

インタフェースの研究開発に取り組んできた<sup>(4)(5)</sup>。さらに本稿では、損傷した脳組織の回復という課題を解決するために、生分解性ポリマーによるドラッグデリバリーシステムを応用した薬剤徐放型柔軟神経プローブの提案<sup>(6)</sup>と製作を行った(図1参照)。

ドラッグデリバリーシステムとは、目的とする場所に、適切な量の薬剤を、適切な期間、送達するのを可能とする技術である。本稿では、グリア瘢痕形成の抑制や損傷した神経のシナプス形成を補助するために Nerve Growth Factor (NGF) という神経成長因子を薬剤として選んだ。そして NGF を一定期間ゆっくりと徐放させるために、PLGA (polylactic-co-glycolic acid) という生分解性ポリマーに包埋し、マイクロスフィアを精製した。次に十分な刺入強度を得るために、polyethylene glycol (PEG) と NGF が包埋されたマイクロスフィアを混ぜ、神経インタフェースのプローブ上に固着させた。大脳皮質に刺入後は、直ちに PEG は溶解し、電極周辺部にマイクロスフィアが拡散する。その後マイクロスフィアは、生分解するとともに適切な期間、電極周辺部に直接 NGF を徐放して、損傷した脳組織の回復を促進することにより、長期間安定した神経活動の多点計測と刺激を可能とする神経インタフェースを実現できると期待される。

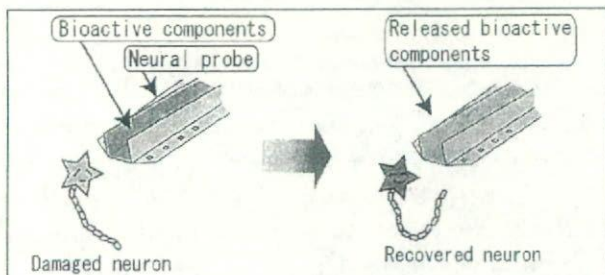


図1 生分解性ポリマーを用いた薬剤徐放型柔軟神経プローブのコンセプト

Figure 1. Concept of effect of released bioactive components on damaged neurons. The left figure shows a neuron damaged by probe's insertion. The right figure shows a neuron recovered by released bioactive components from the neural probe.

## 2. 方法

(2.1) 神経電極の作製 生体適合性が高く柔軟な材料であるパリレンを基盤とし、プローブ先端に電極を多点配置した薬剤徐放型柔軟神経プローブを製作した。PEG と NGF が包埋されたマイクロスフィアを固着させるため、プ

ローブ中心に溝を設けた。

神経プローブの製作は次の手順で行った(図2参照)。まず、シリコン基板にパリレン C を  $10\mu\text{m}$  蒸着した。つぎに、金を  $0.4\mu\text{m}$  蒸着し、電極配線をパターンニングした。そしてプローブ中心に溝をつくるために、厚膜レジストを  $30\mu\text{m}$  蒸着してパターンニングした。さらにパリレン C を  $10\mu\text{m}$  蒸着した。保護膜としてアルミニウムを  $0.3\mu\text{m}$  蒸着した後、プローブの形状に  $\text{O}_2$  プラズマエッチングを行った。そしてアルミニウムと厚膜レジストを取り除き、神経プローブをピンセットで剥離した。最後に、神経プローブにコネクタを接続した。

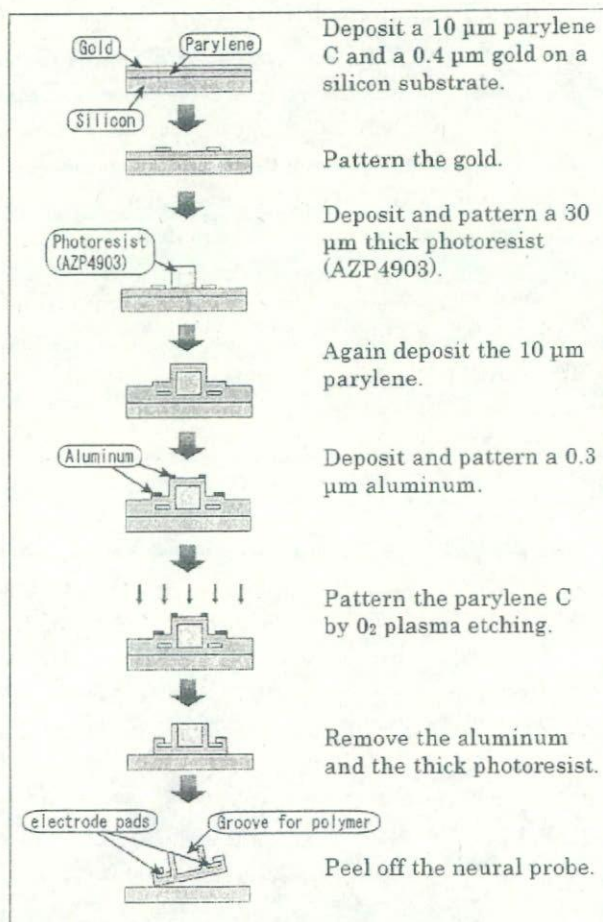


図2 神経プローブの製作過程

Figure 2. Schematic of the neural probe fabrication.

