The Measurement of Compound Neural Action Potential in Sciatic nerve Using Microelectrode Array

Chungkeun Lee, Yongho Kim, Hangsik Shin, Yongjun Kim, and Myoungho Lee

***Abstract*—As a method of observing regeneration of damaged nerves, research is being conducted on analyzing the electric signals of nerve fibers that are damaged and regenerating by implanting a microelectrode array between those nerves. Microelectrode arrays possess high impedance and a unique phase characteristic according to their structural features, thus it requires a phase linearity test and an impedance test to prevent neural signal distortion.**

Therefore, this paper analyzes the features of microelectrode array and designs a bioamplifier. We also measured signals from sciatic nerves in rats with microelectrode array.

I. Introduction

When peripheral nerves are severed and damaged, regeneration of the nerve fibers in the nerves start from the proximal stump of the damaged nerves to the distal stump. For observing the regeneration of damaged peripheral nerves, such methods as function test, biopsy, and nerve conduction test were used.

Although function tests can most clearly observe the level of recovery because it discriminates by examining the results of functions such as gait analysis, a detailed examination is difficult. Biopsy provides precise information by structurally observing sections of the damaged area nerves using electronic microscopes or SEMs, but it cannot observe the subsequent progress of the sections because they have been severed for observation. Nerve conduction examination electrically stimulates the muscles around the damaged nerves and discriminates by evaluating the changes in the conduction speed of the stimuli and the size of the waves, but because the muscles are being stimulated, problems arise from the noise caused by muscle contractions, movements, or muscle disorders[1].

We are grateful to professor Kangmin Ahn of ASAN Medical center, for his help with surgical operation. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG1-CH07-0001)

Chungkeun Lee is with the Electrical & Electronics Engineering Department, University of Yonsei, Seoul, Korea(e-mail : micon78@yonsei.ac.kr)

Yongho Kim is with the Mechnical Engineering Department, University of Yonsei, Seoul, Korea(e-mail : yonghokim@yonsei.ac.kr)

Hangsik Shin is with the Electrical & Electronics Engineering Department, University of Yonsei, Seoul, Korea(e-mail : glority@yonsei.ac.kr)

Yongjun Kim is with the Mechnical Engineering Department, University of Yonsei, Seoul, Korea(e-mail : yjk@yonsei.ac.kr)

Myoungho Lee is with the Electrical & Electronics Engineering Department, University of Yonsei, Seoul, Korea(corresponding author to provide phone: +82-2-2123-4946 ; fax : +82-2-312-2770 ; e-mail : mhlee@yonsei.ac.kr)

To supplement this, flexible and organically appropriate Microelectrode Arrays (MEA) are planted between damaged nerves, and a new method of allowing regenerating nerve fibers to pass through MEA and evaluating by observing the electrical signals of nerve tissues is being researched [2].

However, because MEA is characterized by high impedance and non-linear phase due to its structural features [3], in order to measure the electrical signals of the nerve fibers that pass through MEA, a biopotential amplifier that considers such features is necessary.

In 1967, Beidler used a disk enclosed with glass and attempted to record the nerve fibers of sheep, and Kovacs et al have done research between 1965 and 1967, but technical problems in their procedures limited them from making any significant progress [2].

However, with the development of semiconductor technology and MEMS technology, Akin constructed the micro-silicon electrode in 1991, and Bradly succeeded in recording neural conduction signals in 1994 and measuring and recording long-term nerve conduction signals in 1997 [4].

Akin et al used the ASIC technology on MEA and recorded nerve signals by integrating the amplifier, but it had the disadvantage of high costs [5]. Banks et al built an MEA with sensors of various dimensions to amplify the optimum signal, and developed a biopotential amplifier suitable to MEA features and measured intracellular potential [6].

In order to measure the intracellular biopotential of nerves, this study analyzed the characteristics of the MEA developed by previous research[7], and developed a suitable biopotential amplifier for it. Also, through animal experiments that implanted MEA on rat's sciatic nerve, the extracellular potential and the intracellular potential were measured for the verification of their functions.

II. Method and materials

a. Design & Fabrication of Microelectrode Array

The design parameter for a sieve-type MEA are the configuration of the sieve part, the size of the via holes, and the number of electrodes. 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 values have been investigated in previous studies[8][9]. The fill factor is defined in equation (1).

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

Table 1.
MEA's Physical parameters

Parameters	Value
diameter of the sieve part	1.5 mm
*Fill factor	19 % to 27.6 %
the via hole size	40 ㎛, 50 ㎛
Number of Electrode	8

It is was reported that the proper range of via hole size is between 40 ㎛ and 65 ㎛. Therefore, we are used via hole size of 40, 50 ㎛ in this study[8].

