Flexible Regeneration-type Nerve Electrode with Integrated Microfluidic Channels

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Abstract

The development of a neural interface that will allow signals from the human nervous system to control external equipment is extremely important for the next generation of prosthetic systems. A novel multichannel regeneration-type nerve electrode designed to record from and stimulate peripheral nerves has been developed to allow the control of artificial hands and to generate artificial sensations. In this study a novel flexible regeneration microelectrode based on the nerve regeneration principle was designed and fabricated using MEMS technologies. The electrode, which was fabricated on a 20-μm-thick Parylene C substrate, has multiple fluidic channels. Each fluidic channel was 100 μm wide x 40 μm high x 1500 μm long and featured multiple electrodes inside them as recording and stimulating sites. They also served as guidance channels for the regenerating axons. The authors are currently attempting to evaluate the probes using the sciatic nerve of rats.

Keywords: Neural Probe, Nerve Regeneration, Fluidic Channels

INTRODUCTION

The development of a brain-machine interface that will allow signals from the human nervous system to control external equipment such as artificial hands is extremely important for the next generation of prosthetic systems. Regeneration-type nerve electrodes are known to hold promise as a key device for realizing these neural interface systems. The principle underlying the regeneration-type electrodes is that the peripheral nerves of vertebrates will regenerate after being severed. In this process, the axons in the distal portion will degenerate, but the axons will regenerate from the proximal portion and will reach the distal end of the severed nerve bundle. Therefore, if a device consisting of many microelectrode holes is implanted between the severed stumps, the axons can regenerate through the holes, and the action potential of the regenerating axons can be measured by the electrode (Fig. 1). This regeneration electrode has many advantages such as 1) long-term and stable input/output due to good electrical and physical compatibility between the electrode and nerve fibers and 2) good selectivity of the nerve fibers (this is because signals from a very small number of nerve fibers can be measured by adjusting the

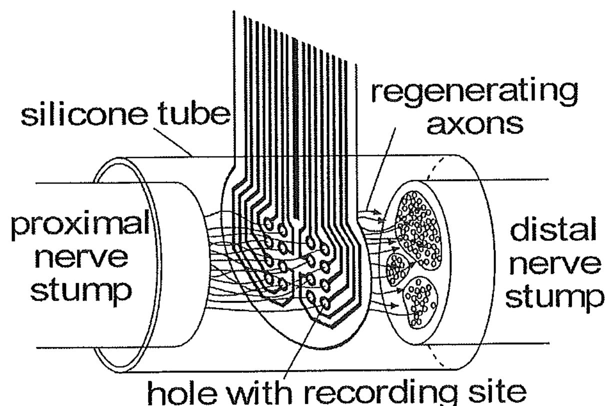


Fig.1: Schematic of the regeneration-type nerve electrode (current model)

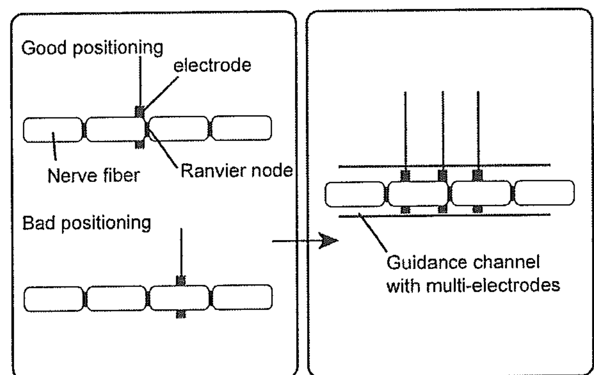


Fig.2: Distance between the recording sites and the Ranvier node of a single nerve fiber

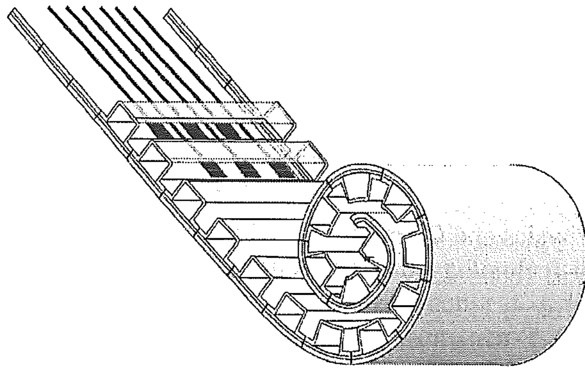


Fig.3: Schematic of the new regeneration-type nerve electrode integrated with multiple guidance channels.

