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Fabrication and Evaluation of a Flexible Sieve-Type Microelectrode Array for Monitoring the Regenerating State of Peripheral Nerves

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This paper reports the design, fabrication and measurement of a flexible microelectrode array for monitoring the regenerating state of peripheral nerves. Polyimide was used so that the device would have biocompatibility, long-term stability and flexibility. Using a 'stress-free etch-release' scheme, it was possible to fabricate a microelectrode array with a smaller feature size because less geometrical deviation took place compared to that using a mechanical peeling-off method. The diameter of the via holes, which provide a path for regenerating nerve fibers, was 40 μm and 50 μm. *In vivo* tests were performed by implanting fabricated microelectrode arrays into the sciatic nerves of a rat and into the peroneal nerves of a rabbit using two different implantation methods. One is a direct inter-fascicular suture technique and the other is a method using a nerve conduit. In both cases, no side effects such as cytotoxicity, neuroma and antigen-antibody reaction were observed. *In vitro* measurement was carried out to confirm the feasibility of measuring nerve signals. The interfacial impedance between body fluids and microelectrodes was measured. The normalized interfacial impedance was 446 Ω/μm^2 at 1 kHz. Signal transfer characteristics were analyzed by applying square waves of various frequencies and magnitudes.

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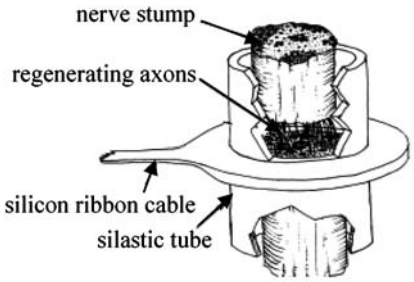
1. Introduction

Nerve injuries and dissections have frequently occurred due to trauma, surgical removal of a tumor and other invasions. A neuron is one of the most differentiated cells in a living body and is not capable of mitosis. Thus, as degeneration of a cell occurs, neurons cannot be regenerated or replaced by other cells. This is true under any circumstances for the central nervous system (CNS). In the peripheral nervous system (PNS), however, a neuron can regenerate axons when a portion of an axon is injured or dissected.[1]

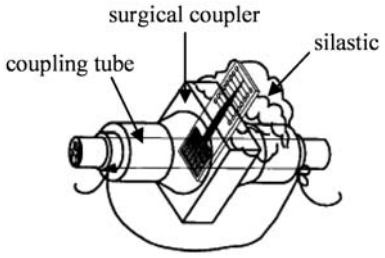
There have been many studies on the recovery of function of injured nerves based on these regenerative phenomena. They can mainly be classified into three approaches. The first method involves an interfascicular suture technique that transplants a new nerve obtained from another site in the individual's body to an injured region. However, it is impossible to obtain a sufficient length for the new nerve without its losing its function at donor site and requiring additional surgical procedures. The second method is to use a conduit that guides regenerating nerve fibers. It is nearly impossible for some specific injured nerves to be regenerated when the length of a transected portion is longer than a certain critical value. According to previous studies, regeneration cannot occur when the dissected length is over 10 mm for a sciatic nerve of a rat (G Lundborg, 1982; LR Williams, 1984) and 6 mm for that of a mouse (BG Uzman, 1983; M Buti, 1996). Furthermore, separation of a conduit from nerve stumps frequently occurs due to their different mechanical properties and structure. For both of these methods, it is impossible to monitor the regenerating state of the nerve fibers. The last method is to use a microelectrode array (MEA) capable of interfacing regenerating nerve fibers. The state of regenerating nerve fibers can be detected by recording nerve signals through microelectrodes. Detection of nerve signals is performed by inserting a sieve-shaped MEA between a proximal and a distal nerve stump of a transected portion. Figure 1 shows typical sieve-type MEAs proposed by previous researchers, R. M. Bradlely *et al.*,[2] Kovacs *et al.*[3] and P. Dario *et al.*[8]

MEAs can be classified into two types according to the substrate materials used, silicon either or polyimide. For types using a silicon substrate,[2–9] it is not easy to obtain a flexible MEA due to the brittleness of silicon. This causes difficulties in satisfying physical compatibility needs because of side effects, such as the scratching of adjacent organs. With a polyimide substrate,[10] the method potentially produces deformation and/or destroys the original shape of the via holes positioned on the sieve, even though flexible MEAs can be used. This problem originates from the step in the fabrication of the sieve in which the small minimum feature size is released by a mechanical 'peeling-off' method.

