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Takafumi Suzuki, Naoki Kotake, Kunihiko Mabuchi, Shoji Takeuchi	Nobel Flexible regeneration-type nerve electrode with integrated micro fluidic channels	Proceeding of International Conference on Microtechnologies in Medicine and Biology		303-305	2006
Yasuhiro Kato, Miharu Nishino, Itsuro Saito, Takafumi Suzuki, Kunihiko Mabuchi	Flexible Intracortical Neural Probe with Biodegradable Polymer for Delivering Bioactive Components	Proceeding of International Conference on Microtechnologies in Medicine and Biology		143-146	2006
加藤康広，鈴木隆文，満渕邦彦	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開発	電気学会研究会資料　医用・生体工学研究会	MBE-06	85-90	2006

Yasuhiro Kato, Takafumi Suzuki, Kunihiko Mabuchi	Development of mesh-structure multichannel flexible neural probe	Proceedings of the 19th International Microprocesses and Nanotechnology Conference		370-371	2006
Yasuhiro Kato, Itsuro Saito, Takayuki Hoshino, Takafumi Suzuki, Kunihiko Mabuchi	Preliminary Study of Multichannel Flexible Neural Probes Coated with Hybrid Biodegradable Polymer	Proceedings of the 28th IEEE EMBS Annual International Conference		660-663	2006
鈴木隆文，小竹直樹，満渕邦彦，竹内昌治	微小流路を利用した神経再生型電極の開発	第21回生体・生理工学シンポジウム論文集		155-158	2006

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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-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

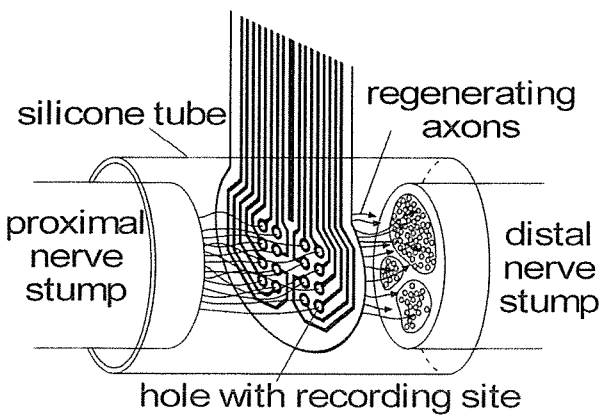

Fig.1: Schematic of the regeneration-type nerve electrode (current model)

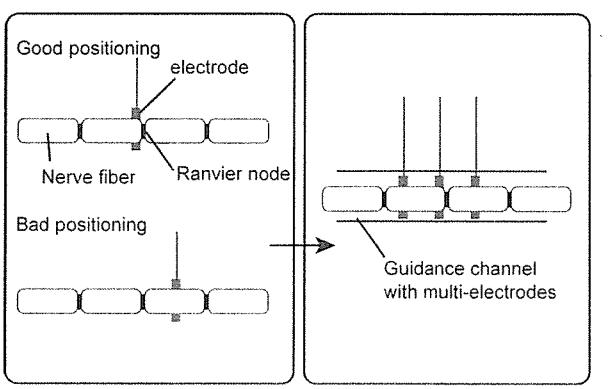

Fig.2: Distance between the recording sites and the Ranvier node of a single nerve fiber

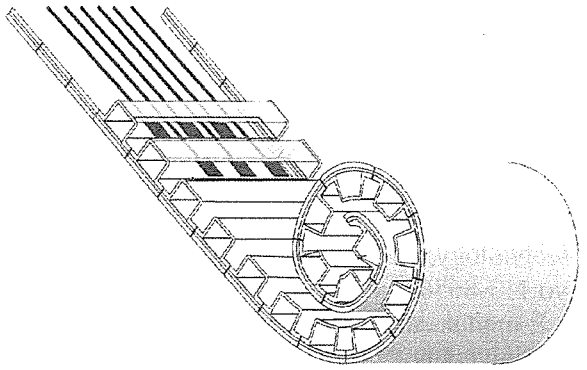

Fig.3: Schematic of the new regeneration-type nerve electrode integrated with multiple guidance channels.

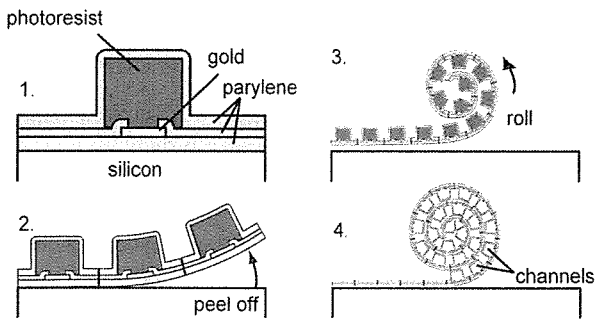

Fig.4: Fabrication process.
The photoresist was removed after rolling the fluidic channel area.

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.