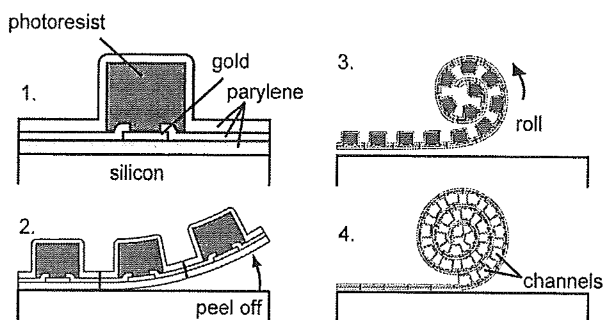


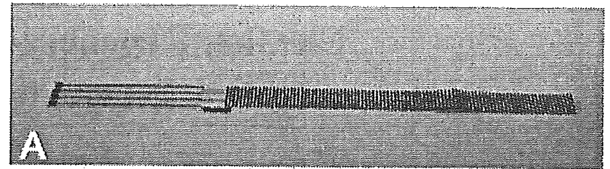
Fig.4: Fabrication process.
The photoresist was removed after rolling the fluidic channel area.

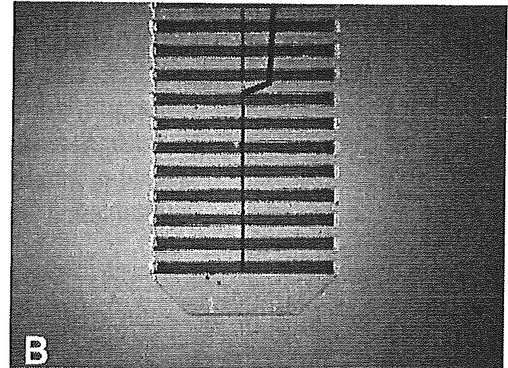
diameter of the holes.) Many studies have been done on regeneration-type nerve electrodes [1-2]. However, in many cases the electrodes were fabricated on silicon substrates or flexible films. When these electrodes have a plane structure, they have problems such as difficulty in recording signals with a good S/N ratio when the position of the recording site is located far from the Ranvier node (Fig. 2), and difficulty in increasing the number of channels due to cabling problems.

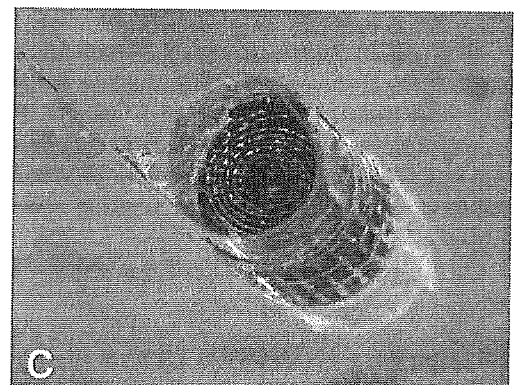
To solve these problems, we have designed and fabricated a novel flexible regeneration-type neural probe integrated with microfluidic channels on a flexible Parylene film base.

METHODS

A schematic of the flexible regeneration-type electrode we fabricated is shown in Fig. 3. Each fluidic channel has one or multiple recording/stimulation sites and serves as a guidance tube for the regenerating axons. The fabrication process is depicted in Fig. 4. This is similar to that of the probe integrated with microfluidic channels that we reported previously [3]. The photoresist worked as an sacrifice layer and was removed after rolling the portion with the fluidic channels.







