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Microelectrode array for bioelectrical signal stimulation and recording

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Abstract: A microelectrode array(MEA) is presented, which is composed of 60 independent electrodes with 59 working ones and one reference one, and they are divided into 30 pairs. Except for the reference electrode, each pair consists of one stimulating electrode and one recording electrode. Supported by the peripheral circuits, four electrode states to study the bioelectrical signal of biological tissue or slice cultured *in-vitro* on the surface of the electrodes can be realized through each pair of electrodes. The four electrode states are stimulation, recording, stimulation and recording simultaneously, and isolation. The state of each pair of working electrodes can be arbitrarily controlled according to actual needs. The MEAs are fabricated in printed circuit board (PCB) technology. The total area of the PCB-based MEA is 49 mm × 49 mm. The impedance measurement of MEA is carried out in 0.9% sodium chloride solution at room temperature by means of 2-point measurements with an Agilent LCR meter, and the test signal for the impedance measurement is sinusoidal (AC voltage 50 mV, sweeping frequency 20 Hz to 10 kHz). The electrode impedance is between 200 and 3 kΩ while the frequency is between 500 and 1 000 Hz. The electrode impedance magnitude is inversely proportional to the frequency. Experiments of toad sciatic nerve *in-vitro* stimulation and recording and signal regeneration between isolated toad sciatic nerves are carried out on the PCB-based MEA. The results show that the MEA can be used for bioelectrical signal stimulation, recording, stimulation and recording simultaneously, and isolation of biological tissues or slices *in-vitro*.

Key words: microelectrode array (MEA); stimulation and recording; extracellular recording; toad sciatic nerve

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The neural system is one of the most complex systems of the human body. For centuries, scientists and researchers have devoted their lives to explore the secrets of neural systems and to reveal their working mechanisms[1]. As the basic components of neural systems, neurons are specialized in the transmission and processing of information[2]. To explore the electrical signal transmission characteristics between neurons through monitoring the electrical activity of neurons *in-vitro* from the microscope has been a hot point in recent years. It can provide a fundamental support for the development of neuroscience, brain science, and cognitive science. So, high spatiotemporal resolution techniques to monitor the neuronal activity are required to understand complex neural processing[3–4]. Neuronal culture preparations *in-vitro* under appropriate conditions preserve a variety of native neuronal network properties including synaptic formation and electrical connectivity. Such systems, distinctive from individually isolated neurons, are applicable in many aspects for physiological studies and pharmacological characterization[5].

The standard methods to measure the electrical activity of neurons are based on two different techniques: the patch clamp or voltage clamp technique, and MEAs [6–7]. But the first technique is invasive and cannot meet the requirements of long-term measurement. Extracellular recording with MEAs provides a noninvasive and long-term recording way to monitor the electrophysiological activity of neurons since their inception in the early 1970s[8]. With their ability to measure complex spatiotemporal neural activity, MEAs can be applied widely in neuroscience and pharmacology research[9–11]. However, because of the restriction of the microelectronic fabrication process, most of the conventional MEA system designs always incorporate an MEA with external signal conditioning electronics and system control realized by discrete off-chip components (Multi-Channel Systems, Reutlingen, Germany; Panasonic, Osaka, Japan; Ayanda Biosystems, Lausanne, Switzerland). Since each electrode requires a connection to the external electronics, the density of the electrodes is limited and signals will be attenuated due to the interconnection of the electrodes to external electronics. The combination of CMOS technology and the MEA overcomes the technical challenges of interconnect restrictions and off-chip circuit integration, and opens a wider field for the development of extracellular recording techniques [2, 4, 12]. By virtue of advanced CMOS technology, not only can high density electrode arrays be fabricated, but also the off-chip circuits can be integrated on one MEA chip.

As a previous work of monolithic integrated CMOS MEA system design, MEAs for extracellular stimulation and recording of biological tissue or slice *in-vitro* are designed and the MEAs are fabricated in PCB technology. Impedance measurement of the working electrodes is conducted, and toad sciatic nerve *in-vitro* stimulation and recording experi-

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ments and neural signal regeneration experiments between isolated toad sciatic nerves are carried out.