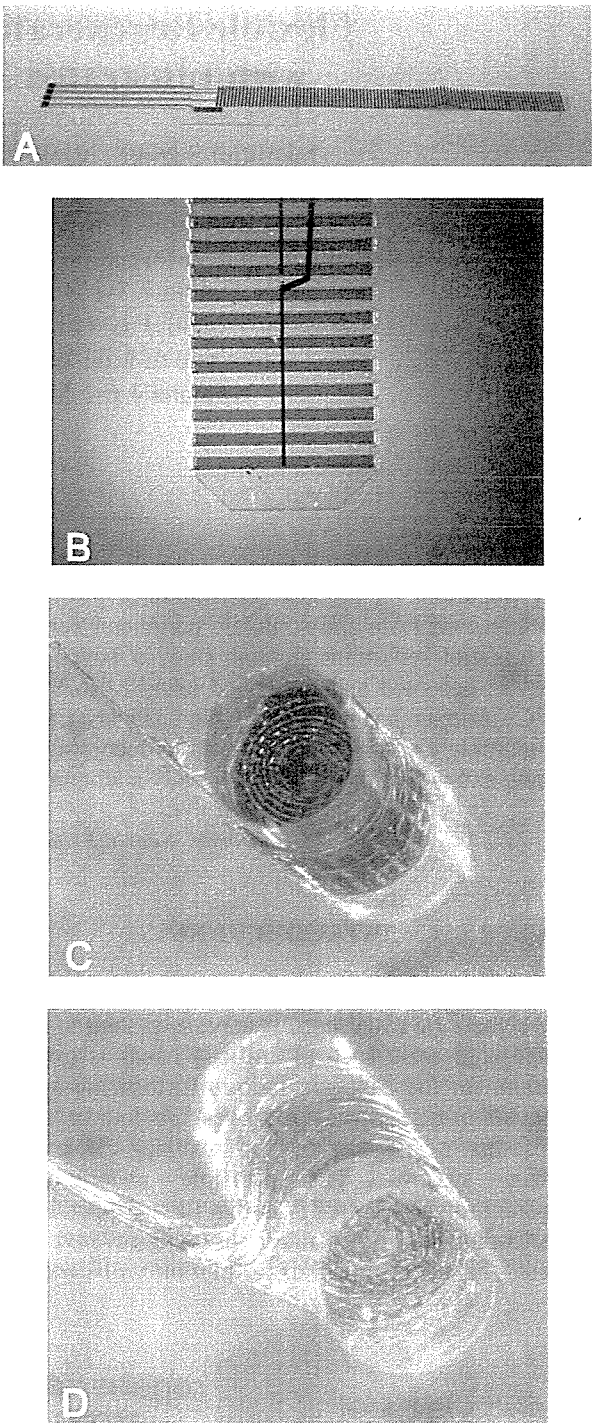

Fig.5: Photo of the fabricated probe. (A) Overview of the probe before rolling. (B) Tip of the probe. Each fluidic channel is W100 μm x H40 μm x L1500 μm (C) After rolling, but before removing the photoresist. (D) After removing the photoresist

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

We thank the VLSI Design and Education Center, the University of Tokyo for the 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, Labour and Welfare of Japan.

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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.

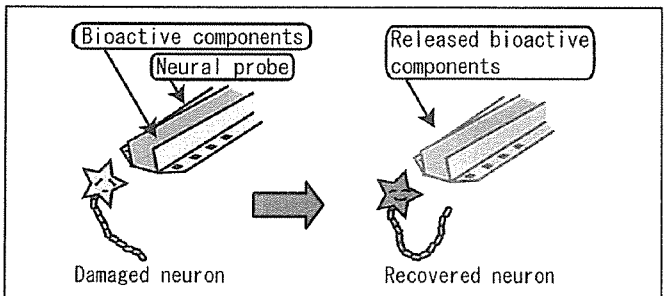

Figure 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the neural probe.

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-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.

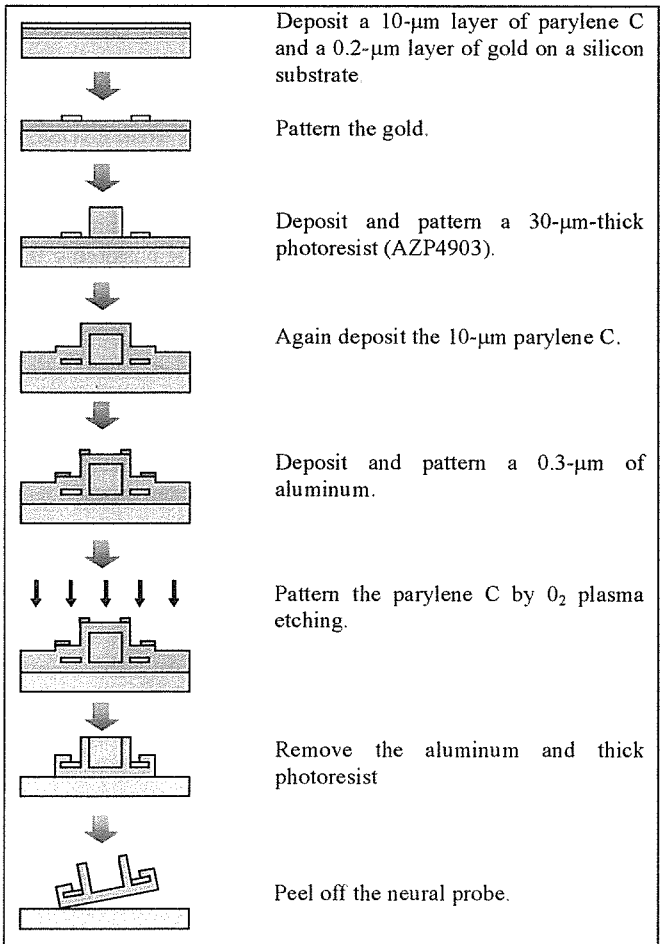

Figure 2. Schematic of neural probe fabrication. □: Silicon, □: Parylene C, □: Gold, □: Thick photoresist (AZP4903), □: Aluminum

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.

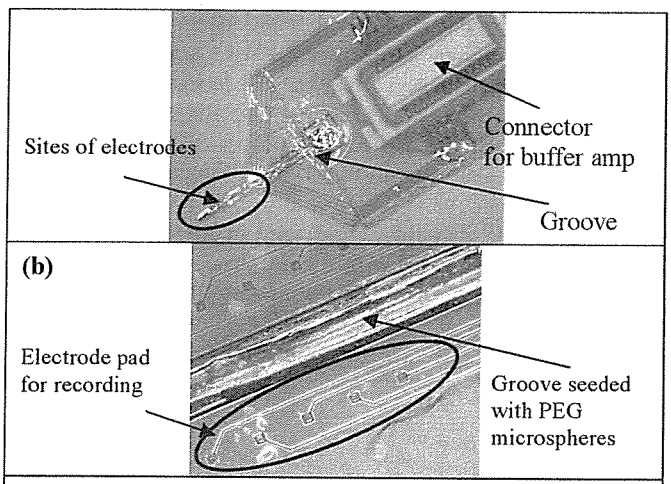

Figure 3. Photos of neural probe. (a) is the neural probe (b) is magnified view of the electrode sites in tip of the neural probe. The biodegradable polymeric microspheres were seeded in the groove.

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.

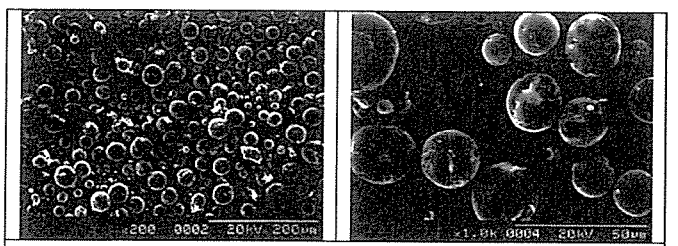

Figure 4. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

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.