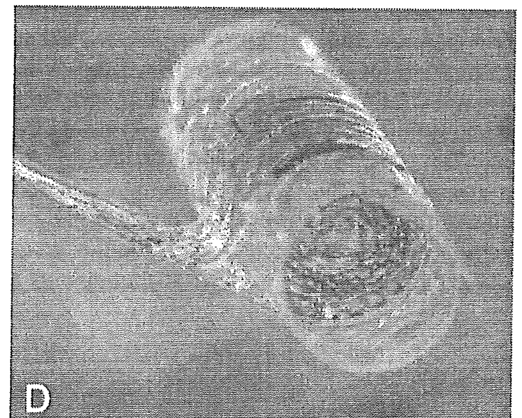


Fig.5: Photo of the fabricated probe. (A) Overview of the probe before rolling. (B) Tip of the probe. Each fluidic channel is W100 μm x H40 μm x L1500 μm (C) After rolling, but before removing the photoresist. (D) After removing the photoresist.

RESULTS and DISCUSSIONS

Figure 5 shows the whole structure of the fabricated neural probe. The length of the probe was 40 mm before

rolling (Fig. 5A), and the width of the probe was 2 mm. We designed several types of probes with between 80 and 200 fluidic channels. Each fluidic channel was 100 μm wide x 40 μm high x 1500 μm long. As the photoresist we used THB-611P or SUNFORT AQ-4059 dry film resist (Asahi Kasei) to obtain a controllable thickness of 40-100 μm, which defines the height of the fluidic channels.

The issues we are currently working on are:
1) *in vivo* experiments using rat sciatic nerves to evaluate the biocompatibility of this probe and the feasibility of the guidance channels.
2) an increase in the number of channels.

ACKNOWLEDGEMENTS

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REFERENCES

[1] A. Mannard, R. B. Stein, D. Charles: Regeneration electrode units: Implants for recording from single peripheral nerve fibers in freely moving animals, Science, 183, 547-549 (1974)
[2] G. T. A. Kovacs, C. W. Storment, J. M. Rosen: Regeneration Microelectrode Array for Peripheral Nerve Recording and Stimulation, IEEE Trans. BME, 39, 893-902 (1992)
[3] S. Takeuchi, D. Ziegler, Y. Yoshida, K. Mabuchi, T. Suzuki: Parylene flexible neural probe with micro fluidic channel, Lab on a Chip, 5, 519-523 (2005)

Flexible Intracortical Neural Probe with Biodegradable Polymer for Delivering Bioactive Components

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Abstract
A flexible intracortical neural probe containing a biodegradable polymer for delivering bioactive components was developed. This was designed to promote regrowth of damaged neural tissues around the implanted neural probe for a long-term recording. The neural probe was based on the flexible and biocompatible material of parylene C incorporated a drug delivery system (DDS). A groove structure of the probe was designed to seed the degradable polymeric microspheres with bioactive components, to promote recovery of the damaged tissues, and improve mechanical stiffness for the probe implantation. The efficacy of released nerve growth factor (NGF) from the microspheres was observed in *in vitro* experiments with PC12 cells. The neural probe was successfully inserted in the cerebral cortex of a rat, and neural signals were recorded. These results have shown the possibility that the flexible intracortical neural probe can be applied for chronic recording along with neural regeneration.

Keywords: Biodegradable polymer, Drug delivery system, Micro Electro Mechanical System, Neural probe

1 INTRODUCTION

In the past decade, a wide variety of neural probes have been applied in Brain-Machine Interfaces, an electrophysiological investigations, and medical treatments. There are, however, no suitable probes for long-term neural recording, nor are there optimal probes to chronically stimulate the brain. One of these significant reasons is the death of brain tissues damaged by the neural probe insertion. Thus, we have been developing a new flexible neural probe to achieve regrowth of damaged neural tissues for chronic recording and stimulation, while integrating a drug delivery system (DDS) based on Micro Electro Mechanical System [1-4].