1 Material and Methods

1.1 MEA design and fabrication

Before the design of the MEA in CMOS technology, MEAs were designed and fabricated in PCB technology first to verify the design idea. Each MEA is composed of 60 independent electrodes with 59 working electrodes and one reference electrode. All the electrodes are divided into 30 pairs. Except for the reference electrode, each pair consists of one stimulating electrode and one recording electrode with a centre distance of 300 μm. According to the electrode size of one MEA produced by a multichannel system (MCS) in Germany and the limitations of the PCB technology, the working electrode size for the MEAs in this paper is designed as 150 μm × 200 μm. The centre distance of each pair of electrodes is 1 mm. With this structure, the state of each working electrode can be arbitrarily controlled according to actual needs, so four states to study the bioelectrical signals of biological tissues or slices *in-vitro* on the surface of the electrodes can be realized through each pair of electrodes. The four states are stimulation, recording, stimulation and recording simultaneously, and isolation. Layout design and verification of the MEAs are completed with DXP2004. The total area of each MEA is 49 mm × 49 mm, which adapts with the MEA adapter (MCS, Germany). The MEAs are fabricated in PCB technology (Gangshun Corporation, Nanjing, China). All the electrodes and pads are gold-plated to ensure the biocompatibility and anti-oxygenic properties.

3-mm high culture chambers (ϕ26 mm) are glued around the electrode area of the realized MEAs with biocompatible and water-resistant medical silica (Elastosil® E43, Germany). The culture chambers are used for the placement of biological tissues or slices *in-vitro*. Fig. 1 shows the photo of one final MEA, schematic of the electrode area, and a micro photo of one pair of working electrodes.

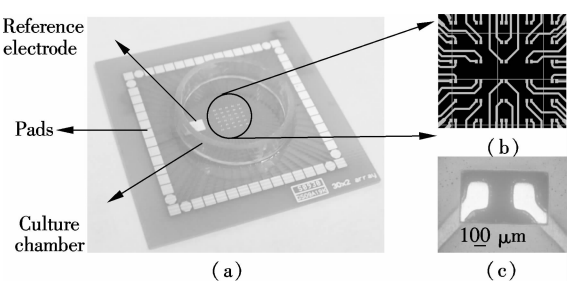

Fig. 1 Photo of one final MEA. (a) One final MEA with a culture chamber glued around the electrode area; (b) Schematic of the electrode area; (c) Micro photo of one pair of working electrodes

1.2 Impedance measurement

Electrode impedance measurement in the physiological solution is an effective analytical method to verify the performance of the MEA [13-14]. Electrode surface performance largely depends on the physical and chemical properties of materials, so the choice of electrode material is critical to stable and reliable measurement results. The impedance measurement is carried out in a 0.9% saline solution at room temperature by 2-point measurements. The impedance between each working electrode and the reference electrode is conducted. The test signal for the impedance measurement (Agilent LCR meter, 4284 A) is sinusoidal (AC voltage 50 mV, sweeping frequency 20 Hz to 10 kHz).

1.3 Neural signal stimulation and recording of isolated toad sciatic nerve

Active potential (AP) can be evoked from all the excitable cells in theory. The activity of the toad sciatic nerve can remain stable for a few hours in proper physiological environments, so the toad sciatic nerve is used as a model in this paper for bioelectrical signal stimulation and recording.

As a control, neural signal stimulation and recording experiments of the isolated toad sciatic nerve are conducted on the 8 × 8 MEA(MCS, Germany) at the same time. The 8 × 8 MEA is composed of 60 gold electrodes with 59 working electrodes and one reference electrode. The diameter of the working electrodes is 100 μm. All the electrodes are arranged in eight lines and eight columns with four vacant corner sites. The experiment is conducted as follows.

1) Toad sciatic nerve preparation

One mature toad is prepared (provided by the experiment animal center of Southeast University, Nanjing, China). The toad is concussed by striking the back of the head and spinal cord. The sciatic nerve is dissected. All the experimental procedures are in accordance with the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals, which comply with international rules and policies.