(2.2) マイクロスフィアの作製 包埋する薬剤として、代表的な神経栄養因子である NGF を用いた。包埋方法は溶媒蒸発法<sup>(6)</sup>を応用して NGF を PLGA に包埋した。またマイクロスフィアの大きさは、プローブ中心の溝に固着させるのに適した、 $50\mu\text{m}$  以下となるように設計した。

マイクロスフィアは次の手順で作製した。まず、4ml の



クロロフォルム溶液に 1mg の PLGA と 200mg のオパールミンを溶解させて 5 分間 8000rpm でホモジナイズした。次に、ホモジナイズされた溶液を 20ml の 1% PVA (Polyvinyl alcohol) 溶液に混合し、再度ホモジナイズした。さらにエマルジョンされた溶液を、300ml の 0.1% PVA 溶液中に 3 時間室温下で分散させた。最後に、遠心分離と蒸留水による洗浄を繰り返した後、オパールミンが包埋された PLGA 微粒子を抽出し、48 時間凍結乾燥した。作製されたマイクロスフィアは、使用まで  $-20^{\circ}\text{C}$  にて保存した。

### 3. 結果と考察

(3.1) 神経電極 生分解性ポリマーを用いた薬剤徐放型柔軟神経プローブ製作し、図 3 に示した。プローブ中心が、マイクロスフィアを設置する溝で、その左右に計測・刺激用の電極が配置されている。白金黒めっき後の電極インピーダンスは、 $100\text{ k}\Omega$  から  $300\text{ k}\Omega$  となった。

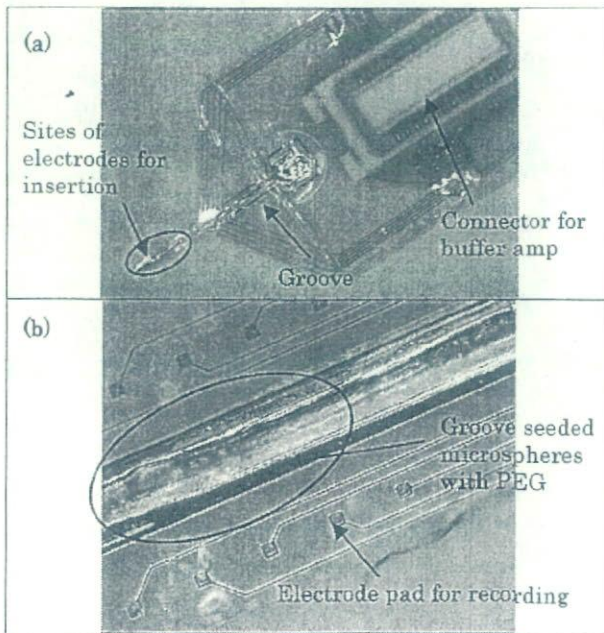


図 3 薬剤徐放型柔軟神経プローブの写真

Figure 3. Photos of the neural probe. (a) is the neural probe. (b) shows the magnification of the electrode's site in the tip of the neural probe. The biodegradable polymeric microspheres were seeded in the groove.

(3.2) 薬剤の包埋 PLGA に NGF を包埋し、マイクロスフィアを作製した。またマイクロスフィアの大きさを、 $10\mu\text{m}$  から  $30\mu\text{m}$  に調整した。図 4 に、作製したマイクロスフィアの走査型電子顕微鏡画像を示した。

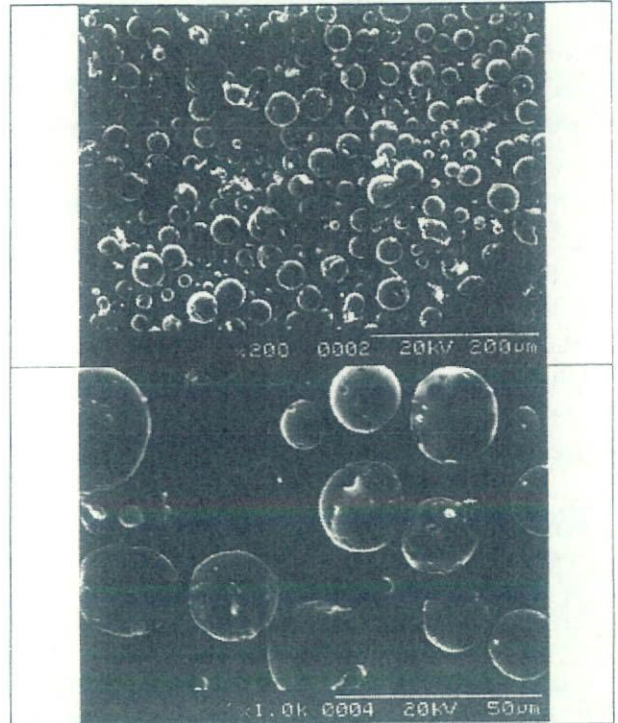


図 4 マイクロスフィアの走査型電子顕微鏡画像

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

(3.3) 薬剤の徐放と活性 マイクロスフィアからの NGF の徐放は、Enzyme-linked Immunosorbent Assay (ELISA) を用いて確かめた。まず凍結乾燥されたマイクロスフィアを、リン酸緩衝生理食塩水に投入し、 $37^{\circ}\text{C}$  でインキュベートした。次に一定時間後、遠心分離して上澄みを抽出した。最後に、抽出した上澄みを NGF ELISA により、NGF の徐放量を測定した。結果、2 週間にわたり NGF は、 $2\sim 6\text{ ng/ml}$  とゆっくり徐放することが確かめられた。