We measured the impedance and phase of MEA using a precision impedance analyzer(HP4230A) to gain the MEA's electrical characteristics. The proposed MEAs are shown in Fig. 1. Fig. 2 presents the impedance and phase features of the MEA. We designed a biopotential amplifier for measuring the CNAP(Compound Neural Action Potential) that considered the proposed MEA.

b. Development of Biopotential Amplifier

We designed a biopotential amplifier for neural signals. The biopotential amplifier consists of a preamplifier, a drive circuit for prevention of powerline interference, a two order 7high pass bessel filter(100 ㎐), a five order low pass bessel filter(10 ㎑), and a variable gain amplifier(2,100 – 10,000). The measured CNAP is converted to digital signal using a Biopac MP150, with a sampling resolution is 16 bits, and a sampling frequency of 100 ㎑. Fig. 3 shows the blockdiagram of the developed bioamplifier.

c. Animal Experiment

Measurements were taken from the mice's sciatic nerve intracelluar potential after artificially causing nerve damage, inserting MEA, then giving them one month of regeneration time.

To eliminate external background noise, a shield box of 1 M × 1 M × 80㎝ dimension was constructed and used for the experiment.

In a electrical stimulus condition, a constant current stimulus of 5 V, 1 ㎃ was given for 0.5 ms, approximately 1 cm from the point of signal measurement. And in prick test, we stimulated the mice's right limb using a needle for 0.5s. After verifying the capacity of the proposed system through this experiment, nerve signals from the mice with MEA inserted in their damaged nerves were measured.

III. RESULT

The sciatic nerve of the mouse was lifted, then the epithelium, intracellular potential with insulating properties, was removed, and the signal from the intracellular area was measured.

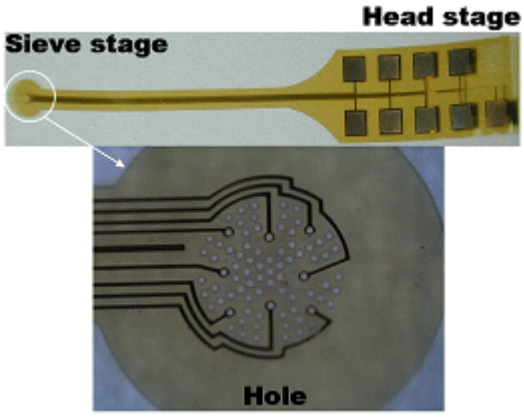
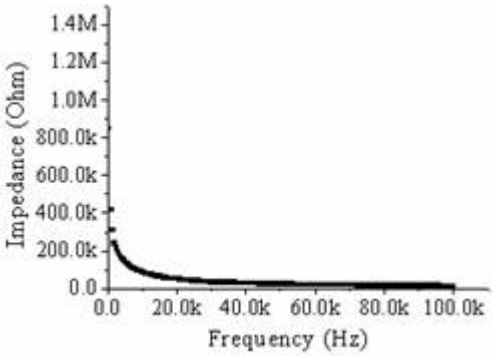
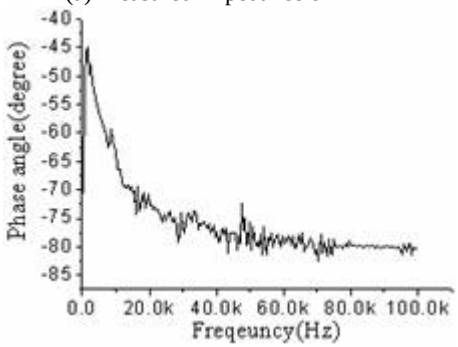


Fig. 1. Proposed MEA



(a) Measured Impedance of MEA



(b) Measured Phase of MEA

Fig. 2. Measurement results for the impedance behavior of the proposed MEA

Fig. 4 shows a sensory action potential recorded from regenerated sciatic nerve in mice taken during prick test. The CNAP duration is about 0.5s and its amplitude is about 110 V. In prick test, regenerated sciatic nerves had an intense energy during ongoing stimulus.

Fig. 5 (a) is the measurement of the intracellular potential reacting to the stimuli and (b) is a magnification of the measurement. Although there was some strain in long-term measurement due to the instability of the connectors in

the process of measuring signals, the signal measurement as a research experiment was successful.