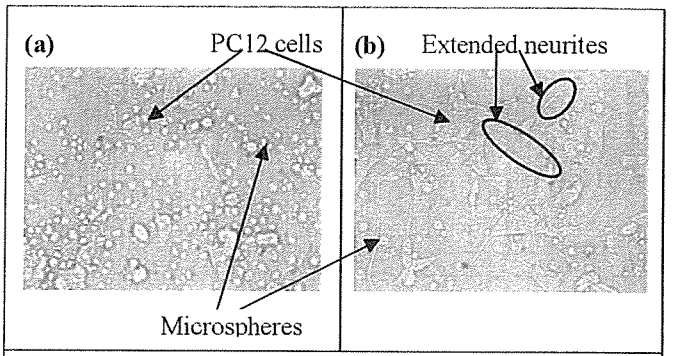

Figure 5. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microsheres extended the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

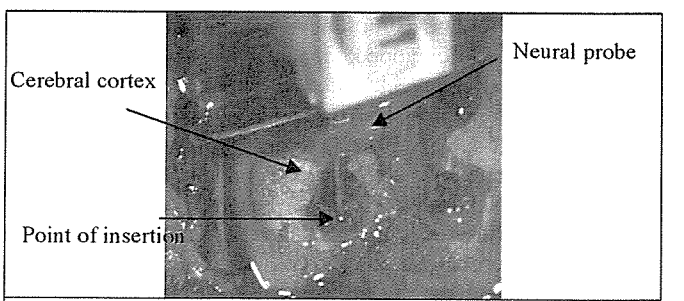

Figure 6. Image of neural probe with PEG microspheres being successfully implanted in cerebral cortex of the rat.

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

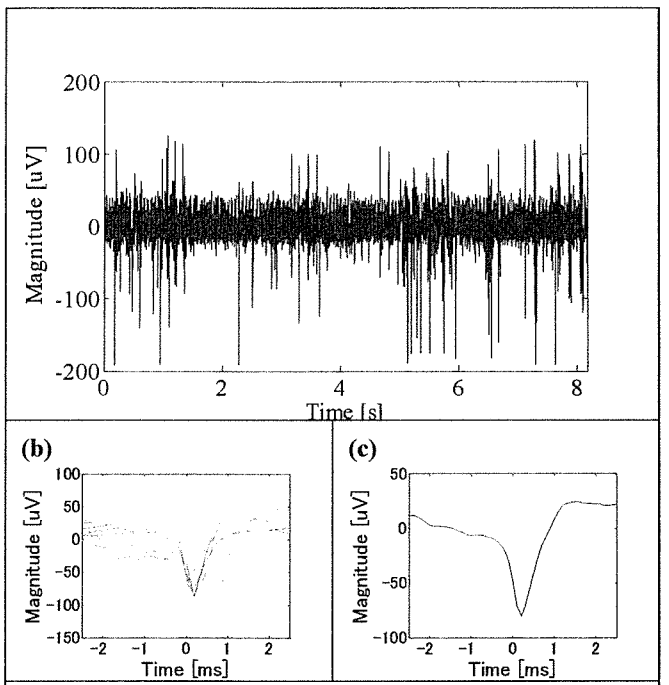

Figure 7. (a) Spontaneous activity from cerebral cortex of the rat recorded by the flexible neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity. (c) The averaged sorted waveform of (b).

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.

5 ACKNOWLEDGEMENTS

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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開発

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Development of a Flexible Neural Probe Composing Biodegradable Polymer for Delivering Bioactive Components

Yasuhiro Kato*, Takafumi Suzuki, Kunihiko Mabuchi (The University of Tokyo)

Abstract

A flexible neural probe containing a biodegradable polymer for delivering bioactive components, such as nerve growth factor (NGF), was developed. This was designed to promote regrowth of damaged neural tissues around the implanted neural probe for a long-term recording and stimulation. The neural probe based on the flexible material of parylene has a groove structure to seed biodegradable polymeric microspheres with NGF, to promote recovery of the damaged tissues and improve mechanical stiffness for the probe implantation. In *in vitro* experiment, NGF from the microspheres was slowly released in 2 weeks. The neural probe was successfully inserted in and recorded neural signals from a cerebral cortex of a rat.

キーワード：生分解性ポリマー、ブレイン・マシン　インタフェース、ドラックデリバリーシステム、微小電気機械システム、神経成長因子、神経インタフェース、神経プローブ

(Biodegradable polymer, Brain-machine interface, Drug delivery system, Micro Electro Mechanical System, Nerve growth factor, Neural interface, Neural probe)

1. はじめに

現在日本には、350万人以上の身体障害者[1]がおり、様々な医療が日々試みられている。しかし、その多くは治療が困難であり、完治に至っていない。そこで、失われたもしくは衰えた身体能力の補綴を目的として、Brain-Machine Interface (BMI)という医用工学技術が必要とされて近年急速に発展してきた。BMIとは、脳と機械を直接接続することにより、脳からの信号を直接取得する、逆に生体外からの信号を直接脳へ伝達するといった、生体－機械間の入出力信号を通して生体を補綴する器機を制御するシステムである。このBMIにおいて、生体の神経信号と生体外の計測・制御信号を直接入出力するデバイスを神経インタフェースという。このような神経インタフェースを完成させBMIを実現することで、人工視覚、人工内耳といった人工感覚生成への応用、そして脊椎損傷患者や筋委縮性側索硬化症、パーキンソン病、てんかんなどの神経学的な障害や身体的問題のある患者に対しての治療や介護において大きな可能性を秘めていると考えらており、開発が進められている。特に、患者の生涯にわたり脳・神経に埋め込み、かつ神経制御をできるような、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の実現が望まれている。しかし、莫大な神経細胞との入出力に必要とされる電極数の不足や、刺入と埋植による脳組織への損傷などの問題により、実現には至っていない。