The neural probe was based on the flexible and biocompatible material of parylene C (poly-monochloro-paraxylylene). The neural probe has a groove structure. This was designed to seed a large amount of microspheres, since the small wells were limited to fill and deliver the volume of bioactive components. Biodegradable polymeric microspheres with bioactive components of nerve growth factor (NGF) seeded in the groove were added with the aim of promoting recovery of damaged tissue around the neural probe. 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 flexible intracortical neural probe and the biodegradable polymeric microspheres were examined.

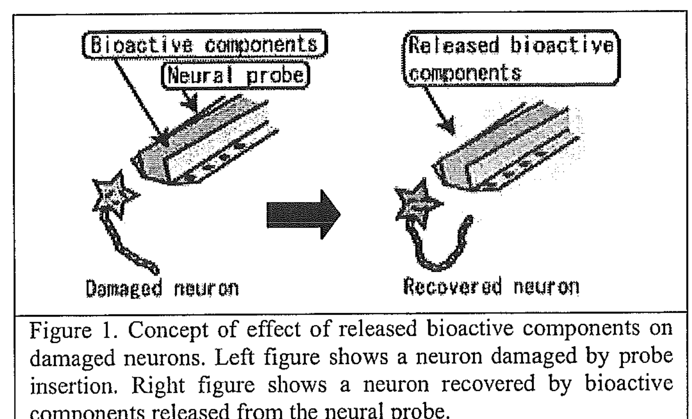

Figure 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the neural probe.

2 METHODS

2.1 Neural Probe Fabrication

The neural probe was constructed using a surface micromachining technique with parylene C and gold layers on

a silicon wafer. The fabrication of the neural probe was as follows. A 10-µm layer of parylene C was deposited (LABCOTER2 PDS2010, Specialty Coating Systems) on a silicon wafer. Then, a 200-nm gold layer was 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 spun 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 removing the aluminum layer, the neural probe was peeled off of the silicon substrate with tweezers. A cross-sectional schematic diagram of the manufacturing process is shown in figure 2.

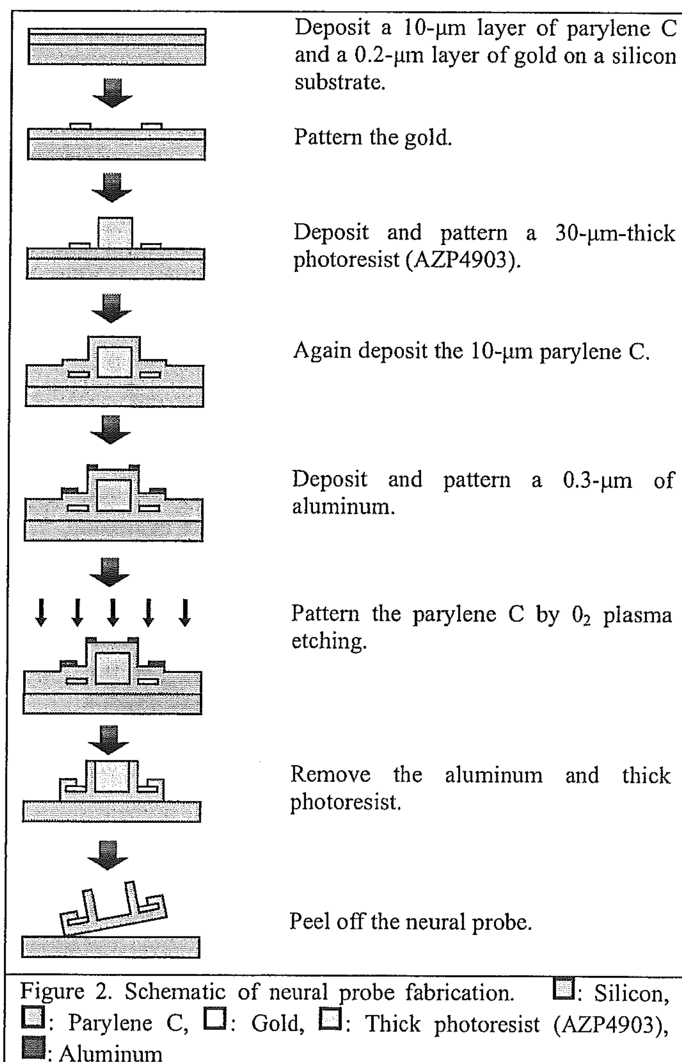

Figure 2. Schematic of neural probe fabrication. □: Silicon, □: Parylene C, □: Gold, □: Thick photoresist (AZP4903), ■: Aluminum

For the insertion and recording of the neural probe, all animal experiments were performed in accordance with the institutional guidelines of the Animal Experiments Committee of the University of Tokyo.