2) Circuits setup

The schematic diagram of the experiment setup is shown in Fig. 2 (a). The prepared toad sciatic nerve is placed on the surface of the MEA. In order to verify the stimulation and recording characteristics of the electrodes, one pair of electrodes in line 6 are assigned as stimulating electrodes, while one pair of electrodes in line 1 are assigned as recording electrodes. Electrode functions are assigned as follows (*mn*: *m* represents line, *n* represents column):

66 represents stimulating electrode connected with the positive pole of the signal generator.

67 represents stimulating electrode connected with the negative pole of the signal generator.

50 represents reference electrode.

17 represents recording electrode connected with the positive pole of the recording circuit.

16 represents recording electrode connected with the negative pole of the recording circuit.

In control experiments, all the electrode function assignments of the 8 × 8 MEA are the same as those of the present MEA.

In order to minimize fatigue and possible metal diffusion

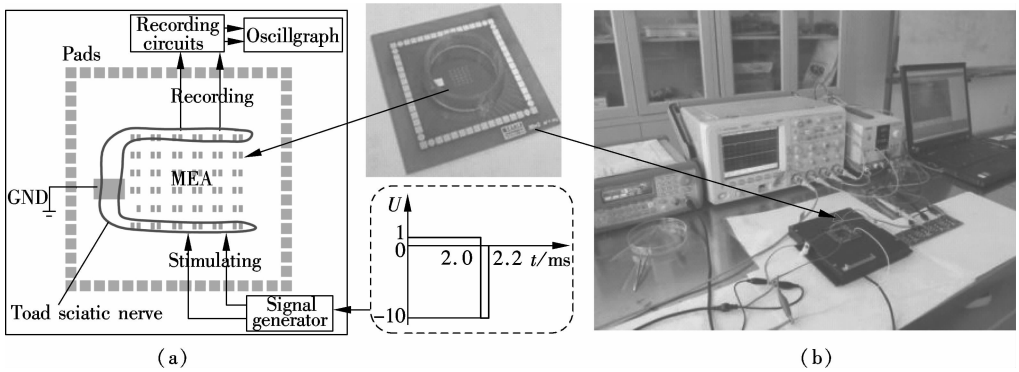

Fig. 2 The experimental setup. (a) Schematic diagram; (b) Photo

to the tissues and decrease the electrochemical damage to the tissues, a biphasic rectangular pulse with a frequency of 1 Hz is used as the stimulation pulse. The duration of the positive pulse is 2 ms, the duration of the negative pulse is 0.2 ms, and the amplitude ratio of the positive and negative pulses is 1∶10. The stimulation pulse is sent to the stimulating electrodes through a signal generator (Agilent 33220, USA), and simultaneously to the oscillograph as a reference. The recorded signal is amplified through the recording circuits 170 times in the experiments. Fig. 2 (b) is the photo of the experimental setup.

1.4 Neural signal regeneration between isolated toad sciatic nerves

The toad sciatic nerve preparation process is the same as the introduction in the above passage.

The schematic diagram of the neural signal regeneration experiment setup is shown in Fig. 3. Two MEAs are used to complete the experiment. The prepared two toad sciatic nerves are placed on the surface of MEAs A and B separately. During the experiment, electrode functions are assigned as follows (*mn*: *m* represents line, *n* represents column):

1) Electrode function assignment in MEA A

66 represents the stimulating electrode connected with the positive pole of the signal generator.

67 represents the stimulating electrode connected with the negative pole of the signal generator.

50 represents the reference electrode.

16 represents the recording electrode connected with the positive input pole of the recording circuit's channel 1.

17 represents the recording electrode connected with the negative input pole of the recording circuit's channel 1.

2) Electrode function assignment in MEA B

66 represents the stimulating electrode connected with the positive output pole of the recording circuit's channel 1.

67 represents the stimulating electrode connected with the negative output pole of the recording circuit's channel 1.

50 represents the reference electrode.

16 represents the recording electrode connected with the positive input pole of the recording circuit's channel 2.

17 represents the recording electrode connected with the negative input pole of the recording circuit's channel 2.