そこで、電極数の増加とその配置など容易にするためにMicro Electro Mechanical System (MEMS) を応用した、ミシガン大学のプローブ型[2]やユタ大学の剣山型[3]のような神経インタフェースが開発された。しかし、ミシガン大学やユタ大学などのMEMSで作製された多くの神経インタフェースは、脳組織に対して硬いシリコン材料を用いた構造により神経に対する侵襲が大きく、目的とする神経細胞が計測・刺激範囲外へ物理的にずれやすいなどの課題を残した。また刺入時による外傷や刺入後の電極のずれなどによる損傷で、計測・刺激対象である脳組織の死滅、また電極部周辺の炎症反応やグリア瘢痕の形成による電極周囲のインピーダンスの変化などにより、長期間安定した神経活動の計測・刺激が困難とされた。

このような課題を解決するために、我々はMEMSを応用し、複数の神経信号を十分な分解能を持って計測・刺激できる微細な電極を多点配置するような設計と加工をするだけでなく、シリコンのような硬い材料に代わり柔らく生体適合性が高いパリレン材料を用いた、複数の神経細胞活動の同時多点計測と刺激を可能とする柔軟な構造を持つ神経

インタフェースの研究開発に取り組んできた[4][5]。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[6][7]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。

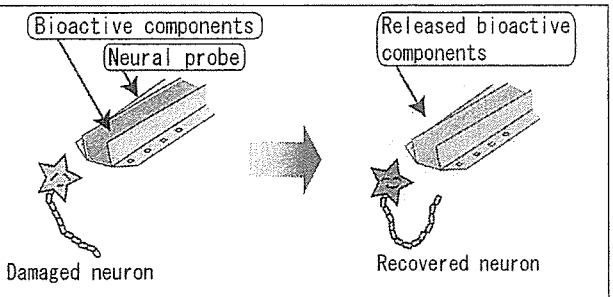

図1　生分解性ポリマーを用いた薬剤徐放型柔軟神経プローブのコンセプト

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 10μm 蒸着した。つぎに、金を 0.4μm 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 30μm 蒸着してパターンニングした。さらにパリレン C を 10μm 蒸着した。保護膜としてアルミニウムを 0.3μm 蒸着した後、プローブの形状に 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。

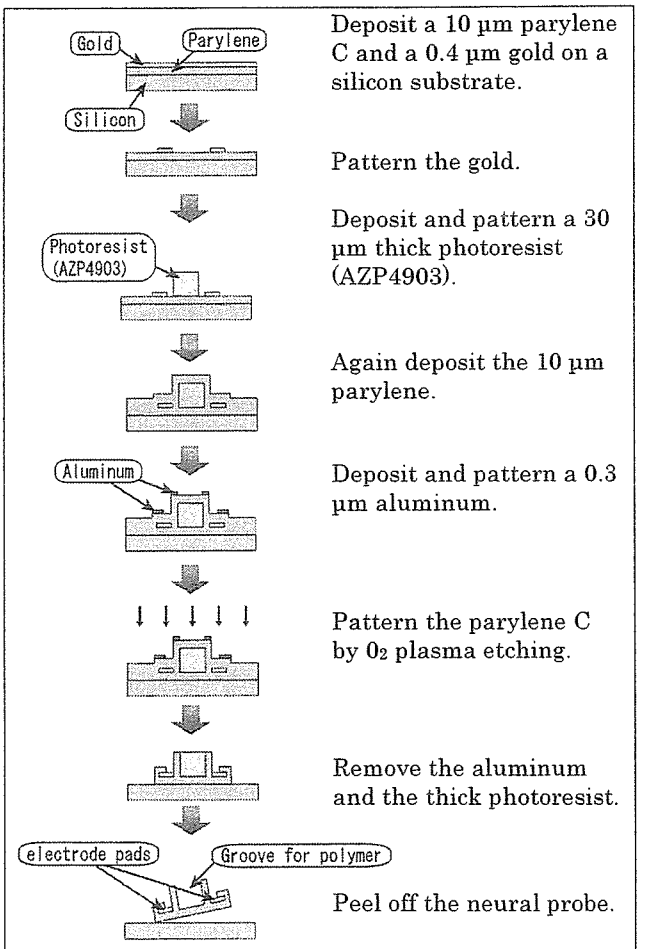

図2　神経プローブの製作過程

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[8]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、50μm 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 のオバルブミンを溶解させて 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、オバルブミンが包埋された 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℃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 k Ω から 300 k Ω となった。

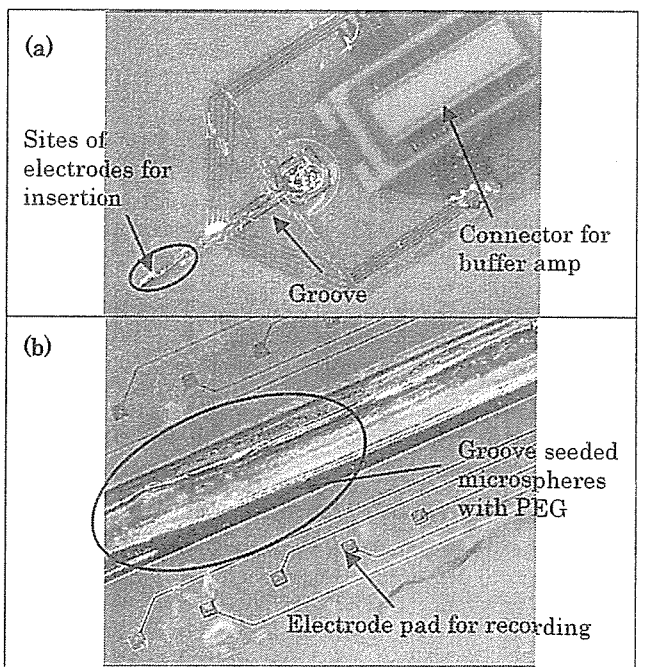

図 3 薬剤徐放型柔軟神経プローブの写真
Figure 3. Photos of the neural probe. (a) is the neural probe. (b) shows the magnification of the electrode's site in the tip of the neural probe. The biodegradable polymeric microspheres were seeded in the groove.