Implantation of the neural probe and recording of the neural signals were performed in a rat (Wistar rat) anesthetized with isoflurane gas. The heart rate was monitored and body temperature was maintained. The anesthetized rat was then fixed to a stereotaxic instrument, and the skin on the head was incised to expose the surface of its skull. After the dura matter was carefully removed, the neural probe with the microspheres containing polyethylene glycol PEG) embedded in the groove was slowly lowered into the cerebral cortex of the rat and recorded 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.

2.2 Microsphere Preparation

NGF with ovalbumin (OVA) was encapsulated in the biodegradable polymer of PLGA by a solvent evaporation method [5]. The method of fabricating the microspheres was as follows. First, 0.2g of OVA and 30µg 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 protein-polymer dispersion was again homogenized in 20 ml of a 1% PVA solution for 5 min at 10000 rpm. The formed emulsion was stirred in 300 ml of a 0.1% PVA solution for 3 hours at room temperature. The microspheres were centrifuged, washed repeatedly with distilled water, freeze dried for 48 hours, and then stored at -20° C prior to use.

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 h, 48 h, 72 h, 168 h, 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 by an Enzyme-Linked Immuno Sorbent Assay. Between 2-6 ng/ml of NGF was constantly released at each time point, and more than 160 ng of NGF had been released after 336 hours.

The bioactivity of NGF released from the microspheres was assessed using 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 with the microspheres without NGF.

3 RESULTS AND DISCUSSIONS

3.1 Neural Probe

The flexible intracortical neural probe was designed and fabricated as shown in figure 3. The device has a single probe

with electrode sites of 20×20 μm, 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 groove was formed, and the bioactive degradable microspheres with PEG were manually seeded in the groove, which provides a route for tissue regeneration around the neural probe after implantation, and improves the mechanical stiffness for insertion.

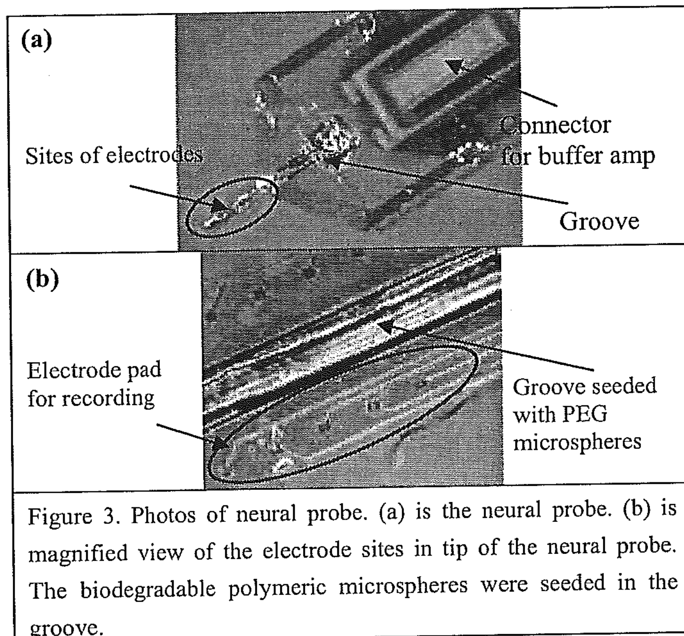

Figure 3. Photos of neural probe. (a) is the neural probe. (b) is magnified view of the electrode sites in tip of the neural probe. The biodegradable polymeric microspheres were seeded in the groove.