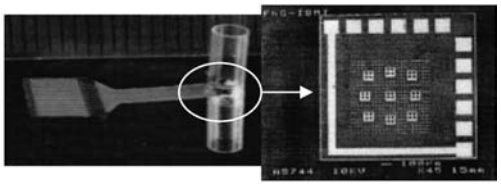
The objective of this study is to fabricate a fully flexible MEA without any possible geometric deviation and to verify its biocompatibility and feasibility for measuring nerve signals. To fabricate a sieve-type MEA with these characteristics, the following methods were investigated. For a fully flexible MEA, polyimide was used as the substrate and passivation layer. To eliminate the possible deformation or failure of via hole configuration, a 'stress-free etch-release' scheme[11] was used, which is depicted in Fig. 2. By chemically removing a sacrificial layer, silicon dioxide in this case, MEAs were obtained. To verify the feasibility of measuring nerve signals, an *in vitro* experiment for the electrical characterization of microelectrodes and the analysis of signal transfer characteristics was performed. To check biocompatibility, *in vivo* experiments were carried out.



(a) R. M. Bradley *et al.*[2]



(b) Kovacs *et al.*[3]



(c) P. Dario *et al.*[8]

Fig. 1. Sieve-type MEAs proposed by other research groups.

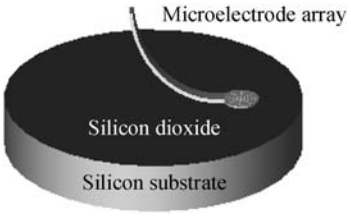


Fig. 2. Schematic of the stress-free etch-release scheme.

2. Design and Fabrication

2.1 *Compatibility issue*

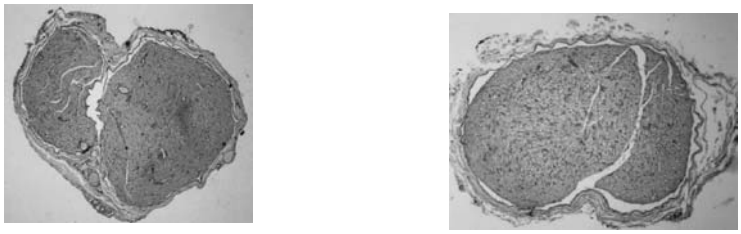
Biocompatibility is a prerequisite for a medical device implanted in a living body. It means that no cytotoxic nor immunological reactions occur when a material or a device is implanted. In this study, polyimide (PI2611, HD Microsystems) was used as a substrate and a passivation layer. Polyimide is a polymeric material obtained by thermal or chemical polymerization of polyamic acid (PAA). It has been widely used in the microelectronics industry because of its favorable characteristics, for example its thermal stability, easy processibility and chemical inertness. Theoretically, it does not react with any other elements after it is polymerized. This characteristic is the major reason it satisfies the requirement of biocompatibility. Gold has also been used as a microelectrode material. Although other biocompatible metals are known, such as platinum and iridium, their processibility is not better than that of gold.

Another aspect to be considered is physical compatibility. This means that the geometrical configuration and mechanical properties of a device are similar to a part of a living body, a nerve stump in this case. If an implanted device is too large or small, it can injure adjacent muscles, organs and nerves. In this study, geometrical information about nerves was obtained from two seven-month-old rats. Figure 3 shows optical photographs of the configuration of cross-sectional areas of dissected and dyed rat sciatic nerves, and Table 1 contains information on their characteristic dimensions.

2.2 *Device design*

The design parameters for a sieve-type MEA are the configuration of the sieve part, the fill factor, the size of the via holes, and the number of electrodes. Although the configuration of a cross-sectional area of a nerve differs from one to another, even for the same kind of animal, it can be approximated as a circle in the case of the sciatic nerves of a rat and the peroneal nerves of a rabbit. Based on dimensional information obtained from sciatic nerves of rats, the diameter of the sieve was designed to have a 1 mm or 1.5 mm active diameter, which is the area where the via holes are positioned. The fill factor and via hole size are critical factors for effective nerve regeneration, and their optimal value has been investigated in previous studies.[12,13] The fill factor is defined in eq. (1).

$$\text{Fill factor (\%)} = \frac{\text{Total area of via holes}}{\text{Area of sieve}} \times 100 \tag{1}$$



(a) First sample (b) Second sample

Fig. 3. Cross-sectional area of dissected and dyed sciatic nerves of a rat.

Table 1
Dimensional information on rat sciatic nerves.

	First sample	Second sample
Maximum length (mm)	0.916	0.83
Minimum length (mm)	0.694	0.56
Area (mm^2)	0.44618	0.403

As fill factor decreases, which means the number of via holes decreases, the sieve obstructs a regenerating path, via holes in this case, for axons. This results in fewer nerve fivers regenerating. On the other hand, as fill factor increases, the number of electrodes inevitably has to be decreased, which equates to decrement in the number of recording channels for nerve signals. Hence, there is trade-off between the fill factor and the number of recording electrodes. The larger the fill factor, which means the larger the number of via holes, the larger the number of paths for regenerating axons. But fill factor cannot be increased infinitely due to the limited resolution of photolithography and the required number of microelectrodes. It is reported that a fill factor of 20–30% showed good muscle contractability,[12] which is one parameter indicating that axons were effectively regenerated. In this research, MEAs with various fill factors from 19% to 27.6% were fabricated.