〈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 μ m から 30 μ 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。

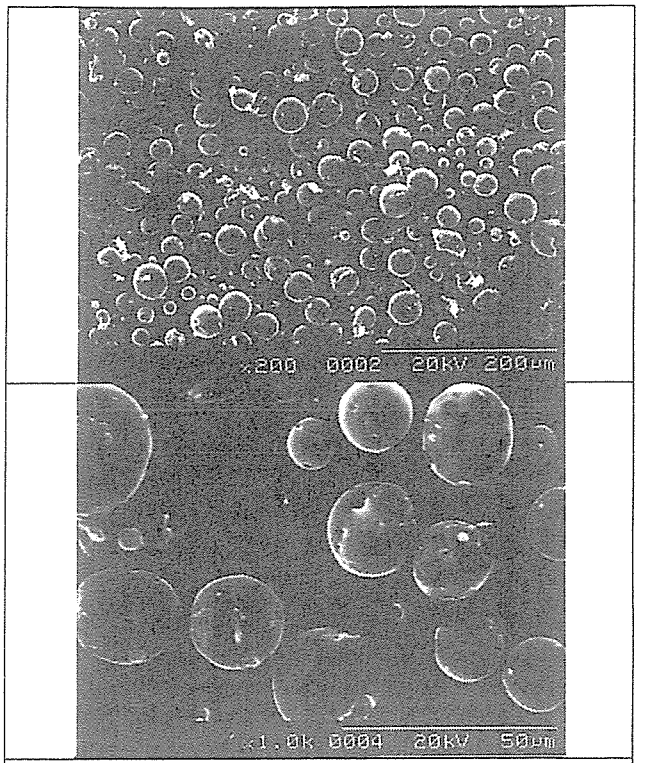

図 4 マイクロスフィアの走査型電子顕微鏡画像
Figure 4. Photos of the size distribution of the biodegradable polymeric microspheres by a scanning electron microscope.

〈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 度でインキュベイトした。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、2〜6ng/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。

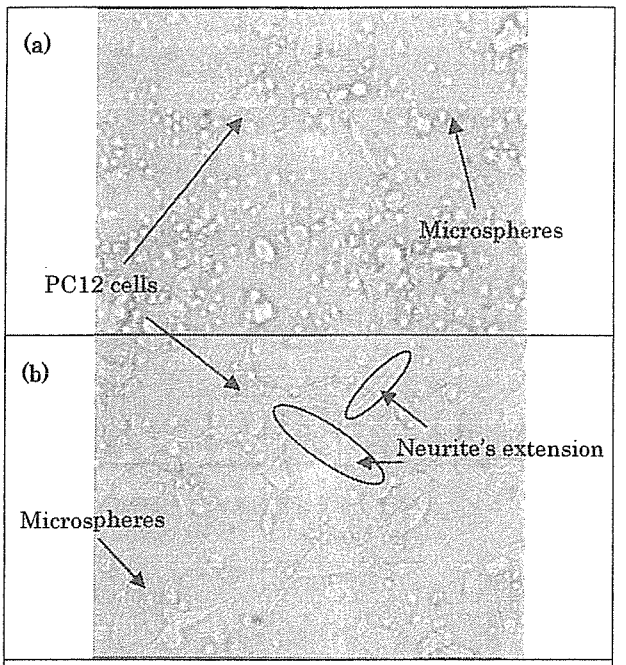

図５　徐放されたＮＧＦによるＰＣ１２神経化の写真

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 microsheres 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。

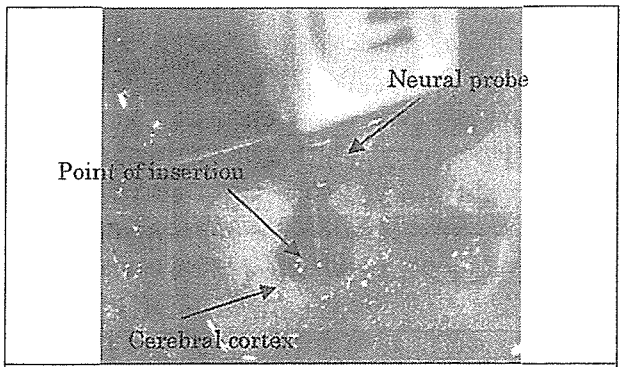

図６　薬剤徐放型柔軟神経プローブの大脳皮質内への刺入写真

Figure 6. Image that the probe with the microspheres and PEG was successfully implanted in the cerebral cortex of the rat.

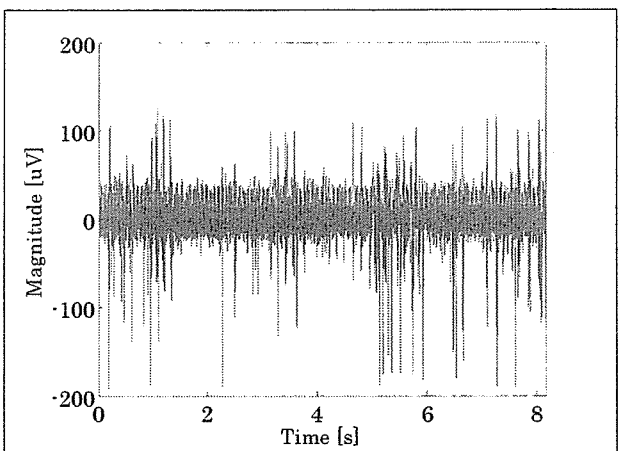

図７　薬剤徐放型柔軟神経プローブにより計測された神経活動の自発発火

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 とドラック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

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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.

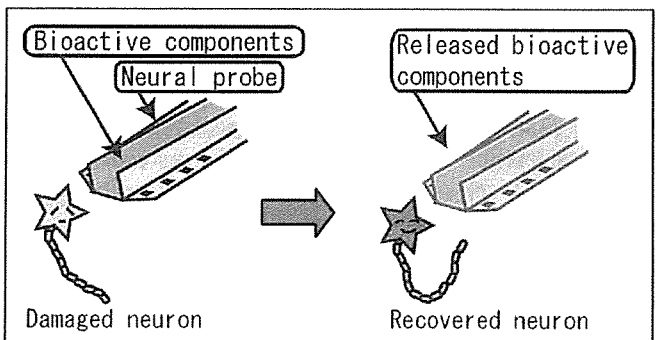

Fig. 1. Concept of effect of released bioactive components on damaged neurons. Left figure shows a neuron damaged by probe insertion. Right figure shows a neuron recovered by bioactive components released from the groove of the type-A neural probe.