次に、徐放された NGF の活性は、ラット副腎褐色細胞腫細胞 (PC12) を用いて観察した。コントロールとして NGF 未包埋のマイクロスフィアがまかれたディッシュ (図 5(a) 参照) と、NGF の包埋されたマイクロスフィアがまかれたディッシュ (図 5(b) 参照) を用意し、無血清下において 8 日間インキュベートした。図 5(a) では、PC12 の神経化はみられないが、図 5(b) では、PC12 の神経化、Neurites の伸展と増加を観察でき、徐放された NGF の活性の保持を確かめられた。



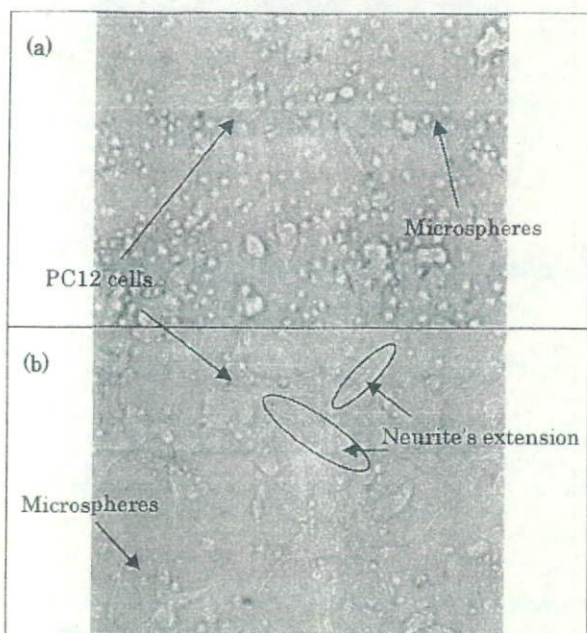


図5 徐放されたNGFによるPC12神経化の写真  
 Figure 5. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) is a control of the microspheres without NGF encapsulation (i.e., there are no NGF in the dish). (b) shows that NGF released from the microspheres extended the neurites of the PC12 cells.

(3・4) 急性実験 全ての実験は、東京大学実験動物委員会の定めるガイドラインに準拠して行った。実験動物として300g前後のWistarラットを使用し、イソフルレンガスの麻酔下において、PEGとマイクロスフィアをプローブ中心に固着させた薬剤徐放型柔軟神経プローブを大脳皮質運動野に刺入した。また術中は、携帯カイロを用いてラットの体温を維持し、圧センサにより心拍のモニターを行った。神経細胞の活動は、500Hzから3kHzを通過帯域とする差動計測を行った。

急性実験により、PEGとマイクロスフィアをプローブ中心に固着させることで、大脳皮質への刺入に必要な強度を持つことが確かめられた(図6参照)。また大脳皮質運動野より神経細胞の自発発火活動が計測され、図7に結果を示した。

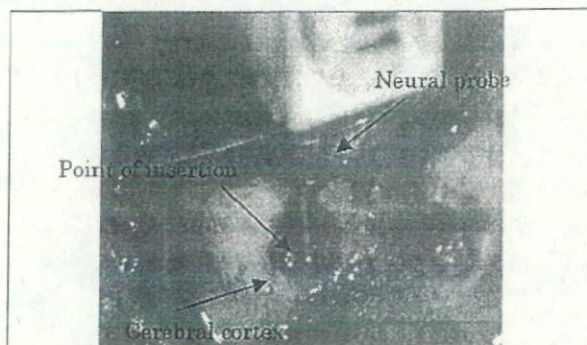


図6 薬剤徐放型柔軟神経プローブの大脳皮質内への刺入写真  
 Figure 6. Image that the probe with the microspheres and PEG was successfully implanted in the cerebral cortex of the rat.

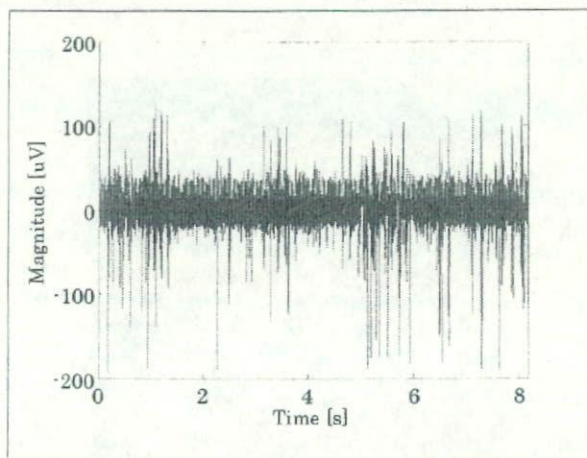


図7 薬剤徐放型柔軟神経プローブにより計測された神経活動の自発発火  
 Figure 7. Spontaneous activity from the cerebral cortex of the rat was recorded by the flexible neural probe.

