the electrodes was about 100k-300 kΩ after being plated with platinum black. The type-B neural probe was formed with five electrode sites of 20×20 μm^2, a probe length for implantation of 2.9 mm, each probe width insulated by parylene C of 16 μm, a widest span of the probes of 186 μm, and a thickness of 10 μm. The impedance of the electrodes was about 100k-600 kΩ, which was not constant. Further, adjustment of the RIE and plating platinum black was required.

The hybrid bioactive degradable polymers were manually seeded in the groove for the type-A neural probe and coated on the type-B neural probe, which was aimed to promote tissue regeneration around the neural probe after implantation, and improve the mechanical stiffness of the flexible probe for insertion.

B. Hybrid biodegradable polymer and NGF Release

The degradable polymeric microspheres were fabricated and optimized to sizes between 10 μm and 30 μm for embedding in the groove or coating on the skeleton-like probe, as shown in Fig. 5. It was succeeded in loading the biodegradable microspheres with bioactive components of NGF with OVA. Also, as measured by NGF ELISA, 2-6 ng/ml of NGF was constantly released at each time point, and more than 160 ng of NGF was released after 336 hours.

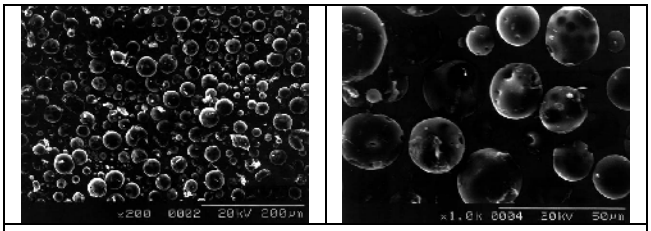

Fig. 5. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

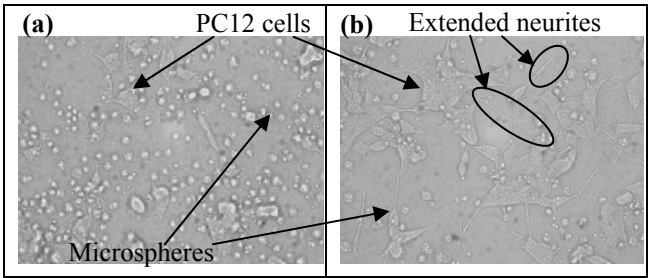

Fig. 6. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microsheres extended the neurites of PC12 cells.

The bioactivity of NGF released from the microspheres was investigated with the PC12 cells. The neurites of the PC12 cells were observed, after the microspheres were incubated in a dish for eight days. Fig. 6 shows the results that the neurites were longer and more numerous than those of the control

neurites. This indicates that the NGF released from the microspheres retained bioactivity.

C. Neural Probe Implantation

Both of the neural probes were successfully inserted in the rat's cortex. No buckling and breakage of the neural probes were observed. The implantation of the type-A and type-B neural probes is shown in Fig. 7. These results have shown that the neural probes have sufficient mechanical stiffness for insertion as well as good flexibility in the brain tissue after the hybrid biodegradable polymer was dissolved.

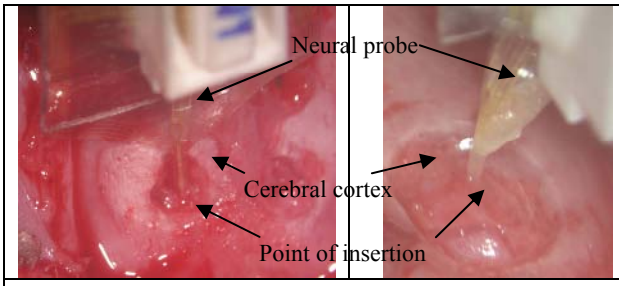

Fig. 7. Images of neural probes with PEG microspheres being successfully implanted in cerebral cortices of the rats.

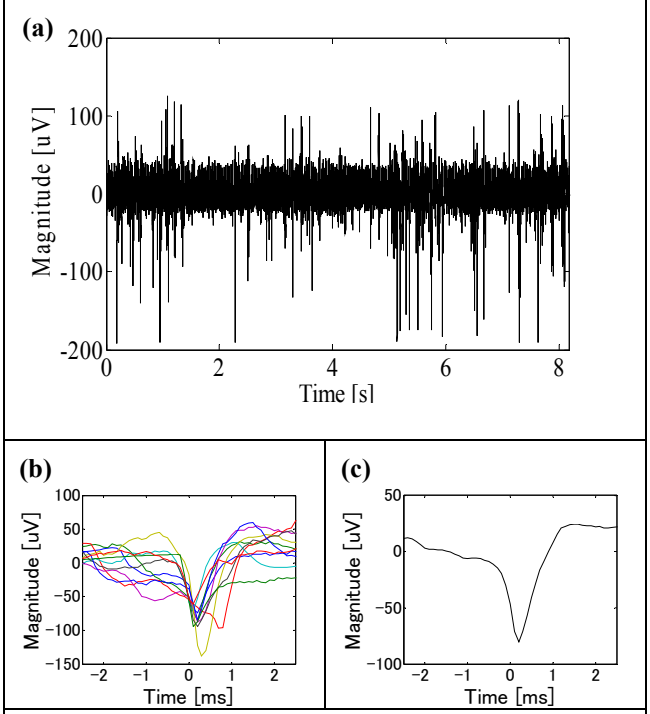

Fig. 8. (a) Spontaneous activity from the cerebral cortex of the rat recorded by the type-A neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity. (c) The averaged sorted waveform from (b).

Records from the rat's cerebral cortex were collected using the type-A neural probe, as indicated in Fig. 7, but the neural signals were not observed from the type-B neural probe. This was assumed due to the large physical distance between the electrode pads and the neuron activated, since the hybrid biodegradable polymer was on the electrode pads whereas there was no polymer on the electrode pads of the type-A probe. Thus, the type-B neural probe may need more sufficient time to dissolve and diffused the polymer from the electrode pad for recording.

The recorded neural signals from the type-A neural probe were sorted to observe the characteristics of the neural activity (Fig.8). A 5-ms portion of ten sorted waveforms was manually selected. It was observed that the recorded signal was from spontaneous activity, since the wavelength was about 1 ms and the wave magnitude was about 100 μVolt.

IV. Conclusion

The type-A and type-B multichannel flexible neural probes containing a hybrid biodegradable polymer were designed and developed. The efficacy of the released bioactive components was observed in the *in vitro* experiment with the neurites' extension and increment of the PC12 cells by the NGF released from the microspheres. In the *in vivo* experiment, it was succeeded in inserting both of the neural probes showing sufficient stiffness, and recording neural signals from the cerebral cortex of a rat using the type-A neural probe. While much work remains, these preliminary results have shown the possibilities that flexible neural probes coated with hybrid polymer can be applied for chronic recording along with neural regeneration.

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