The stimulation pulse used in the experiment is the same as that used in the neural signal stimulation and recording experiments of the isolated toad sciatic nerve, which has been described in the above section.

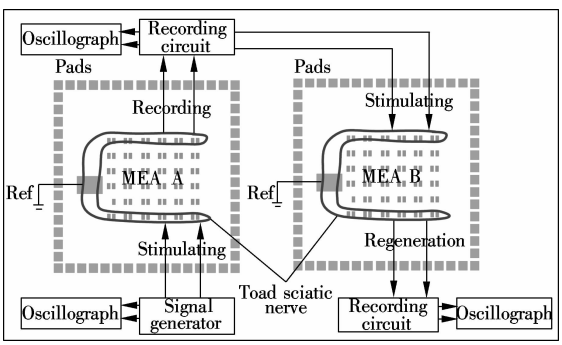

Fig. 3 Schematic diagram of experiment of neural signal regeneration between isolated toad sciatic nerves

2 Results and Discussion

2.1 Impedance Measurement

The electrode impedance measurement results are shown in Fig. 4.

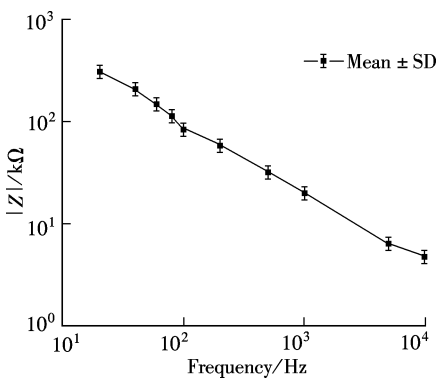

Fig. 4 Impedance magnitude of gold working electrodes in 0.9% sodium chloride solution ($n = 59$)

From Fig. 4, it can be found that the electrode impedance magnitude is inversely proportional to the frequency, which complies with the results of the impedance measurement results of the silicon-based MEA[15-16] and the CMOS MEA[17]. The impedance measurement results show excellent consistency, which indicates that the gold electrodes are fabricated uniformly. Fig. 4 indicates that the electrode im-

pedance is between 200 and 3 kΩ while the frequency is between 500 and 1 000 Hz, which can meet the requirements of bioelectrical signal stimulation and recording.

2.2 Extracellular stimulation and recording of toad sciatic nerve *in-vitro*

Fig. 5 shows the neural signal stimulation and recording experimental results recorded by the 8 × 8 MEA. Fig. 6 shows neural signal stimulation and recording experimental results recorded by the MEA presented in this paper. The results show that the evoked extracellular neural signal of the toad sciatic nerve can be recorded by the 8 × 8 MEA when the amplitude of the stimulation pulse is 500 mV, as shown in Fig. 5 (a). From Fig. 6(a), it can be seen that the presented MEA can record the evoked neural signal of the toad sciatic nerve when the amplitude of the stimulation pulse is 300 mV. The results indicate that the threshold voltage to evoke the extracellular neural signal of the toad sciatic nerve of the presented MEA is lower than that of the 8 × 8 MEA. It can be seen from Fig. 6 (b) that the recorded signal of the presented MEA is a standard AP with a duration of about 2 ms and an amplitude of about 9.4 mV when the stimulation pulse amplitude is 500 mV. The recorded amplitude is higher than 6.6 mV recorded by the 8 × 8 MEA when the stimulation pulse amplitude is 1 V. The results prove that the sensitivity of the presented MEA is higher than that of the 8 × 8 MEA.

Fig. 7 is the curve of the amplitude of the recorded signal vs. the amplitude of the stimulation pulse. It can be seen from Fig. 7 that the amplitude of the recorded signal arises along with the amplitude increase in the stimulation pulse from 300 to 500 mV, but keeps no change when the amplitude of the stimulation pulse is between 0.5 and 3 V. The results indicate that the presented MEA can be used for extracellular stimulation and recording of the toad sciatic nerve *in-vitro*.

