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An implantable optrode composed of fiber and flexible thin-film electrode*

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An implantable optrode composed of fiber and multi-channel flexible thin-film electrode is developed. The flexible recording electrode is made from polyimide and is wrapped around the optical fiber. The front end of the fiber is tapered by wet etching. With the tapered shape, the light can leak from the sidewall of the fiber, and the tapered tip makes it easy to be implanted. The flexible electrode is attached with its recording sites aligning to the tapered part on the fiber. With this method, the fiber acts as an optical waveguide, as well as a support probe for flexible thin-film electrode. This novel device simplifies the fabrication process and decreases the size of the optrode. The device works well in vivo and the optical caused spike can be recorded with signal-to-noise ratio of 6:1.

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Electrode is an important mean of obtaining neural signals for the treatment of neurological diseases[1]. Electrode can not only record the electrical signal of neurons, but also affect the activity of neurons by stimulation. Electrical stimulation and light stimulation are mainly two methods to stimulate neurons currently. Light stimulation is based on optogenetic technology. Compared with electrical stimulation, light stimulation has a higher spatial resolution and can be electrically recorded while stimulated[2]. The earliest optrode structure is the metal wire directly attached to the fiber[3]. This structure is simple, but the manual operation is difficult to maintain the consistency of the electrode. In order to solve this problem, the electrode is directly integrated into the optical fiber surface using micro-electromechanical systems (MEMS) technology[4]. However, there is only one recording site on a single optical fiber, which limits the recording range of the optrode. In order to increase the density of recording site, many studies have been investigated to use MEMS technology to prepare a silicon-based thin-film microelectrode array. V-shaped groove was reserved on the electrode array to fix the fiber[5]. The Numikko team[6] inserted a tapered fiber into the center of the Utah array to achieve a high recording density optrode. However, hard wire electrodes and silicon-based electrodes are mechanically mismatched with soft biological tissue. They can lead to a strong immune response, which is disadvantageous to long-term recording. Therefore, flexible polymer materials, such as polyimide, chitosan, silk protein and parylene, are widely used in the electrode[7]. However, the soft nature of flexible electrodes also brings a problem that it is difficult to be implanted. The current solution is to use hard materials which are dissolved or removed after implantation to assist insertion[8]. The material of assisted implantation does not play a functional role and will not cause mechanical damage if removed.

In this paper, a fiber-based multi-channel optrode is designed and fabricated. It achieves light stimulation and electrical recording simultaneously. The polyimide-based flexible recording electrode is wrapped on the outside of the optical fiber. The fiber acts as an optical waveguide and support material for the implantation of flexible electrode. In order to reduce the damage caused by implantation, the front end of the fiber is tapered by wet etching. The flexible recording electrode is prepared by

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MEMS process, integrating eight recording sites on one optrode. The assembled optrode is tested in vitro and in vivo, respectively. The feasibility of this optrode in optogenetics is verified by the experiment.

The preparation of optrode is divided into three parts, which are the preparation of tapered fiber tip, the preparation of flexible recording electrode and the assembly of optrode.

The preparation of tapered fiber tip is a common fiber processing method. Tapered tips are used extensively in biochemical and clinical applications[9-11]. The damage caused by implantation can be reduced by changing the front end of the fiber into a tapered shape[12]. It also facilitates the assembly with the flexible recording electrode. There are mainly two types of methods to fabricate tapered tips, namely chemical etching method[9] and fused biconical taper (FBT) method[13]. Chemical etching is a method of placing an optical fiber in an etching solution for corrosion. The fused biconical taper includes a discharge taper method, hydrogen-oxygen flame heating method and a CO_2 laser irradiation method. The discharge taper is a high-voltage discharge on the optical fiber, and then the fiber tip is pulled into a cone shape. This method has high technical requirements, so it is difficult to achieve. The hydrogen-oxygen flame heating method uses a hydrogen-oxygen flame as a heating source to fabricate a tapered fiber. It needs to control the gas flow rate and the fiber pulling speed. Though the manufacturing process can be precisely controlled, it is easily influenced by the external environment. The CO_2 laser irradiation uses an infrared laser to heat the optical fiber, and the optical fiber is drawn to obtain a tapered tip, which usually requires expensive equipment. Compared with the latter three methods, the etching method removes the cladding of the optical fiber. When the light is transmitted from the fiber to the tapered tip, the former light leaks from the fiber earlier. In this way, the recording sites are close to the illuminated neuron, so the light response of the neurons is more easily recorded. Therefore, this study chooses the chemical etching method that does not require special equipment.