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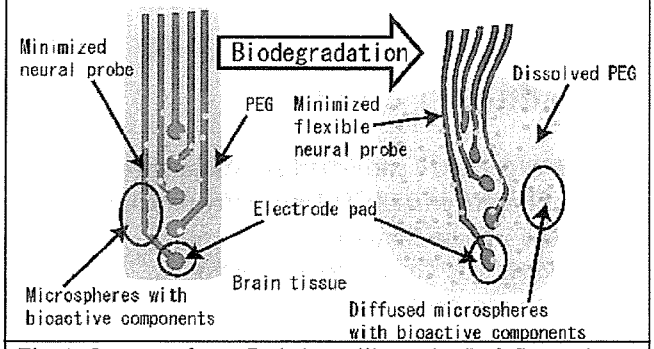

Fig. 2. Concept of type-B skeleton-like probe. Left figure shows implanted skeleton-like neural probe coated with hybrid biodegradable polymer of microspheres and PEG. Right figure shows that each minimized probe is flexible to brain tissue and the hybrid polymer is dissolved to promote recovery of damaged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

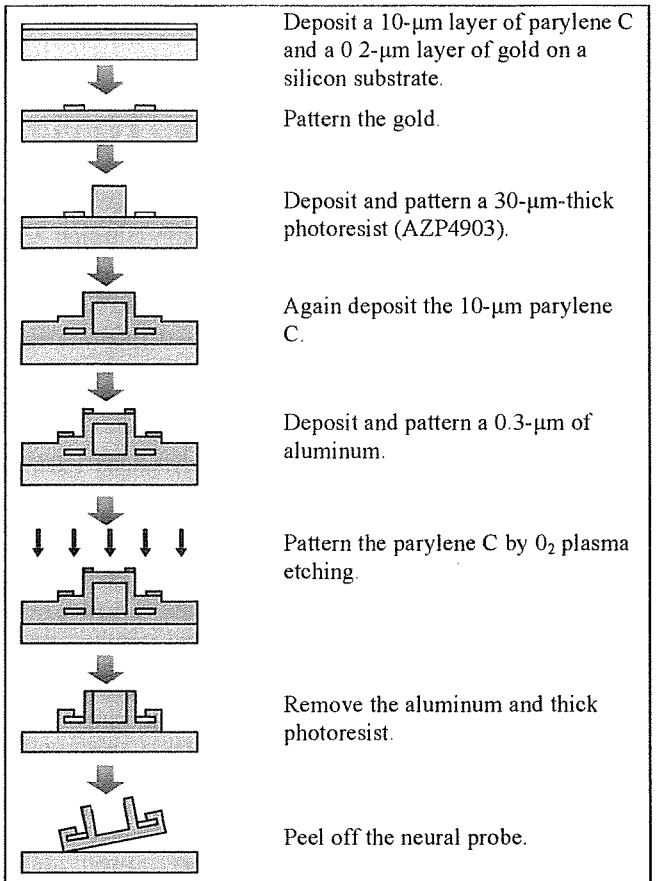

Fig. 3. Schematic of type-A neural probe fabrication. ☐: Silicon, ☐: Parylene C, ☐: Gold, ☐: Thick photoresist (AZP4903), ☐: Aluminum

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.

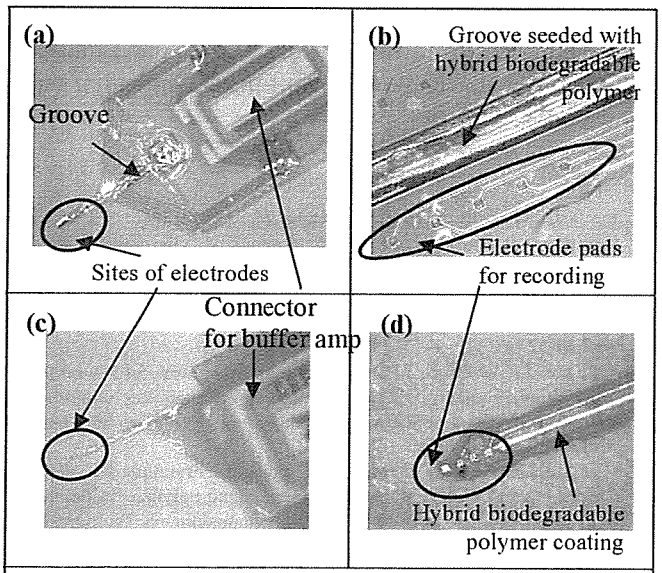

Fig. 4. Photos of neural probe. (a) is the type-A neural probe. (b) is magnified view of the electrode sites in tip of the neural probe. The hybrid biodegradable polymeric microspheres were seeded in the groove. (c) is the type-B neural probe. (d) is magnified picture of the electrode sites and each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

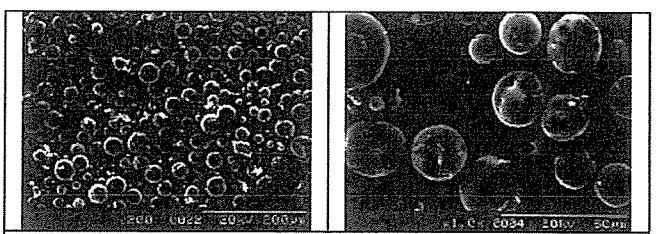

Fig. 5. Photos of size distribution of biodegradable polymeric microspheres by scanning electron microscope.

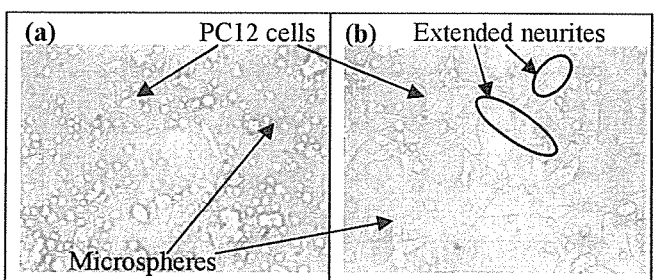

Fig. 6. Photos showing PC12 cells with extended neurites on a collagen dish eight days after NGF was released from the microspheres. (a) shows a control of the microspheres without NGF encapsulation (i.e., there was no NGF in the dish). (b) shows that NGF released from the microsheres extended the neurites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

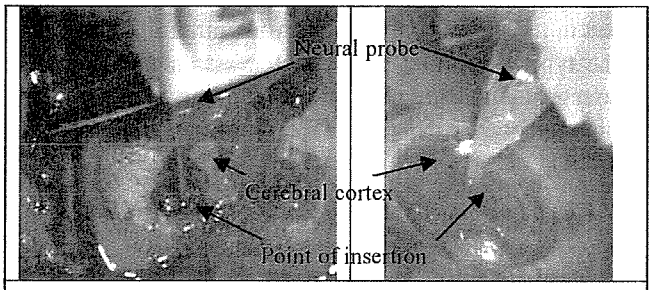

Fig. 7. Images of neural probes with PEG microspheres being successfully implanted in cerebral cortices of the rats.