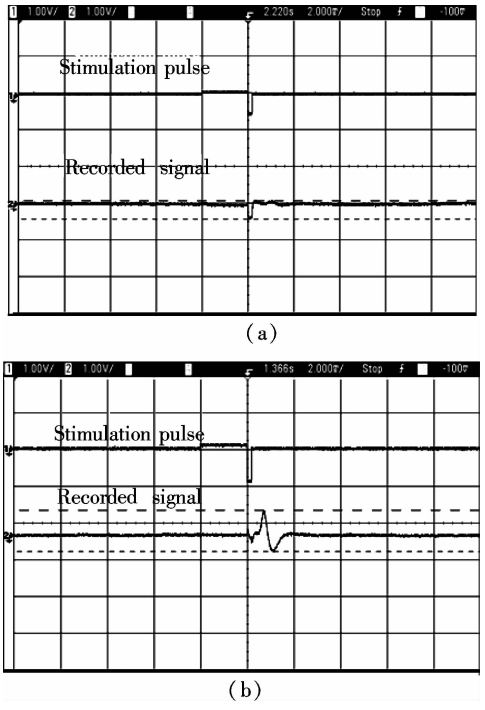

Fig. 5 Neural signal stimulation and recording experimental results recorded by 8 × 8 MEA. (a) Recorded results with 1 Hz, 500 mV stimulation pulse; (b) Recorded results with 1 Hz, 1 V stimulation pulse

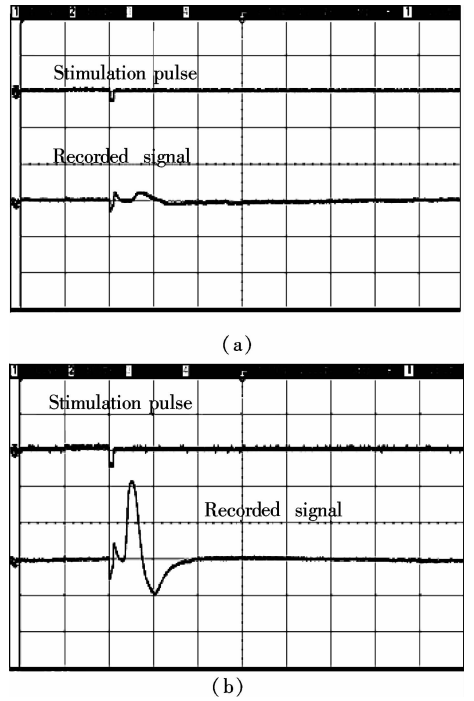

Fig. 6 Neural signal stimulation and recording experimental results recorded by presented MEA. (a) Recorded results with 1 Hz, 300 mV stimulation pulse; (b) Recorded results with 1 Hz, 500 mV stimulation pulse

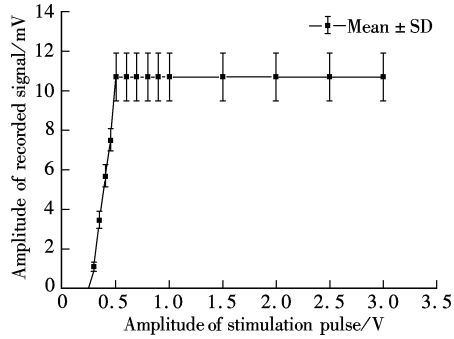

Fig. 7 Amplitude of recorded neural signal curve vs. the amplitude of stimulation pulse ($n = 3$)

2.3 Neural signal regeneration between isolated toad sciatic nerves

Fig. 8 gives the recorded results of neural signal regeneration experiment between isolated toad sciatic nerves recorded by the presented MEA. The first signal of each figure is the stimulation pulse, the second is the recorded signal of MEA A and the third one is the recorded signal of MEA B. Fig. 8(a) shows the recorded neural signal when the amplitude of the stimulation pulse is 300 mV, and Fig. 8 (b) shows the recorded neural signal when the amplitude of the stimulation pulse is 500 mV. It can be seen from Fig. 8(a) that the MEA A can record the envoked neural signal of the

first toad sciatic nerve when the stimulation pulse amplitude is 300 mV, but the MEA B can record nothing. As shown in Fig. 8(b), the MEA B can successfully record the evoked signal of the second toad sciatic nerve when the stimulation pulse amplitude rises to 500 mV. The phase delay between the recorded signals of MEAs A and B indicates that the signal recorded by the MEA B is an evoked neural signal of the second toad sciatic nerve but not an artificial signals of the first toad sciatic nerve. The results indicate that neural signals between two isolated toad sciatic nerves can be successfully regenerated with MEAs A and B.