3.2 Microspheres

The degradable polymeric microspheres were fabricated. It was succeeded in loading the biodegradable microspheres with bioactive components of NGF with OVA, and optimized the size between 10μm and 30 μm for embedding in the groove, as shown in figure 4.

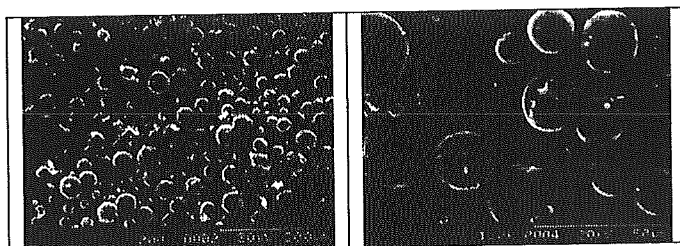

Figure 4. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

3.3 NGF Release

The bioactivity of NGF released from the microspheres was investigated. After the microspheres were incubated in a dish for 8 days, it was observed that the neurites of the PC12 cells were longer and more numerous than those of the control neurites. This indicates that the NGF released from the microspheres retained bioactivity.

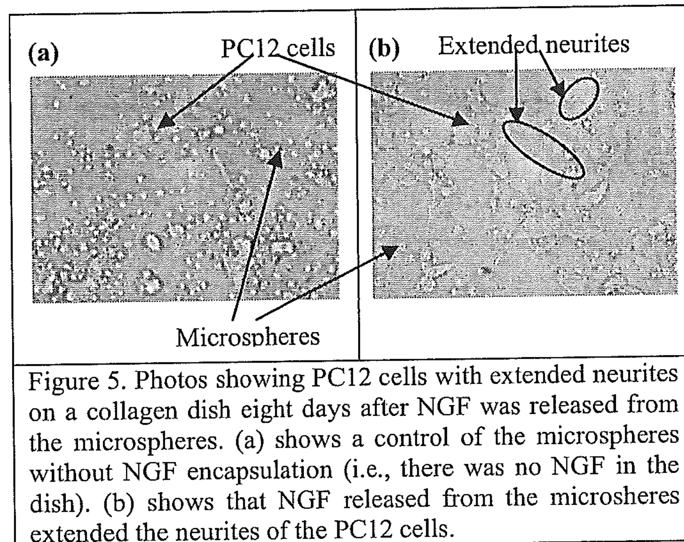

Figure 5. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microsheres extended the neurites of the PC12 cells.

3.4 Neural Probe implantation and Recording

The neural probe was successfully inserted in the rat's cortex. No buckling and breakage of the neural probe was observed. Figure 6 shows the neural probe implantation. This result has shown that the neural probe has sufficient mechanical stiffness for insertion as well as good flexibility in the brain tissue after the PEG is dissolved.

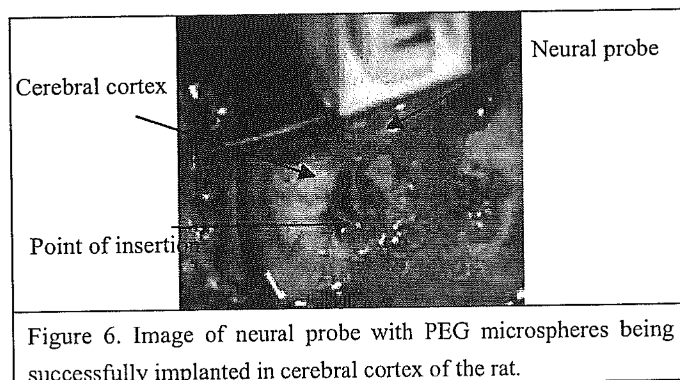

Figure 6. Image of neural probe with PEG microspheres being successfully implanted in cerebral cortex of the rat.

Records from the rat's cerebral cortex were successfully collected using the neural probe, as indicated in figure 7. The recorded neural signals were manually sorted to observe the characteristics of neural activity. In this study, a 5-ms portion of ten sorted waveforms was randomly selected. It was observed that the recorded signal was from spontaneous activity, since the wave length was about 1 ms and the wave magnitude was about 100 μVolt.