The diameter of mammalian nerve fibers differs based on the type of nerve, as shown in Table 2. For peripheral nerves, the diameter of fibers ranges from 12 μm to 20 μm. The diameter of a via hole should be larger than that of the nerve fibers because the via hole provides a path for regenerating nerve fibers. Thus a via hole size of 40 μm or 50 μm was used in this study; the selection of these ranges is supported by a previous study,[13] in which it was reported that a suitable range of via hole size is between 40 μm and 65 μm.

A trade-off exists between the number of microelectrodes and the number of via holes. Ideally, the number of microelectrodes will be equal to the number of regenerating nerve fibers, which means that the signal from the individual nerve fibers can be monitored. However, an increase in the number of microelectrodes inevitably decreases the number of via holes; mainly due to limitations in the resolution of photolithography. In this research, eight microelectrodes for recording nerve signals were placed on the periphery of the via holes and one ground microelectrode was placed on the periphery of the sieve.

2.3 *Fabrication*

Figure 4 shows simplified fabrication steps for a sieve-type MEA, and Fig. 5 shows fabricated devices for two different implantation schemes. One is a direct interfascicular suture technique and the other is a method using an assembly of an MEA and a nerve

Table 2
Nerve fiber types and their dimensions.

Fiber type		Function	Fiber diameter (μm)
A	α	Proprioception, somatic motor	12–20
	β	Touch, pressure	5–12
	γ	Motor to muscle spindles	3–6
	δ	Pain, temperature, touch	2–5
B		Preganglionic automic	less than 3
C	Dorsal root	Pain, reflex responses	0.4–1.2
	sympathetic	Postganglionic sympathetics	0.3–1.3

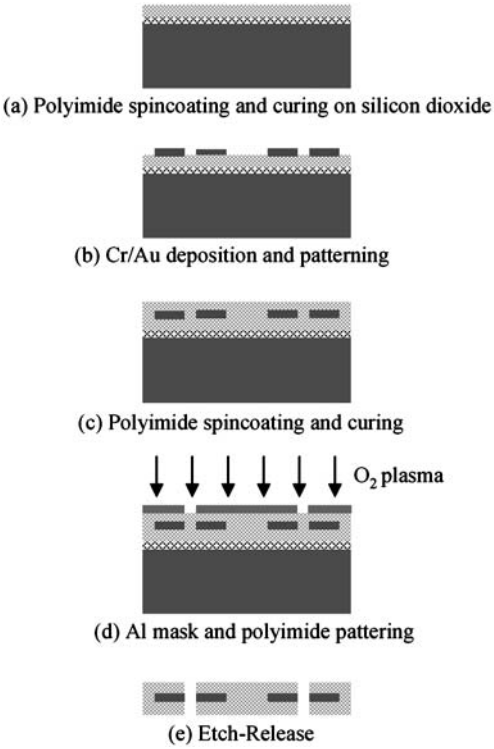
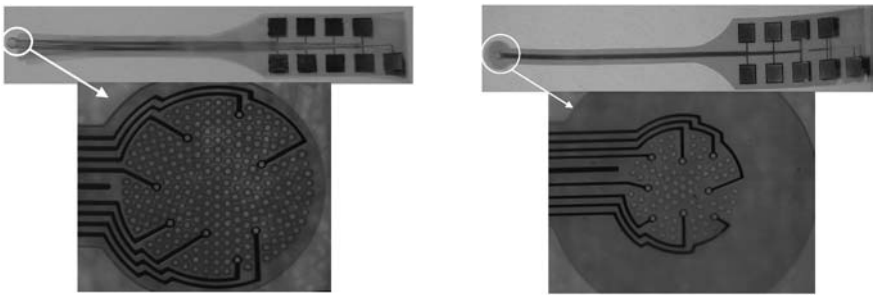


Fig. 4. Simplified fabrication steps for a sieve-type MEA.



