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RESEARCH PAPER

Application of Planar Microelectrode Array Modified by Nano-structure Titanium Nitride on Dual Mode Neural Information Recording

JIANG Ting-Jun[1,2], LIU Chun-Xiu[1], SONG Yi-Lin[1], XU Sheng-Wei[1], WEI Wen-Jing[1, 2], CAI Xin-Xia[1,2,*]

[1] State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100190, China

[2] University of Chinese Academy of Sciences, Beijing 100190, China

Abstract: The nano-structure TiN was modified on the laboratory self-made planar microelectrode array pMEA by magnetron sputtering method. The performance of modified pMEA was investigated. The research on neuroelectrical and neurochemical recording was studied in vitro. The impedance of the modified pMEA was decreased almost one order of magnitude, and the background noise level was reduced to ±6 μV. In the same testing environment, the signal-to-noise ratio (SNR) of modified electrodes was 1.7 times of bare electrodes. The SNR of neuroelectrical recording on the brain slice of SD rats reached 10:1, and the weak signal such as ±12 μV was separated easily. For neuroelectrical recordings, the detection limit of dopamine (DA) solution reached 50 nM with the 2:1 SNR. In the concentration range of 0.05–100 μM, the linearly dependent coefficient of the DA oxidation currents was 0.998. The modification of nano-structure TiN on pMEA reduced pMEA impedance and background noise level, meanwhile the SNR was increased. The weak signals of neuroelectrical and neurochemical recording were successfully recorded.

Key Words: Microelectrode array; Titanium nitride; Neuroeletrical, Neurochemical, Dopamine

1 Introduction

Neural diseases are one of the greatest dangers to modern society. The pathogenic mechanism of most of the neural diseases is related to abnormal firing and transfer of neural signals in cerebral network. Cerebral network, a complex system consisted of huge amount of neurons and their networks, has a function of connecting external environment and adjusting internal activities. Two kinds of signals, electrical and chemical signals, are the main ways for signals transfer among neurons. Electrical signals can be obtained by extracellular electrophysiological testing; while chemical signals are obtained by electrochemically detecting release of neurotransmitters. By combining these two detection models, it is more effective for study and analysis of neural diseases. Therefore, correctly and simultaneously detecting electrophysiological and neurotransmitters chemical signals become the basis of studying and treating of neural diseases[1–4].

Microelectrode array (MEA) provides a high-throughput, high spatial and temporal resolution and high sensitivity measuring device for neural signals detection and recording. Currently, MEA consists of planar MEA (pMEA)[5,6], complementary metal oxide semiconductor (CMOS) integrated MEA[7] and 3-dimentional MEA[8,9], and most of them are used for detection of electrophysiological signals. pMEA has several advantages, such as simple preparation, high mass production yield, easy and diverse surface treatments, and direct cell culture. Especially for the last one, it does no destruction to neurons, so pMEA has a wide application in neural signals detection *in vitro*. For extracellular electrophysiological detection of nerve cells, the attitude of signals is rather weak (about μV), and the release of neurotransmitters is trace (nM to μM). So to prepare a practical pMEA, one should overcome the increase of resistance and Nyquist noise caused by the decreasing electrode size. Modifying nanostructure onto pMEA surface is

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* Corresponding author. Email: xxcai@mail.ie.ac.cn
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a good way for these issues[10–13]. Compared with Pt black, Pt-Ir and grapheme nanostructures modified by electrochemical deposition method, magnetron sputtering titanium nitride electrode surface (TiN) has a structure of nanoscale and can stick on MEA firmly. Meanwhile it can be used repeatedly and is low-cost on bulk production.

For the demand of study and treatment of neural diseases, a pMEA[14] made by magnetron sputtering nanostructural TiN provided an effective tool for neural signals dual-mode detection. The pMEA had a high reusability and is easy to make mass production. The performance of TiN modified pMEA was verified by AC impedance spectroscope and background noise testing. The pMEA was also applied to detect electrophysiological and neurotransmitters electrochemical signals, so as to further investigate its dual-mode detection performance.