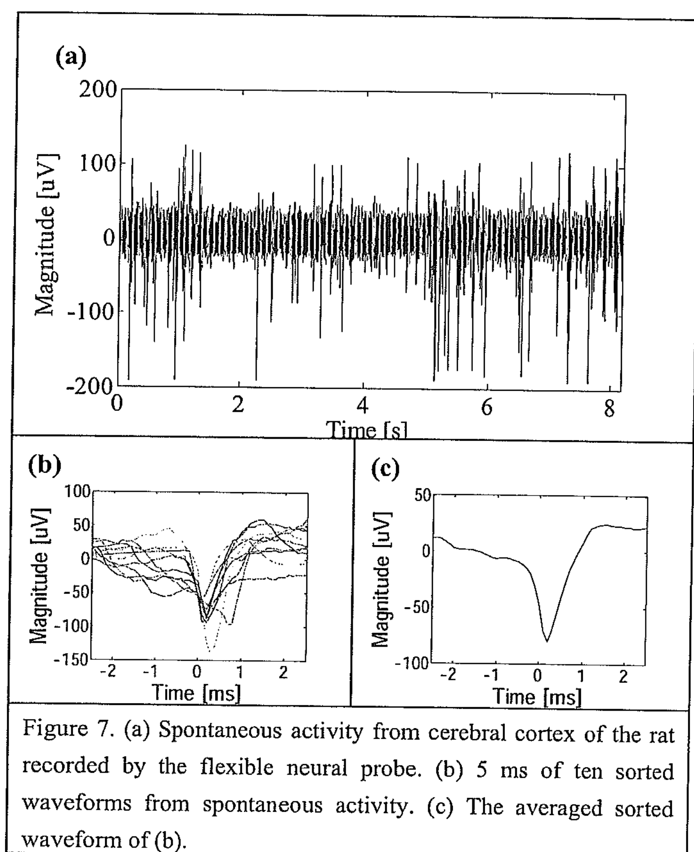

Figure 7. (a) Spontaneous activity from cerebral cortex of the rat recorded by the flexible neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity. (c) The averaged sorted waveform of (b).

4 CONCLUSIONS

The flexible intracortical neural probe containing the biodegradable polymer for delivering bioactive components was designed and developed. In the *in vitro* experiment, the efficacy of the released bioactive components was observed, since the neurites of the PC12 cells were extended and increased by the NGF released from the microspheres. In the *in vivo* experiment, it was succeeded in inserting the neural probe and recording neural signals from the cerebral cortex of the rat. Thus, these experiments have shown the possibilities of the neural probe in that it provides an optimal implant environment, extends the longevity of the tissue-electrode interface, and enables neural regeneration.

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6 BIBLIOGRAPHICAL REFERENCES

[1] S. Takeuchi, T. Suzuki, K. Mabuchi, H. Fujita "3D flexible multichannel neural probe array", Journal of Micromechanics and Microengineering, Vol.14, No.1, pp.104-107 (2004)
[2] S. Takeuchi, D. Ziegler, Y. Yoshida, K. Mabuchi, T. Suzuki "Parylene flexible neural probe with micro fluidic channel", Lab on a Chip, Vol.5, pp.519-523 (2005)
[3] Y. Kato, T. Suzuki, K. Mabuchi "Preliminary study of a neural probe using biodegradable polymer for delivering bioactive components", Journal of Japanese Society for Medical and Biological Engineering, Vol.43, suppl.1(2005)
[4] Y. Kato, M. Nishino, T. Suzuki, K. Mabuchi "Preliminary Model of a Flexible Neural Probe Containing Biodegradable Polymer for Delivering Bioactive Components", 20th Annual Symposium on Biological and Physiological Engineering, pp.17-18(2005)
[5] X. Cao, MS. Shoichet "Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders", Biomaterials, Vol.20, No.4, pp.329-39(1999)