(a) (b)

Fig. 5. Optical photographs for the MEAs fabricated for different implantation methods. (a) MEA for the implantation of the assembly of MEA and a conduit. (b) MEA for the direct interfascicular suture.

conduit. Fabrication starts with a sacrificial layer deposition of 5000-Å-thick silicon dioxide. Polyimide (PI2611, HD Microsystems) that serves as a flexible substrate was spincoated at 1000 rpm and polymerized in two steps. The first step was performed at 200°C for 2 h with a temperature gradually rising from ambient temperature. The second

step was carried out at 350°C for 1 h. The thickness of the substrate was approximately 8 μm. A microelectrode layer, 5000-Å-thick Au, was deposited on an adhesion layer, 100 Å-thick Cr, by thermal evaporation. Microelectrodes were formed with conventional photolithography techniques. The Au layer was etched by a solution of KI, I_2 and DI water, and the Cr layer was patterned using a commercial solution, CR-7. Table 3 shows composition of the Au etching solution. The second polyimide layer was formed using the aforementioned method. This layer is for passivation and electrical insulation of microelectrodes from the harsh environment inside a living body. An Al mask was defined for opening contact pads, which was carried out etching the second polyimide layer using O_2 reactive ion etching. Dry etching was carried out under conditions of 50 sccm O_2, a pressure of 100 mTorr, and a power of 100 W for 50 min. After removing the first Al mask, another Al mask was deposited and patterned. Dry etching was again performed to define the overall configuration and via holes under the same conditions mentioned above. The last fabrication step is the removal of the sacrificial layer, the silicon dioxide layer in this case. The process was performed by a stress-free etch-release method instead of a mechanical peeling-off method, to release the sieve-type MEA from the silicon substrate. Stress-free etch-release does not apply any mechanical stress to the device, because it is a chemical process. This step was performed in 25% dilute HF for 45 min. No dimensional change in the via holes was observed. The thickness of the fabricated devices was approximately 15 μm.

3. Measurements and Results

Biophysical compatibility and long-term stability are prerequisites for medical devices that are implanted in a living body. For biocompatibility and long-term stability, the materials composing a device should not release any toxic components and should not undergo any chemical reactions in the body. Physical compatibility is also a crucial factor to avoid harmful effects on adjacent organs, muscles and nerves. For satisfactory physical compatibility, a device must have similar mechanical properties, such as flexibility, to the tissue they are replacing.

In vivo tests were performed to verify the biological compatibility and long-term stability of the fabricated devices by implanting fabricated MEAs in the sciatic nerves of a rat and the peroneal nerves of a rabbit. *In vitro* tests were carried out to confirm the feasibility of measuring nerve signals and signal transfer characteristics through microelectrodes in body fluids. Flexibility was tested by repetitive bending and twisting of the fabricated MEAs.

Table 3
Composition of the Au etching solution.

Component	Quantity
K1	200 g
I_2	50 g
DI water	2 L

3.1 In vivo *tests*

The objective of this experiment was to verify the biocompatibility and long-term stability of the fabricated sieve-type MEA. For this aim, a healthy seven-month old rat and a rabbit were anesthetized. The right and left sciatic nerves of the rat and the peroneal nerves of the rabbit were dissected. Afterward, the fabricated MEAs were implanted into the animals using two different implantation methods. One was a method using a conduit that guides regenerating nerves and minimizes relative movement of implanted MEA with respect to nerve stumps. Conduits were fabricated with 100% polyglycolic acid (PGA, Albany International, Inc.), which is a biodegradable non-woven mesh. The diameter of each fiber is 12 μm and its inherent viscosity is 1.23 dl/g. PGA meshes were reinforced using 75:25 polylactic-co-glycolic acid in chloroform solution. A Teflon cylinder was covered with a reinforced PGA mesh to make a cylindrically-shaped conduit. The fabricated conduits had been dried in a vacuum oven for 3 days to remove the remaining solvent and then sterilized at 37°C. They were cut to a length of 6 mm to reduce nerve defects just before implantation of the assembly. Figure 6 shows a fabricated assembly of an MEA and a conduit. The other method was an interfascicular suture technique in which a device is directly sutured to a nerve stump. This method shortens the required regeneration time of dissected nerve fibers. Figure 7 depicts schematics of both implantation methods. For the implantation to the sciatic nerves of the rat, both implantation methods were applied. For the case of the peroneal nerves of the rabbit, implantation using an interfascicular suture method only was carried out to reduce the required time for nerve regeneration. All surgical procedures were performed under the microscope, and Fig. 8 shows part of the surgical operation. For the device implanted in the sciatic nerves of the rat, no side effects, such as neuroma and antigen-antibody reactions, were observed for 4 months after implantation. In the case of the rabbit, similar results were obtained during an implantation period of 8 weeks. Figure 9 shows the devices implanted in the sciatic nerves of the rat, and Fig. 10 is a photograph of the device implanted in the peroneal nerves of the rabbit.