Commercial multimode bare fiber is composed of three layers. They are core, cladding and polymer coating from inside to outside with dimensions of 50 μm, 125 μm and 250 μm, respectively. The optical fiber was corroded by wet etching as shown in Fig.1. The specific steps are as follows.

Step1: Optical fiber end was processed. A fiber cutter was used to cut the fiber into the required length.

Step2: Optical fiber was corroded by acid solution. The optical fiber was fixed on the jig, so that the optical fiber was strictly perpendicular to the hydrofluoric acid solution (HF, 49%). A xylene layer with thickness of 5 mm covered the hydrofluoric acid surface to prevent its volatilization. The etching solution would gradually corrode the optical fiber, forming a tapered tip on the optical fiber.

Step3: The optical fiber coating was removed. The optical fiber was removed from the HF solution after soaking for 90 min. Then the fiber was washed by acetone, ethanol and deionized water several times and dried with nitrogen. Finally, polymer coating was stripped with a fiber-optic plier. The corroded optical fiber was fabricated completely.

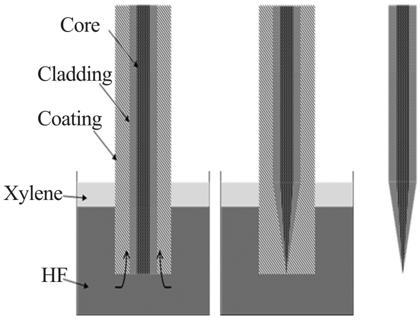

Fig.1 The process of optical fiber corrosion

The electrode is generally designed as polyimide-metal-polyimide sandwich structure. Polyimide is used as the upper and lower insulating layers. It not only plays the role of insulation protection, but also has excellent biocompatibility. The preparation steps are as follows.

Step1: The substrate was processed, and the lower insulating layer was deposited. A standard 4-inch silicon was used as substrate. The silicon wafer was immersed in sulfuric acid solution and hydrogen peroxide solution with volume ratio of 4:1. Heat treatment was performed at a temperature of 120 °C for 30 min to remove surface organics and native oxide layers. Then the wafer was cleaned by RCA standard cleaning methods. Polyimide was spin-coated and cured by stepwise heating to form the lower insulating layer.

Step2: The reactive ion etching (RIE) with P=300 W, $p(O_2)$=10 pa, $f(O_2)$=20 mL/min and t=1.5 min was used to roughen the underlying insulating layer. The purpose of the process was to enhance the adhesion of metal to polyimide.

Step3: A patterned metal layer (Ti/Au/Ti=13/200/13 nm) was formed by photolithography, sputtering and lift-off processes. The titanium layer was used to enhance the adhesion of the gold and polymer layers[14].

Step4: The same procedure as in Step1 was implemented to coat with polyimide as the upper insulating layer.

Step5: Recording sites and bonding pads were exposed by photolithography and RIE. The recording site with diameter of 15 μm was able to record the signal of individual cells[15].

Step6: The electrode was immersed in a 2% HF acid solution for about 5 s to remove the titanium on the recording sites. Then the Au interface would be exposed.

Step7: The electrode structure was released by a tweezer in deionized water.

Symmetrical structure of the electrode is illustrated in Fig.2. The middle of the hole is used for the insertion of the tapered tip. Eight recording sites are arranged on both sides of the hole. Tetrode arrangement helps to locate neurons[16]. The diameter of the recording site and the center distance between two recording sites of each tetrode are 15 μm.