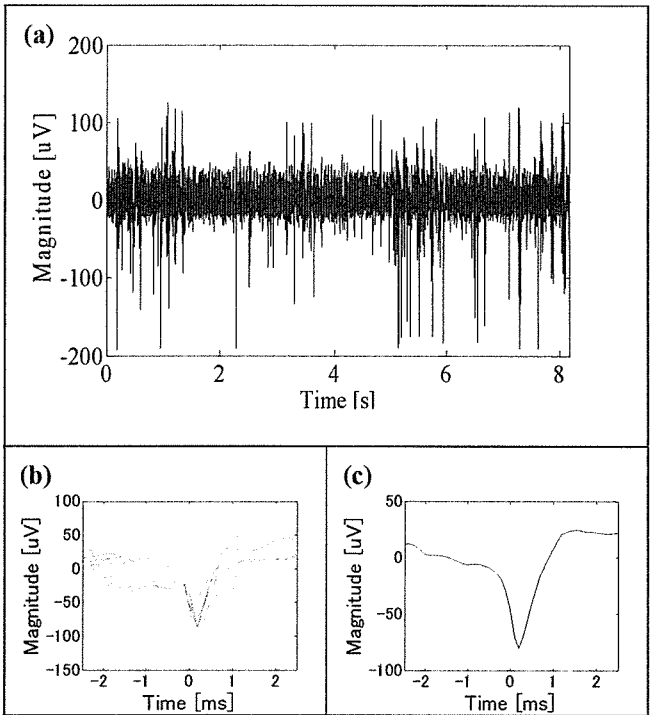

Fig. 8. (a) Spontaneous activity from the cerebral cortex of the rat recorded by the type-A neural probe. (b) 5 ms of ten sorted waveforms from spontaneous activity (c) The averaged sorted waveform from (b).

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.

Acknowledgment

We thank Dr. Nakagawa of the Chinzei 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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微小流路を利用した神経再生型電極の開発

Novel Regeneration-type Nerve Electrode with Integrated Microfluidic Channels

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Abstract Neural interface devices 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 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 regeneration-type nerve electrode based on the nerve regeneration principle was designed and fabricated using MEMS technologies. The electrode, which was fabricated on a 25-μm-thick Parylene C substrate, has multiple fluidic channels. Each fluidic channel was 100 μm wide x 30 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.

1. 背景

1.1 神経インタフェースデバイス

近年，生体の神経系と直接の情報入出力を行う神経インタフェースデバイスの開発が国内外で活発に行われている．神経系に対する多チャンネルかつ長期間安定した信号入出力が可能となれば，運動指令信号による義手などの補綴機器の制御や，自律神経系の情報を利用した人工臓器制御，あるいは逆に外部から感覚信号を入力することによる人工触覚等の人工感覚生成などが実現できるものと期待されている．

神経系と情報の入出力を行う箇所としては，中枢神経系と末梢神経系が考えられる．中枢神経系，特に脳を対象とする場合，埋め込み型のデバイスは頭蓋骨の中に位置するため，運動等の影響をうけにくく，比較的安定した信号入出力が期待できる半面，万が一の感染等の事故の場合には，影響が重篤となる可能性を有する．一方，末梢神経系を対象とする場合には，事故の影響が限局的になり得る上，計測した神経信号の解釈も中枢での場合よりも容易であることが期待できる．その一方で，埋め込み箇所と筋肉とが隣接するため運動の影響を受け易く，埋め込んだデバイスを神経組織へと，ずれることなく長期間安定して固定することが大きな課題となる．このような末梢神経を対象とする埋め込み型の神経インタフェースデバイスとして，末梢神経の再生能力を利用した神経再生型電極が提案され開発が進められている[1-2].

1.2 神経再生型電極

神経再生型電極の原理，特長について説明する．末梢神経系を構成する神経細胞の軸索（神経線維）を切断すると，細胞体から見て切断箇所より遠位の部分ではシュワン細胞を残して軸索が変性(Waller 変性)していくが，近位側では切断箇所（の１～数個近位側のランビエ絞輪）から先へと軸索を再生させていく．その際に再生軸索が遠位側の断端の方に誘導されることが知られている．

そこで神経束を切断して，断端間に多数の小孔を備えた電極を置いて再生を待つと，電極上の小孔を通って軸索が再生する(Fig.1)．各小孔はそれぞれ独立した電極として機能させる．以上が神経再生型電極の原理である．神経再生型電極は原理的に以下のような特徴を有している．

1. （再生軸索が電極孔を通過しているため）電極と神経束が物理的，電気的にしっかりと固定されるため，長期間の安定した計測，刺激が可能である．（針型の電極のようにずれることが無い．）
2. 小孔の径を調整することによって１～数本の神経線維に対する計測，刺激が実現する可能性を有する．
3. 従来の金属電極，ガラス管電極では困難な多チャンネル入出力が可能になる．

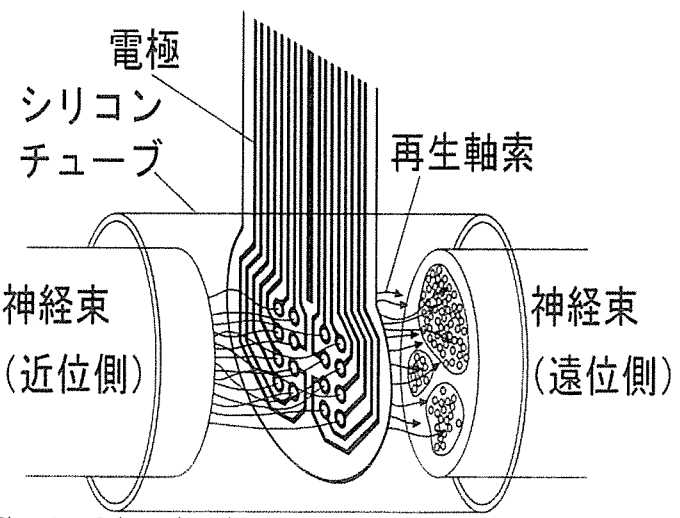

Fig. 1 Schematic of the regeneration-type nerve electrode (conventional model).