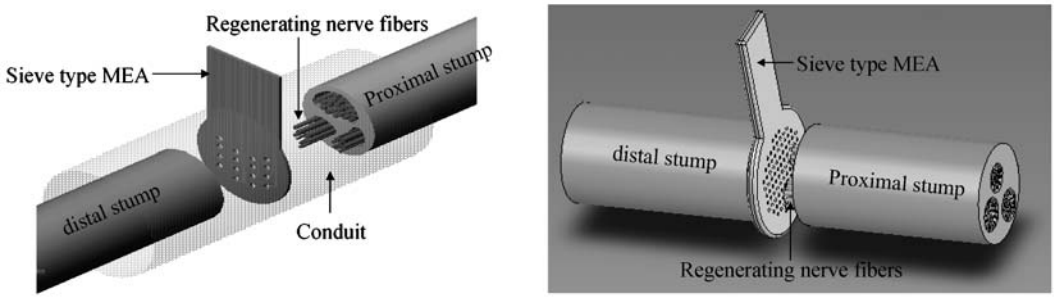
3.2 In vitro *tests*

3.2.1 *Impedance characterization*

According to *in vivo* test results, it was verified that the proposed device is biophysically compatible and stable in the long-term. To check the feasibility of measuring nerve signals, the interfacial impedance (z) between a microelectrode and a body fluid was measured using a precision impedance analyzer (PN4294A, Agilent) with a frequency range from 40 Hz to 100 kHz. Normal saline solution, 0.9% NaCl, was used as the body fluid. The measured data, including interfacial impedance and phase angle, are shown in Fig. 11. Interfacial impedance was approximately 420 kΩ at 1 kHz and normalized interfacial impedance over the microelectrode area, 942 μm^2, was 446 $\Omega/\mu m^2$. The normalized interfacial impedance is lower than that reported by E. Valderrama *et al.*, 3200 $\Omega/\mu m^2$,[5] at the same frequency. As equivalent circuit parameterizing impedance characteristics can be modeled as shown in Fig. 12, interfacial impedance is described by eq. (2),

$$Z = \frac{R_{eq}}{1+\left(wCR_{eq}\right)^2} - j\frac{R_{eq}^2 wC}{1+\left(wCR_{eq}\right)^2}, \tag{2}$$

Fig. 6. A fabricated assembly of MEA and a conduit.

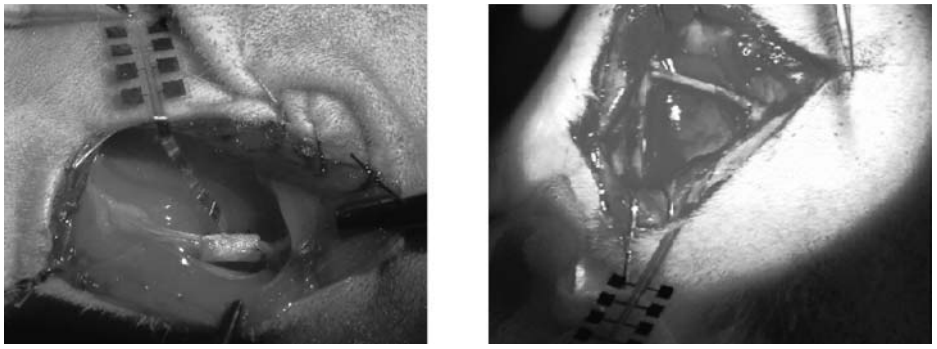


(a) (b)

Fig. 7. Schematics of two different implantation methods. (a) Scheme using a conduit. (b) Direct implantation scheme.

Fig. 8. One scene in the operation.

where Z is the interfacial impedance between the probe tip and the microelectrode, R_{eq} is the equivalent resistance between the probe tip and the microelectrode, $(R_{int\text{-}1}+R_{bs}+R_{int\text{–}1})$, w is the measurement frequency and C is the capacitance between the probe tip and the microelectrode.



(a) (b)

Fig. 9. Photographs of MEAs implanted in the sciatic nerve of a rat using two different implantation methods. (a) Assembly implantation. (b) Implantation by interfascicular suture.

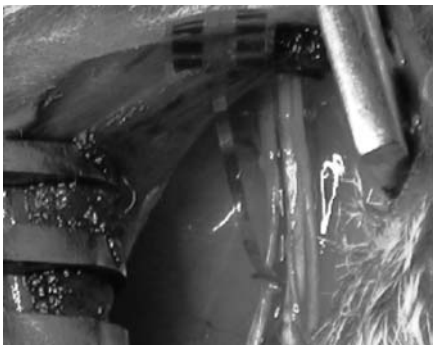


Fig. 10. Photographs of an MEA implanted in the peroneal nerve of a rabbit using the interfascicular suture technique.

The measured phase angle has a large negative value, which means the product of resistance and capacitance is lower than that reported in previous studies.[3,5] It is obviously advantageous to measure nerve signals.