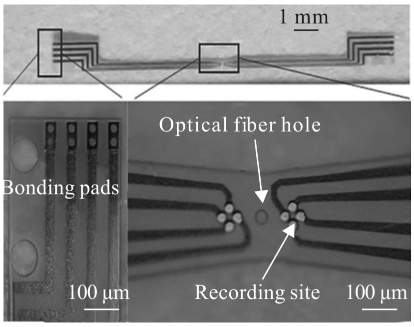

Fig.2 Flexible recording electrode of polyimide substrate

The symmetrical flexible electrodes were folded in half. Pads at both ends were bonded with PCB by gold wire. Then the connector was welded on the PCB. The PCB acts as a holder for the optrode and connects the amplifier.

The packaged electrode was combined with the corroded optical fiber. First, the tapered tip of the optical fiber was inserted into the small hole in the middle of the flexible electrode. And then it was fixed with a small amount of ultraviolet (UV) glue to ensure the bonding between the electrode and the optical fiber. The maximum cross-sectional area of the implanted portion of the assembled optrode is 0.3 mm×0.3 mm, and the total weight of the electrode is 0.3±0.02 g (n=10). The assembled optrode and partial enlargement of accessing to light (532 nm) are shown in Fig.3.

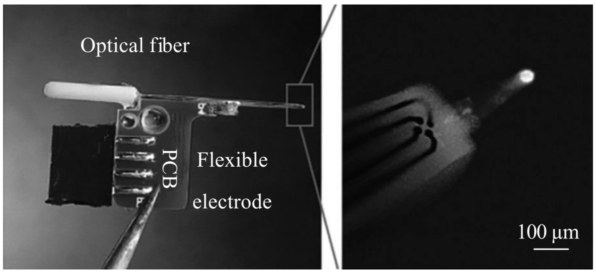

Fig.3 The assembled optrode and partial enlargement of accessing to light (532 nm)

The general fiber etching method is to etch after peeling off the coating layer. In this paper, two methods of optical fiber corrosion are compared. In Fig.4(a) the optical fiber was set aside hydrofluoric acid solution for 90 min after the coating layer was peeled off, and in Fig.4(b) the coating layer was peeled off after putting optical fiber in hydrofluoric acid solution for 90 min. The upper liquid of the etching solution was 5-mm-thick xylene.

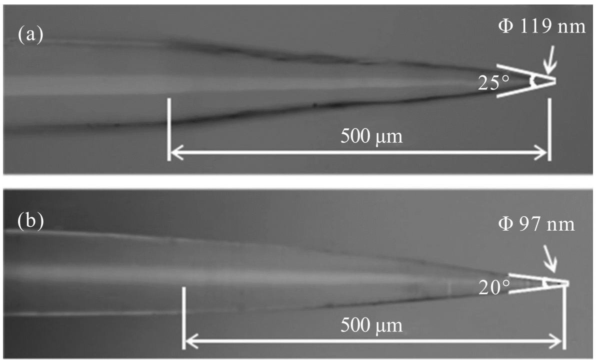

Fig.4 The optical fibers obtained through (a) corrosion after stripping the optical fiber coating layer and (b) stripping the fiber coating layer after corrosion

The average fiber taper angle of fiber (b) is 20±3°, the consistency is good, and the surface is smooth. However, the average fiber taper angle of fiber (a) is 25±10°, the consistency is poor, and the surface is rough. The main reason is that the optical fiber is not uniformly etched in a hydrofluoric acid solution. Bubbles and sol can be generated when the fiber is dissolved[17,18]. As the reaction progressed, the corroded products accumulate on the surface of the fiber, thus hindering hydrofluoric acid from reaching the surface of the fiber for reaction. At this time, the reaction rate will slow down. It is the accumulation of reactants and changes in corrosion rate that easily leads to rough fiber surfaces.