2 Experimental

2.1 Instruments and materials

USB-ME16-FAI-System 16-channel data filtering amplifier and acquisition system (Multi Channel Systems Company), Autolab AUT85039 electrochemistry station (Metrohm), M205C stereomicroscopy (Leica), S-3500 scanning electron microscopy (Hitachi), LEAD15-48 peristaltic pump (Lange), self-made stimulator, MW-D20 ultra-pure water system (Michem), and BSA124S electrical scale (Sartorius) were used in the experiment.

Dopamine (DA, Sigma), 0.9% saline (Shijiazhuang No.4 Pharmaceutical Company) and Ag|AgCl paste (Dupont Company) was used in the experiment. Artificial cerebrospinal fluid (ACSF) consisted of NaCl of 125.0 M, KCl of 2.5 M, $CaCl_2$ of 2.0 M, $MgCl_2$ of 1.3 M, NaH_2PO_4 of 1.3 M, $NaHCO_3$ of 25.0 M, Glucose of 25.0 M, *L*-sodium ascorbate of 1.3 M and sodium pyruvate of 0.6 M with pH 7.4 (keep pumping mixed gas of 95% O_2 and 5% CO_2 during use). SD rat hippocampus slices were provided by the group of Prof. Xingguo Gang in Institute of Neuroscience, Peking University. All the domestic chemicals used in the paper were analytical grade, and the water was deionized.

2.2 Preparation, modification and characterization of pMEA

A self-made 60-channel pMEA was made by our laboratory. It consisted of following components: glass slice as substrate, Pt as conducting material, and TiN as insulating layer. The pMEA contained electrode array, electrode leads contacted external circuit, reference electrode for neural electrophysiological detection, reference electrode for electrochemical detection of neurotransmitters, and counter electrode. The electrochemical reference electrode whose surface was coated with Ag|AgCl paste was made by thin film technology and lithography in Micro-electro-mechanical systems (MEMS). More detailed information can be found in Ref. [14]. Before modifying TiN, oxygen plasma was introduced to remove possible impurities absorbed on pMEA, and the area which was ready for modification was exposed by lithography technique. After magnetron sputtering in a flow of Ar/N_2 (15:1) for 30 min, the residual photoresist was washed away and the nano-TiN modified pMEA was obtained. The morphology of TiN structure was characterized by SEM.

2.3 Testing method

The pMEA and 10-channel data filtering amplifier system were connected by self-made external circuit interface, and the tissue fluid environment was simulated by physiological saline. A same stimulating pulse signal was introduced by self-made stimulator to simulate the neural physiological process. Supporting software was used to observe and record the background noise data of pMEA with the TiN electrode and a bare electrode, and the average data, RSD and response to same stimulating signal of each channel were collected and analyzed. SD-rats hippocampus slice was placed into the culturing circle, and with help of stereomicroscope, the slice was placed onto certain electrodes area. After that, a slice anchor was applied to make sure the brain slice sticks with electrode closely. Pumping ACSF into the system, the physiological information of neurons on the slice were observed and recorded.

By using Autolab electrochemistry station as the measuring platform for electrochemical experiments and Ag|AgCl electrode as the reference electrode, impedance spectroscopy scanning for both bare, the TiN electrodes in physiologic saline was carried out and the data were recorded. Meantime, the responses of different concentrations of DA on the two electrodes were investigated by cyclic voltammetry and *i-t* curve method. The experiment data was observed by Autolab Nova, and data was fitted and charted by Origin.

All the experiments were performed in Faradic Cage, and the whole system was connected to ground to minimize external interference.

3 Results and discussion

3.1 Nano-morphology of TiN and its improvement on pMEA

3.1.1 Nano-morphology of TiN

Combined with both Magnetron sputtering and MEMS lithography, TiN was modified onto pMEA electrodes Pt surface. As shown in the SEM image in Fig.1A, a layer of triangular pyramidal nanostructure was observed after the