3.2.2 *Signal transfer characterization*

As a nerve signal moving along nerve fibers originates from the concentration difference between Na^+ and K^+, the signal through regenerated nerves can be considered a voltage source. Thus, a stimulating signal in the voltage mode was applied to a microelectrode through a probe tip with very sharp edge. Recorded signals through the microelectrode were transferred to an oscilloscope (LECROYLT-344) through an interconnection line. Figure 13 shows the conceptual process used to analyze signal transfer characteristics, and Fig. 14 shows the actual setup for the measurement. The signal measurement was

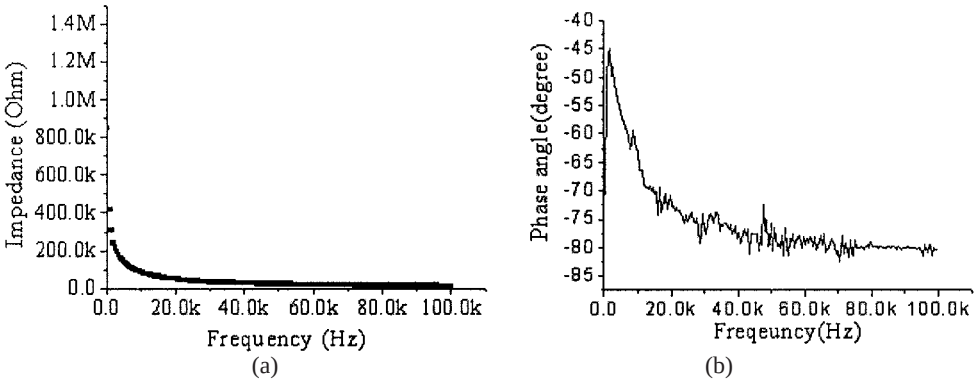


Fig. 11. Measurements of the impedance behavior of the proposed microelectrodes. (a) Measured impedance. (b) Measured phase angle.

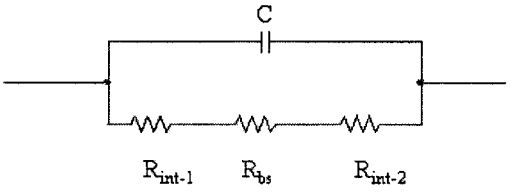


Fig. 12. Equivalent circuit for the impedance model of a probe tip and a microelectrode.
C: capacitance between the probe tip and the microelectrode
R_{int-1}: interfacial impedance between the probe tip and body fluid
R_{bs}: resistance of body fluid
R_{int-2}: interfacial impedance between body fluid and the microelectrode

performed using different magnitudes of sine and square waves of various frequencies. Figure 15 shows data measured for square waves of different magnitudes at a frequency of 500 Hz. The magnitude of the signals measured through the microelectrodes is nearly equal to that of the input signal, but it contains a large amount of noise, such as noise at 60 Hz and high frequency noise. From the measured results, it is expected that nerve signals can be recorded when a proper circuitry for suppressing that noise is introduced.

3.2.3 *Flexibility tests*

Repetitive bending and twisting tests for the fabricated MEAs were performed, and then signal transfer tests were conducted to verify whether the interconnection lines were still working. The signal transfer test was performed by applying a 400 mV sine wave at a frequency of 10 kHz. Figure 16 shows a bending and a twisting test, and Fig. 17 shows a signal transfer test.

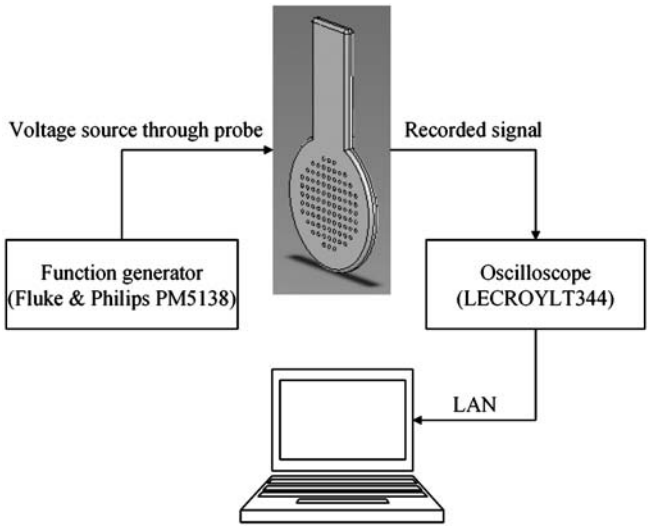


Fig. 13. Measurement flow to analyze signal transfer characteristics of the microelectrodes.