#### 4. おわりに

本稿では、MEMSとドラッグデリバリーシステムの技術の融合による、生分解性ポリマーを用いた薬剤徐放型柔軟神経プローブの提案と製作を行った。作製した神経プローブを大脳皮質内に刺入し、神経活動が計測された。またマイクロスフィアからNGFの徐放と活性が確かめられた。これにより、損傷した脳組織の回復を促進する機能を賦与する、より低侵襲で長期間安定した神経活動の多点計測と刺激が可能な神経インタフェースの実現可能性を示した。今後の課題として、マイクロスフィアへのNGF包埋の濃縮、

神経プローブに設置されたマイクロスフィアから徐放された NGF の評価、そして慢性実験での神経活動の計測などが必要と思われる。

## 謝辞

本神経プローブ製作に関して、東京大学先端科学技術研究センター生命大部門人工生体機構分野の鎮西恒雄助教授、斎藤逸郎助手、並びに中川英元客員研究員に装置利用と有用なアドバイスを頂き感謝致します。またマスク作成に際しては、東京大学大規模集積システム設計教育センター(VDEC)より御支援を頂いた。本研究の一部は、文部科学省科学研究費補助金(基盤研究(A), 課題番号 17206022), 及び厚生労働科学研究費補助金(萌芽的先端医療技術推進研究事業 H17-ナノ-010)の助成を受けた。ここに記して謝意を表す。

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## 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- $\mu\text{m}$ -thick Parylene C substrate, has multiple fluidic channels. Each fluidic channel was 100  $\mu\text{m}$  wide x 40  $\mu\text{m}$  high x 1500  $\mu\text{m}$  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

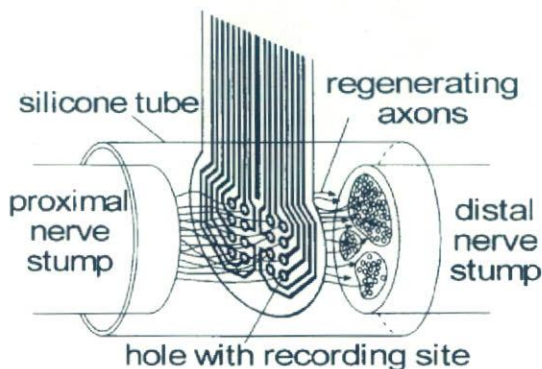


Fig.1: Schematic of the regeneration-type nerve electrode (current model)

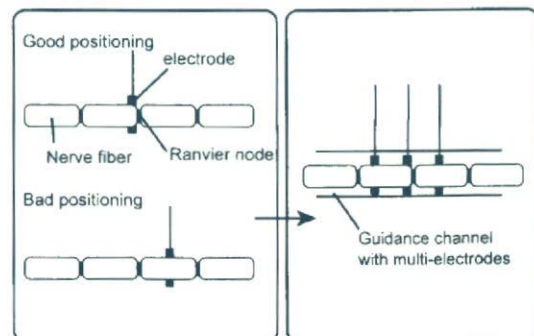


Fig.2: Distance between the recording sites and the Ranvier node of a single nerve fiber



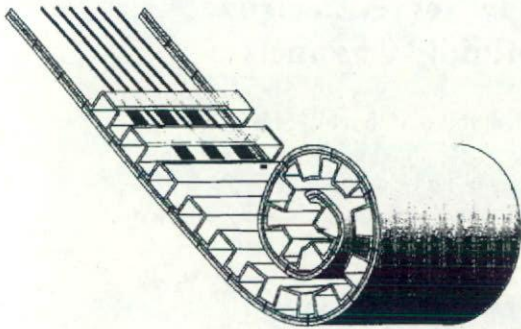


Fig.3: Schematic of the new regeneration-type nerve electrode integrated with multiple guidance channels.

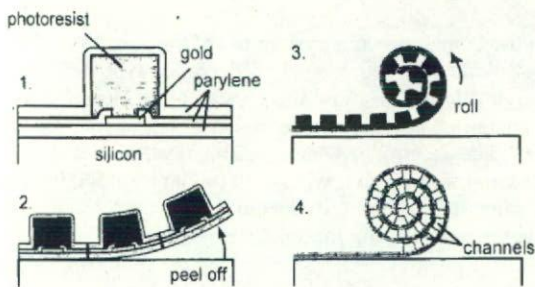


Fig.4: Fabrication process. The photoresist was removed after rolling the fluidic channel area.

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.