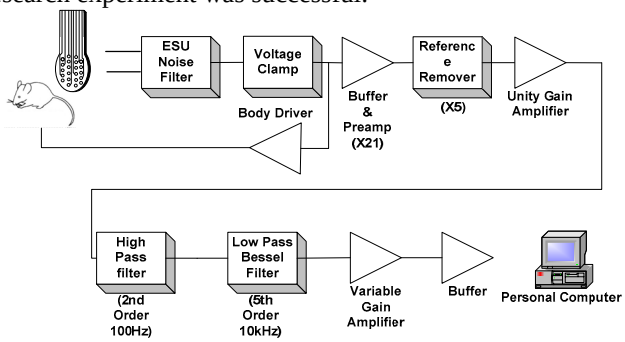


Fig. 3 Blockdiagram of developed biopotential amplifier

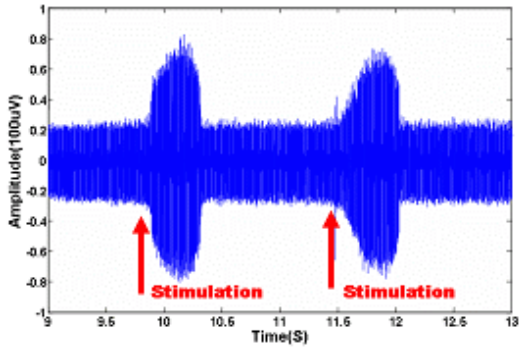
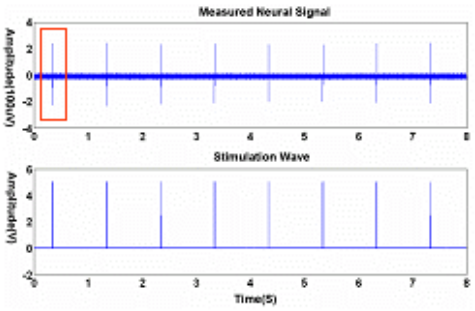
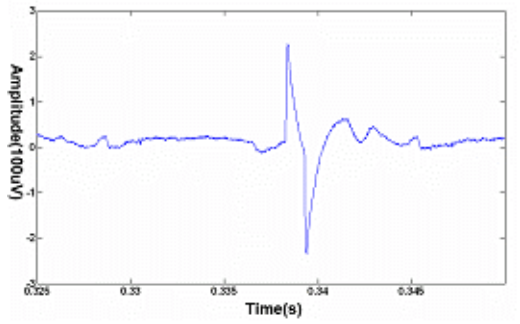


Fig. 4. Measurement results in prick test



(a) Measured neural signal from MEA



(b) One Neural Signal

Fig. 5. Measurement results in electrophysiological test

IV. Conclusion

This study observed the regeneration of damaged nerves through the use of proposed MEA. Signals were measured from damaged nerves with MEA implants. However, the challenge of long-term measurement due to unstable connections, which shows the necessity of considering stable connectors remain. It is expected that once the limitations of the current system are supplemented and analyses are made through both function tests and tissue tests, it will be able to contribute to the electrophysiology research of nerve regeneration.

Reference

[1] Paolo Dario, Paolo Garzella, "Neural Interfaces for Regenerated Nerve Stimulation and Recording", IEEE Transactions on rehabilitation engineering, VOL. 6, NO. 4, DECEMBER, 1998

[2] P. Heiduschka, S. Thanos, "Implantable bioelectronic interfaces for lost nerve functions", Progress in Neurobiology Vol. 55, pp. 433-461, 1998

[3] J. Webster, Medical Instrumentation : Application and Design, Houghton Mifflin, 1992

[4] Bradley, R. M., Cao, X., Akin, T. and Najafi, K., "Long term chronic recordings from peripheral sensory fibers using a sieve electrode array", J. Neurosci. Meth. 73, pp. 177-186,1997

[5] Tayfun Akin, Khalil Najafi, Robert M. Bradley, "A Wireless Implantable Multichannel Digital Neural Recording System for a Micromachined Sieve Electrode", IEEE JOURNAL OF SOLID-STATE CIRCUITS, VOL. 33, NO. 1, JANUARY 1998

[6] D. J. banks, W. Balachandran, P. R. Richards and D. Ewins, "Instrumentation to evaluate neural signal recording properties of micromachined microelectrodes inserted in invertebrate nerve", Physiol. Meas., 23, 437-448, 2002.

[7] Yong-Ho Kim, Chung-Kun Lee, Yong-Jun Kim, and Myoungho Lee, "Fabrication and Evaluation of a Flexible Sieve-Type Microelectrode Array for Monitoring the Regenerating State of Peripheral Nerves," Sensors and Materials, Vol. 17, No. 3, 2005, pp. 139-153

[8] L. Wallman, Y.Zhang, T.Laurel and N. Danielsen, “The geometric design of micromachined silicon sieve electrodes influences functional nerve regeneration”, Biomater, vol. 22, pp. 1187-1193,2001

[9] X. Navarro, S. Calvet, M.Buti, N. Gomez, E.Cabruja, P.Garrio, R. Villa and E.Valderrama, “Peripheral nerve regeneration through microelectrode arrays based on silicon technology.”, Restor Neurol Neurosci 1996, vol. 9, pp.151-160, 1996