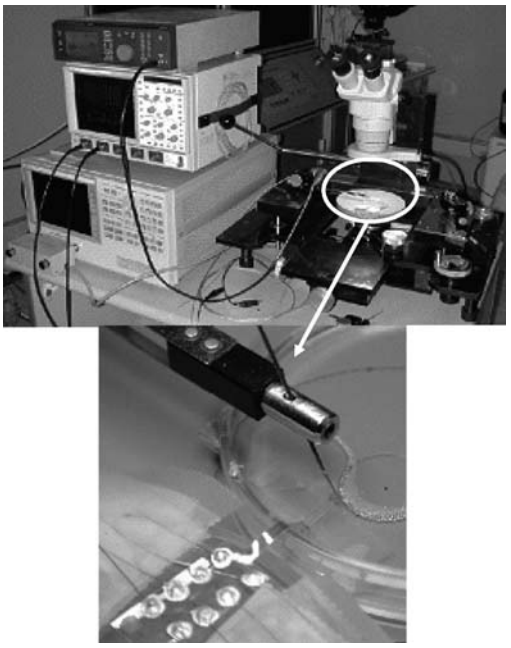
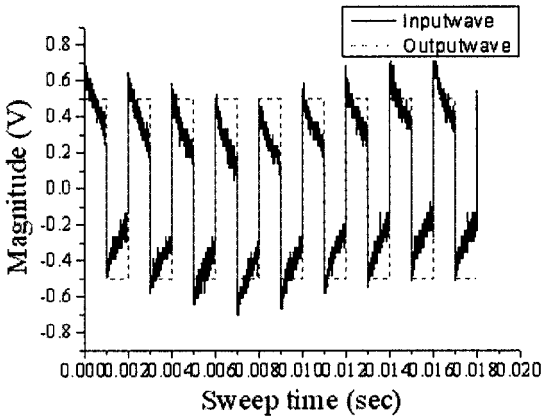


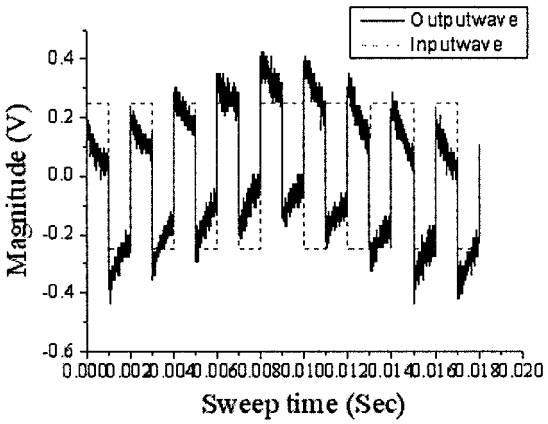
Fig. 14. Measurement apparatus to measure signal transfer characteristics of the microelectrodes.

4. Conclusion

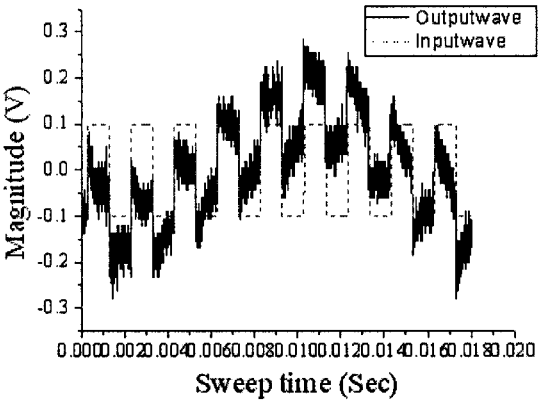
A fully flexible sieve-type microelectrode array has been proposed to monitor the state of regenerating nerves. Using a stress-free etch-release scheme, it was possible to fabricate



(a) Applied voltage: 1 V



(b) Applied voltage: 500 mV

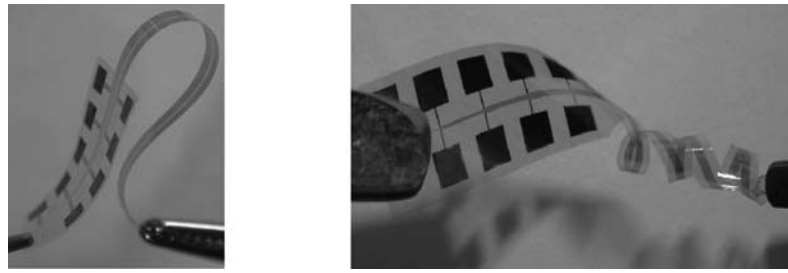


(c) Applied voltage: 200 mV

Fig. 15. Data measured for square waves with an input voltage of different magnitudes at frequency of 500 Hz.

a multilayer structure because of the enhanced adhesion between the substrate and the polymer layer. Furthermore, a device with a smaller feature size was able to be fabricated because less geometrical deviation occurs compared to devices for which the mechanical peeling-off method is used. Polyimide was used because of its biophysical compatibility, long-term stability and flexibility.