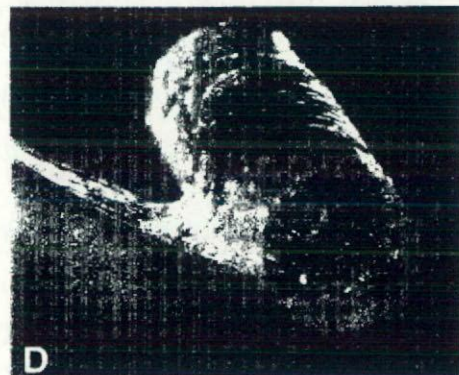
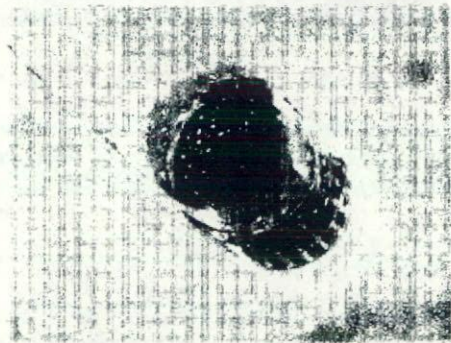
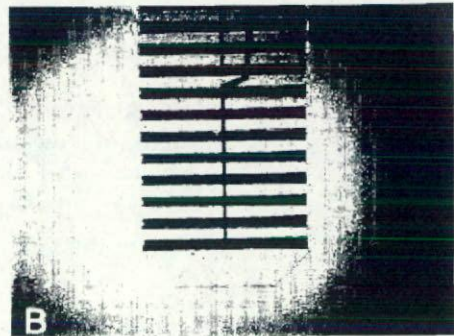


Fig.5: Photo of the fabricated probe. (A) Overview of the probe before rolling. (B) Tip of the probe. Each fluidic channel is  $W100\ \mu\text{m} \times H40\ \mu\text{m} \times L1500\ \mu\text{m}$  (C) After rolling, but before removing the photoresist. (D) After removing the photoresist.

### 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  $\mu\text{m}$  wide x 40  $\mu\text{m}$  high x 1500  $\mu\text{m}$  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  $\mu\text{m}$ , 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.

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## 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.

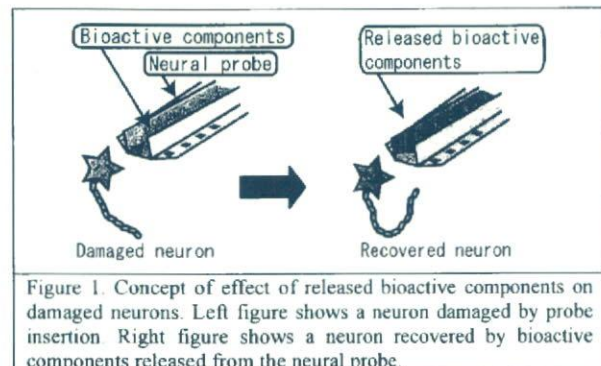


Figure 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the neural probe.

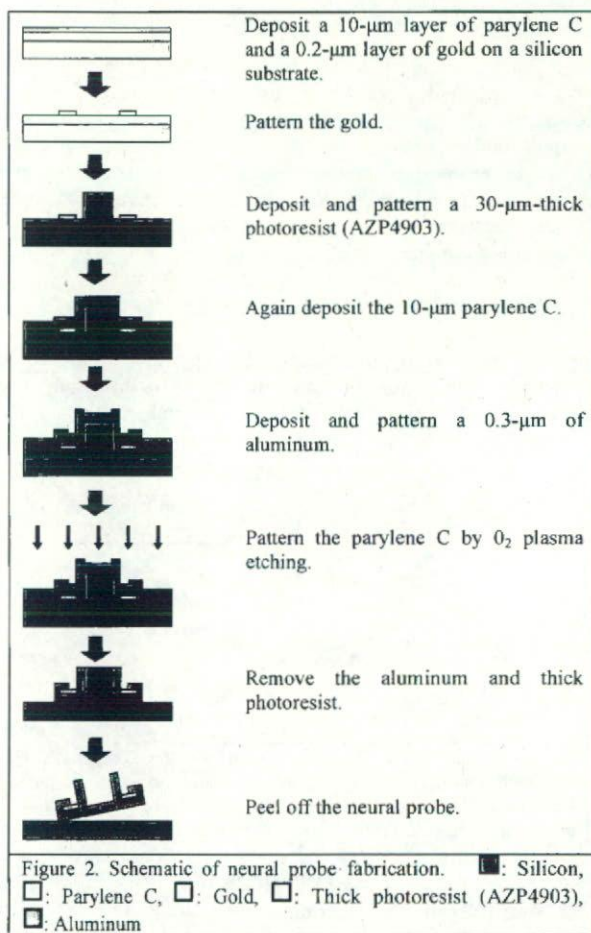
### 2 METHODS

#### 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- $\mu\text{m}$  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  $\mu\text{m}$ . A 10- $\mu\text{m}$  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  $\text{O}_2$  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.



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 30 $\mu\text{g}$  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  $-20^\circ\text{C}$  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  $37^\circ\text{C}$ . 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  $20 \times 20 \mu\text{m}$ , a probe length of 3.7 mm, a probe width of  $554 \mu\text{m}$ , a groove width of  $120 \mu\text{m}$ , and a thickness of  $20 \mu\text{m}$ . The impedance of the electrodes was about  $100\text{k}-300 \text{k}\Omega$  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.