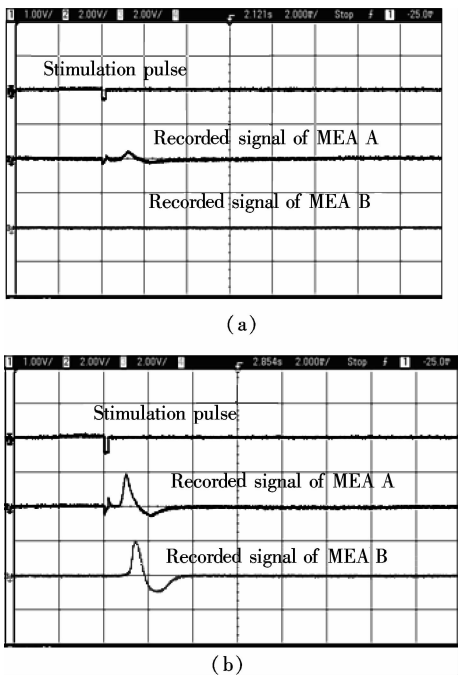

Fig. 8 Results of neural signal regeneration experiment. (a) Recorded results with 1 Hz, 300 mV stimulation pulse; (b) Recorded results with 1 Hz, 500 mV stimulation pulse

3 Conclusion

MEAs for extracellular stimulation and recording of biological tissue or slice *in-vitro* are designed and the MEAs are fabricated in PCB technology. The impedance measurement results of the working electrodes indicate that they can meet the requirements of bioelectrical signal stimulation and recording. Toad sciatic nerve *in-vitro* stimulation and recording experiments indicate that the presented MEA can successfully stimulate and record evoked neural signals of the toad sciatic nerve. Compared with the 8 × 8 MEA, the presented MEA can record evoked neural signals of the toad sciatic nerve with lower threshold voltage, and the presented MEA has higher sensitivity. The neural signal regeneration experiments between isolated toad sciatic nerves on the surfaces of the presented MEAs show that the presented MEAs can be used for bioelectrical signal stimulation, recording, stimulation and recording simultaneously, and isolation of biological tissue or slice *in-vitro*. All the conducted work will be a good basis of the future design of monolithic integrated CMOS neurochip composed of MEAs with large scale electrodes, neural signal stimulation circuits, and neural signal recording circuits.

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生物电信号激励与探测用微电极阵列

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摘要:介绍了一种微电极阵列(MEA),该 MEA 包含 60 个独立的电极,其中 59 个为工作电极,一个为参考电极. 工作电极成对排列,每一对工作电极分别包含一个激励电极和一个探测电极. 结合外围电路,每一对工作电极的组合都可以实现对电极表面培养的生物组织或切片进行 4 种状态(激励、探测、同时激励和探测、隔离)的控制. MEA 采用 PCB 工艺制作完成,总面积为 49 mm × 49 mm. 采用 LCR 测试仪,在 20 Hz ~ 10 kHz,50 mV 交流信号下对工作电极进行了阻抗测试,测试在 0.9% 的 NaCl 溶液中进行. 测试结果表明,频率在 500 ~ 1 000 Hz 之间时,工作电极的阻抗在 200 ~ 3 kΩ 之间,电极的阻抗幅值与频率成反比. 采用 MEA 进行了蟾蜍离体坐骨神经干电信号激励与探测实验,及坐骨神经干之间的神经信号再生实验. 实验结果表明,MEA 可实现对体外培养的生物组织或切片进行激励、探测、同时激励和探测、隔离 4 种状态的控制.

关键词:微电极阵列(MEA);激励与探测;胞外记录;蟾蜍坐骨神经
中图分类号:TN43