In vivo tests were performed to verify biophysical compatibility and long-term stability by implanting fabricated MEAs into the sciatic nerves of a rat and the peroneal nerves of a rabbit. The fabricated devices were implanted using two different methods. One used a conduit made from PGA and the other, an interfascicular suture technique. For implantation into the sciatic nerve of the rat, both implantation methods were applied. For the case



(a) (b)

Fig. 16. Flexibility test of the sieve-type MEAs fabricated. (a) Bending test. (b) Twisting test.

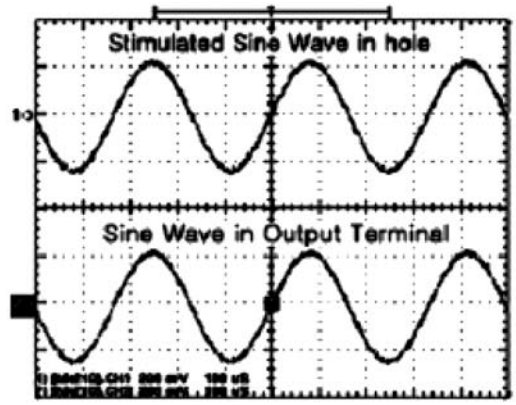


Fig. 17. Signal transfer test after repetitive bending and twisting of the MEAs.

of the peroneal nerves of the rabbit, an interfascicular suture method was applied to reduce the required time for nerve regeneration. For both implantations, no side effects were observed for 4 months in the case of the rat and 8 weeks for the rabbit. According to these results, it was verified that the fabricated microelectrode arrays can be implanted into a living body with biocompatibility and long-term stability.

In vitro tests were conducted to confirm the feasibility of measuring nerve signals. The interfacial impedance characteristic between microelectrodes and body fluid also was measured. The normalized interfacial impedance was 446 $\Omega/\mu m^2$ at 1 kHz, and the measured phase had a large negative value, which indicates a low product of resistance and capacitance. Signal transfer characteristics were analyzed for square waves of various magnitudes and frequencies. The magnitude of the measured signals through the microelectrodes was nearly equal to that of the input signal, except for some noise.

According to these results, it is expected that the regenerating state of nerves can be monitored with the proposed microelectrode array due to its low magnitude of interfacial impedance, and good phase and signal transfer behavior, if proper noise suppressing circuitries are introduced.

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References

1 W. T. Lee and K. A. Park: Medical Neuroanatomy (Korea Medical Book Publisher, Seoul, 1996) Chap. 2
2 R. M. Bradlely, X. Cao, T. Akin and K. Najafi: J. Neuro. Meth. **73** (1997) 177.
3 G. T. A. Kovacs, C. W. Storment and J. M. Rosen: IEEE Trans. Biomed. Eng. **39** (1992) 893.
4 C. C. D. Santina, G. T. A. Kovacs and E. R. Lewis: J. Neuro. Meth. **72** (1997) 71.
5 E. Valderrama, P. Garrido, E. Cabruja, P. Heiduschka, A. Harshc and W. Gopel: The 8th International Conference on Solid-State Sensors and Actuators and Eurosensors IX Digest of Technical Papers 1995 (IEEE, Stockholm, 1995) p. 63.
6 L. Wallman, A. Levinsson, J. Schouenborg, H. Holmberg, L. Montelius, N. Danielsen and T. Laurell: IEEE Trans. Biomed. Eng. **46** (1999) 1065.
7 Q. Zhao, J. Drott, T. Laurell, L. Wallman, K. Lindstrom, L. M. Bjursten, G. Lundborg, L. Montelius and N. Danielsen: Biomat. **18** (1997) 75.
8 P. Dario, P. Garzella, M. Toro, S. Micera, M. Alavi, U. Meyer, E. Valderrama, L. Sebastiani, B. Ghelarducci and P. Pastacaldi: J. Micromech. Microeng. **7** (1997) 233.
9 P. Dario, P. Garzella, M. Toro, S. Micera, M. Alavi, U. Meyer, E. Valderrama, L. Sebastiani, B. Ghelarducci, C. Mazzoni and P. Pastacaldi: IEEE. Trans. Rehab. Eng. **6** (1998) 353.
10 T. Stieglitz, H. Beutel, R. Keller, M. Schuettler and J. U. Meyer: Proceedings of the 7th International Conference on Microelectronics for Neural, Fuzzy and Bio-inspired Systems 1999 (IEEE, Granada, 1999) p. 112.
11 E. S. Hwang, Y. J. Kim and B. K. Ju: Sen. Act. A **111** (2004) 135.
12 L. Wallman, Y. Zhang, T. Laurel and N. Danielsen: Biomater. **22** (2001) 1187.
13 X. Navarro, S. Calvet, M. Buti, N. Gomez, E. Cabruja, P. Garrido, R. Villa and E. Valderrama: Restor. Neurol. Neurosci. **9** (1996) 151.
14 W. F. Ganong: Review of Medical Physiology (Lange, California, 1985) Chap. 2.