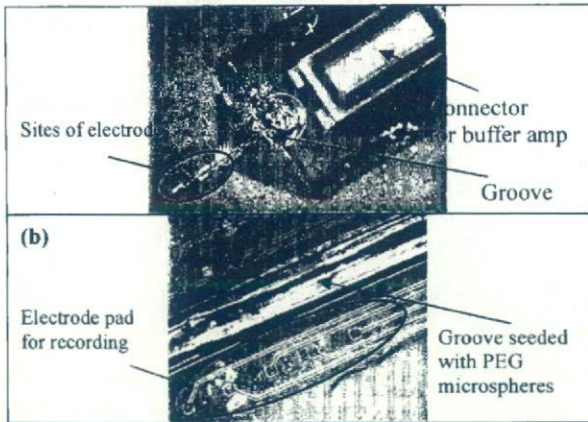


Figure 3. Photos of neural probe. (a) is the neural probe. (b) is magnified view of the electrode sites in tip of the neural probe. The biodegradable polymeric microspheres were seeded in the groove.

### 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  $10 \mu\text{m}$  and  $30 \mu\text{m}$  for embedding in the groove, as shown in figure 4.

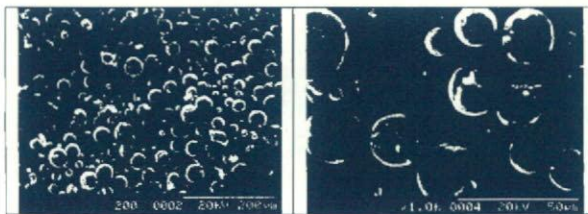


Figure 4. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

### 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.

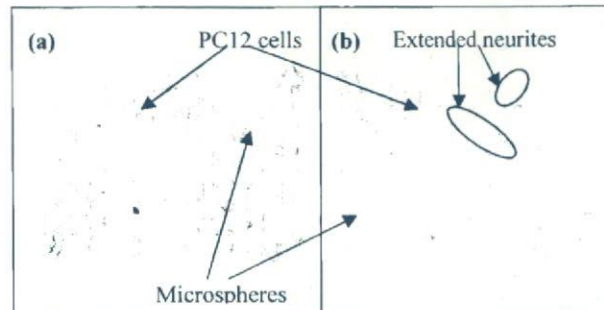


Figure 5. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microspheres extended the 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  $100 \mu\text{V}$ olt.



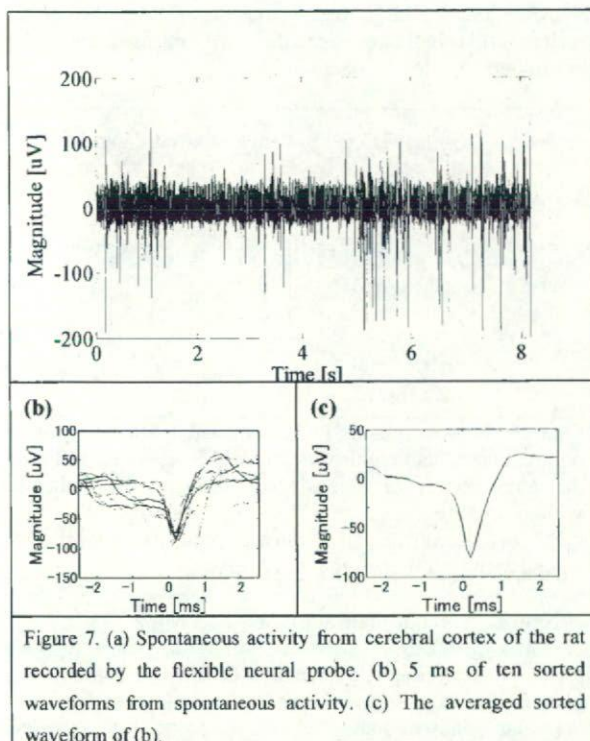


Figure 7. (a) Spontaneous activity from cerebral cortex of the rat recorded by the flexible neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity. (c) The averaged sorted waveform of (b).

#### 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.

#### 5 ACKNOWLEDGEMENTS

We thank Dr.Saito and Dr.Nakagawa of the Chinzai Laboratory in Research Center for Advanced Science and Technology at the University of Tokyo for their practical suggestions and a probe fabrication. We also thank the VLSI Design and Education Center at the University of Tokyo for a mask fabrication. This work was supported by a Grant-in-Aid for Scientific Research (A) 17206022 from the Ministry of Education, Science, and Culture of Japan, and Research on Advanced Medical Technology H17-NANO-010 from the Ministry of Health and Welfare of Japan.

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

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**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.