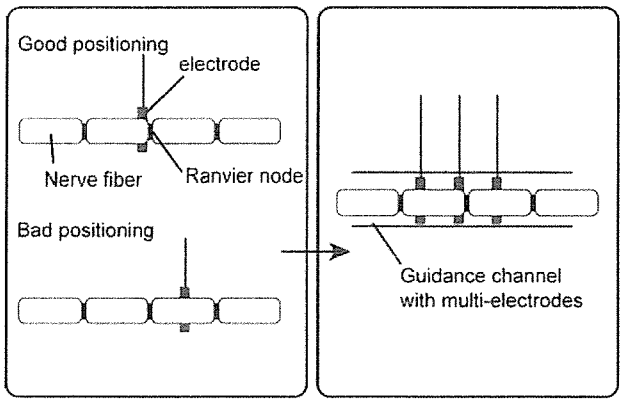

Fig. 2: Distance between the recording sites and the Ranvier node of a single nerve fiber.

こうした神経再生型電極の研究は従来，主にシリコンを基板材料として開発が行われてきたが，近年，ポリイミド等の高分子材料も利用されるようになってきた．しかしながら，いずれの電極も，Fig. 1 に示されるような平面構造のものであり，一つの平面状の基板の上に，電極孔と配線とが配列されていた．このため，電極孔を通過した各神経線維と電極とは一点でのみ接することになり，有髄の神経線維の場合には，ランビエ絞輪と電極孔との位置関係によっては，SN 比の悪化が避けられないと考えられる(Fig.2 左).

1.3 本研究の目的

こうした問題を解決するため，本研究では流路を束ねた形状の三次元構造を有する神経再生型電極を新しく提案する．さらに薬液注入可能な別の流路によって，軸索の再生誘導機能を付加することも提案する．著者らはこれまでに，ポリイミドやパリレン C（ポリクロロパラキシリレン）などの高分子材料を利用して，柔軟型神経電極や，流路を備えた柔軟神経電極を開発しているが[3-5]，本研究で提案する新しい神経再生型電極は，これらの技術を応用したものである．

本研究では，この電極の設計，製作，および基礎的な評価を行ったので，報告する．

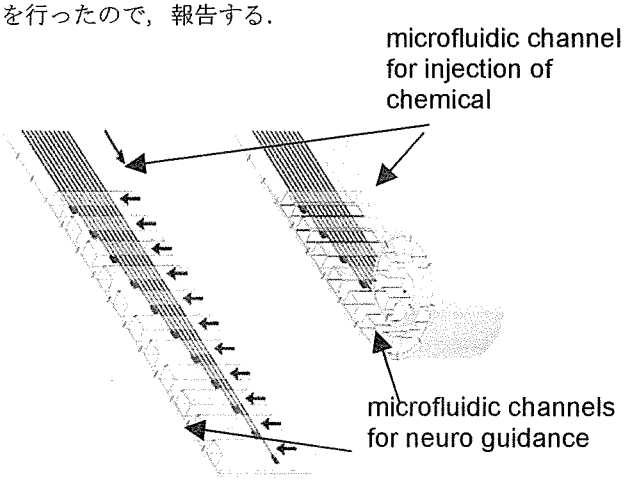

Fig. 3: Concept sketch of the regeneration nerve electrode in this work
The electrode has a microfluidic channel for injection of chemicals and microfluidic channels for neuro guidance.

2. 方法

2.1 概念

本研究で提案する神経再生型電極の概念図を Fig. 3 に示す．フィルム基板上に流路を並べ，それを巻くことで流路の束を形成する．軸索はこの流路の中を再生していくようにする．各流路の内部には電極を複数個並べる．これによって，ランビエ絞輪に近く SN 比の良い電極を選択して使用することが可能となる(Fig.2 右)．また流路構造自体が，細胞外計測における神経スパイクの高さの減衰を抑えることも期待できる．

流路の遠位側には，従来型の神経再生型電極のように切断した神経束の遠位端を置くこともできるが，Fig.3 のように薬液注入用の流路を付加することにより，NGF（神経成長因子）などの再生を促進する物質を各流路内に注入することも可能となると考えられる．

2.2 作成方法

本電極の作成方法を Fig 4 に示す．作成はシリコンウェハ上で行う．

① ウェハ上にパリレン C を 10μm コートして，この上に電極及び配線用の金を約 300nm 蒸着し，パターニングを行う．
② 第二層のパリレン C を 5μm コートし，電極部とコネクタ部が露出するようにパターニングを行う．
③ 厚膜レジスト (THB-611P または SUNFORT AQ-4059 ドライフィルムレジスト (旭化成)をコートして，パターニングを行う．これは流路を形成するための犠牲層となる．
④ さらに，第三層のパリレン C を 10μm コートした上で，外形の切り出しと流路の出口やコネクタ部等の露出のためにアルミマスクによる酸素プラズマエッチングを行う．(Fig.4-1)
⑤ 流路部をはがしてから，あるいははがしながら巻いていく．その際，エポキシ接着剤を間に入れる．(Fig.4-3)
⑥ 巻き終わった後で電極をアセトンに入れ，犠牲層となる厚膜レジストを除去する．

これらのプロセスは，巻くことを除いては，基本的に著者らによる流路を備えた柔軟神経電極[5]の作成方法と同様のものである．

3. 結果

3.1 試作結果

作成した電極の写真を Fig.5 及び Fig.6 に示す．Fig. 6 は薬液注入用流路の無いタイプのものについて，作成の各段階での経過を示すものである．Fig.6 の A 図と C 図に示されるように，巻き終わりの 1 周分については，流路長よりも電極基板の幅が大きく(2mm)なるようにしてあり，これが従来型の神経再生型電極における神経束接続用のシリコンチューブの役割を果たす．各流路のサイズは，幅 100 μm ，高さ 30 μm 長さ 1500 μm.である．薬液注入用流路の有無だけでなく，神経再生用流路の数についても 80 本から 200 本のいくつかの種類の電極を試作した．厚膜レジストのコーティング厚を（スピンコータの速度調節等により）調整することで，流路の高さを変えることができる．