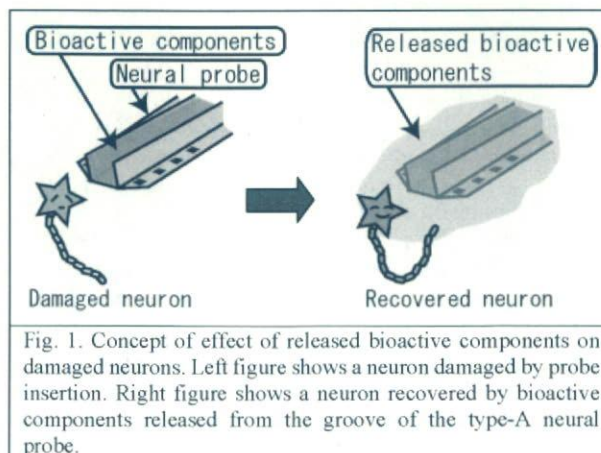
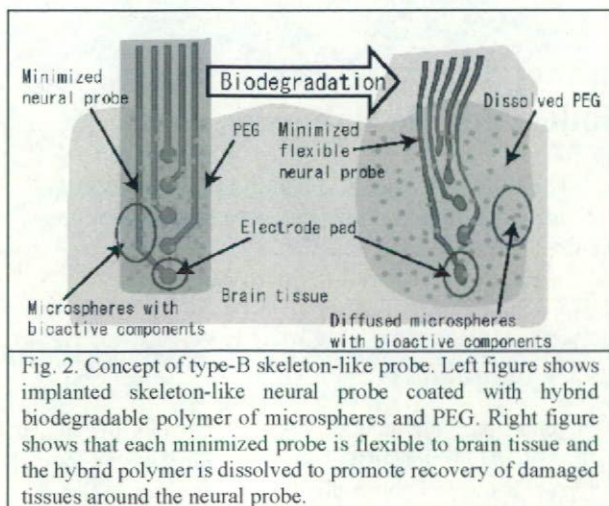


Fig. 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the groove of the type-A neural probe.

This work was supported by a Grant-in-Aid for Scientific Research (A) 17206022 from the Ministry of Education, Science, and Culture of Japan, by a Grant-in-Aid for Research on Advanced Medical Technology H17-NANO-010 from the Ministry of Health, Labour and Welfare of Japan, and by a grant from Tateishi Science and Technology Foundation.





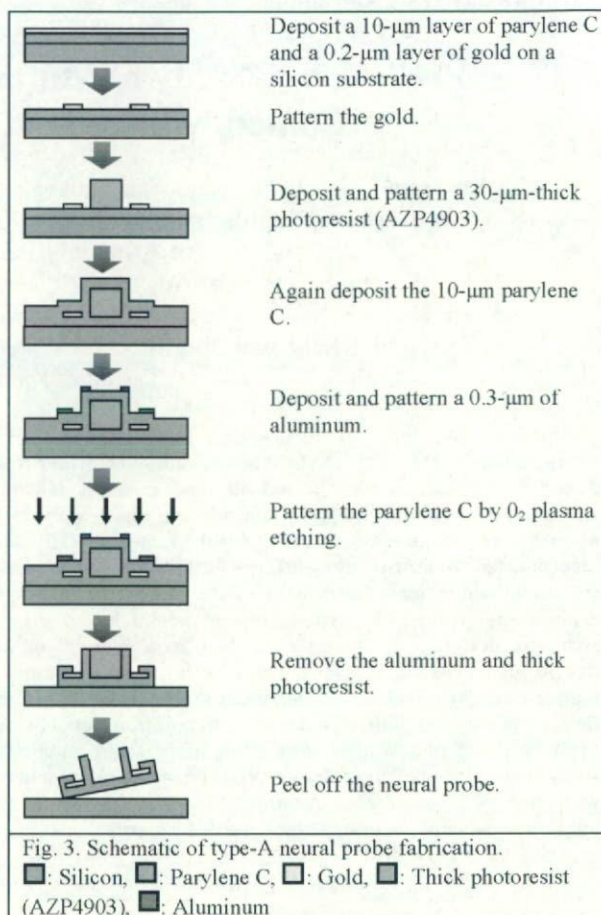
## II. METHODS

### 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- $\mu\text{m}$  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  $\mu\text{m}$ . A 10- $\mu\text{m}$  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  $\text{O}_2$  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- $\mu\text{m}$  layer of parylene C and a 200-nm gold layer were deposited. An additional 5- $\mu\text{m}$  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  $\text{O}_2$  RIE. After dissolving the aluminum layers by a mixed acid aluminum etchant, the neural probe was easily released from the silicon substrate.



### 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  $\mu\text{g}$  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  $-20^{\circ}\text{C}$  prior to use. Before coating the neural probe for implantation, the hybrid biodegradable polymer was made by mixing the biodegradable microspheres and PEG.

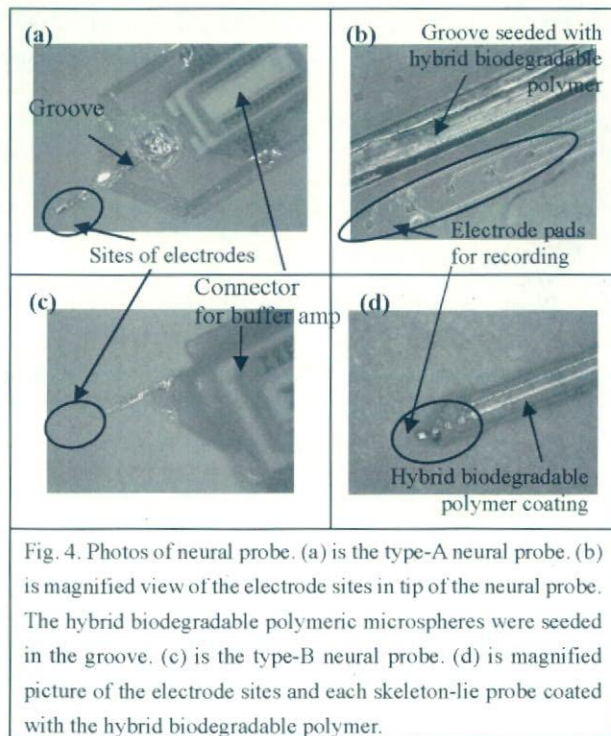
In an experiment on the NGF release from the biodegradable polymeric microspheres, the 0.2 g of the microspheres were immersed in 10 ml of a phosphate-buffered saline solution (PBS) and incubated at  $37^{\circ}\text{C}$ . At each time point (24, 48, 72, 168, and 336 h), the suspension of the microspheres 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.



The type-A neural probe has a single probe with ten electrode sites of  $20 \times 20 \mu\text{m}^2$ , a probe length of 3.7 mm, a

probe width of  $554 \mu\text{m}$ , a groove width of  $120 \mu\text{m}$ , and a thickness of  $20 \mu\text{m}$ . The impedance of the electrodes was about 100k-300 k $\Omega$  after being plated with platinum black. The type-B neural probe was formed with five electrode sites of  $20 \times 20 \mu\text{m}^2$ , a probe length for implantation of 2.9 mm, each probe width insulated by parylene C of  $16 \mu\text{m}$ , a widest span of the probes of  $186 \mu\text{m}$ , and a thickness of  $10 \mu\text{m}$ . The impedance of the electrodes was about 100k-600 k $\Omega$ , 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  $10 \mu\text{m}$  and  $30 \mu\text{m}$  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.

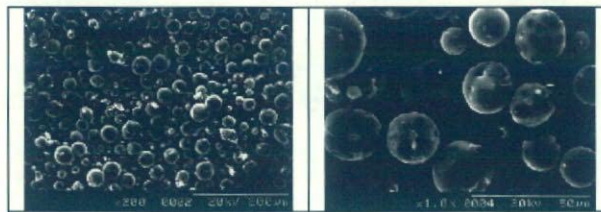


Fig. 5. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

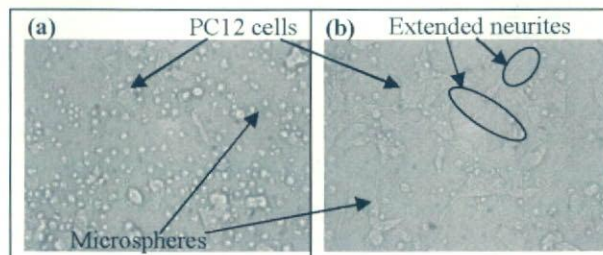


Fig. 6. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microspheres extended the neurites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

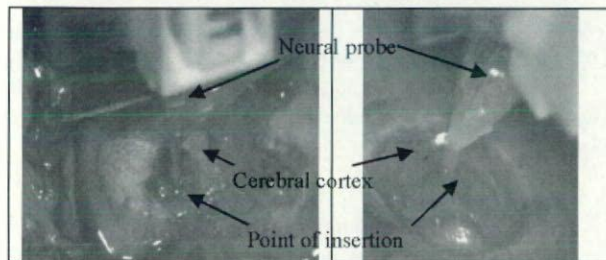


Fig. 7. Images of neural probes with PEG microspheres being successfully implanted in cerebral cortices of the rats.

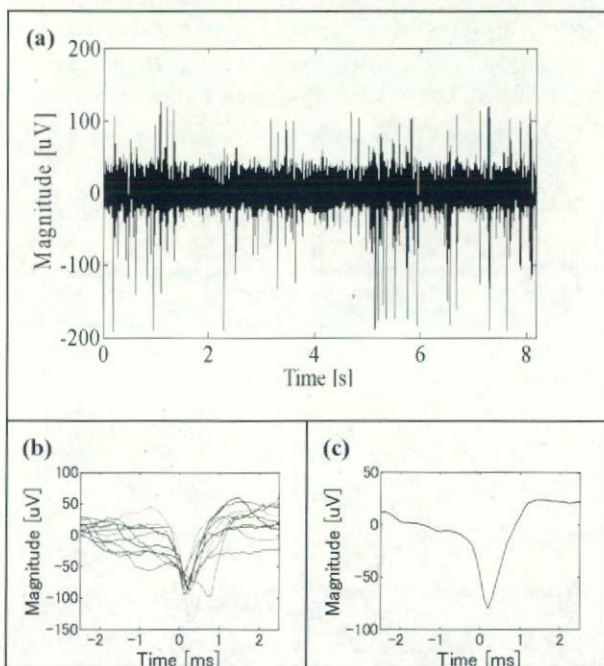


Fig. 8. (a) Spontaneous activity from the cerebral cortex of the rat recorded by the type-A neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity. (c) The averaged sorted waveform from (b).

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  $\mu$ Volt.

## 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.

## ACKNOWLEDGMENT

We thank Dr. Nakagawa of the Chinzai Laboratory in Research Center for Advanced Science and Technology at the University of Tokyo for his practical suggestions of the probe fabrication. We also thank the VLSI Design and Education Center at the University of Tokyo for helping with a mask fabrication.

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