The electrical test in vitro allows a reasonable assessment of the performance of the optrode prior to animal experiments in vivo. Electrochemical impedance spectroscopy is an important method to evaluate the performance of electrodes[4,15]. Therefore, this paper uses the electrochemical workstation to measure the electrochemical impedance of the optrode.

A commercial Ag/AgCl electrode was used as reference electrode. Both optrode and the reference electrode were immersed in 0.1 mol/L phosphate buffered saline (PBS) solution (36 °C, pH=7.3). The sine wave voltage of 10 mV was used as the excitation signal to test the frequency response from 1 Hz to 1 MHz. The impedance response and the phase response are shown in Fig.5. The impedance of the optrode is 300 kΩ at the typical frequency of 1 kHz for neural activity. The thermal noise of the electrode is mainly related to the impedance, and the lower the impedance, the smaller the thermal noise[19]. The impedance of the gold interface, which is commonly used for electrophysiological recordings, is between 300 kΩ and 1.2 MΩ. This optrode has quite low impedance. As a consequence, the anti-noise capability of this optrode is excellent. In addition, the phase of 75° shows that the electrode is the ideal gold interface[4].

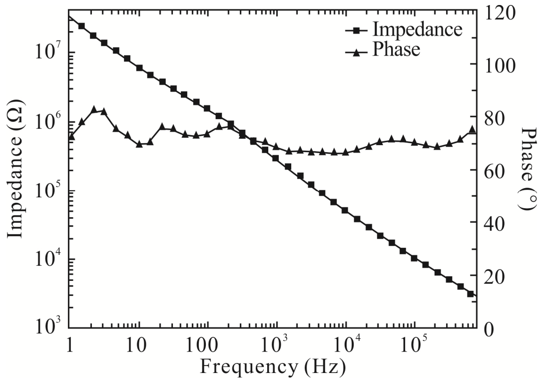

Fig.5 Impedance response curve and phase response curve of optrode (The impedance of the optrode is 300 kΩ at 1 kHz.)

The vivo experiment was performed with ChR-2 transgenic mice. The mouse's head was fixed by a stereo adjustment stand. Then the optrode was slowly implanted into motor cortex of the mouse. Blue light source at 473 nm was controlled by the TTL trigger, and could be adjusted by MATLAB program. Light was applied a square pulse (5 ms) train with the power density of 5 mW/mm^2 at 10 Hz. Neural signal was recorded by TDT multi-channel neural signal processor. The filter range is 0.3—5 kHz, analog-to-digital converter sampling frequency is 25 kHz, and the result is shown in Fig.6. It can be seen that the electrical signal of neurons has a direct response to light stimulation, and the signal-to-noise ratio of the electrodes is about 6:1. The signal-to-noise ratio is an important parameter that needs to be measured in the recording of the electrode signal. The higher signal-to-noise ratio is, the more effective neuron information that is occupied from the obtained signal[20]. The signal-to-noise ratio of the optrode is high enough to obtain the information of the target neuron. It shows that the optrode has an important application prospect in optogenetics.

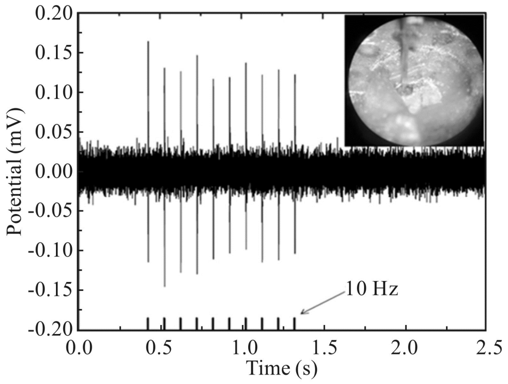

Fig.6 The result after the optrode being implanted into the motor cortex of awake mice (The optical caused spike is obtained under light pulses for 1 s at 10 Hz.)

This study designs a multi-channel optrode composed of fiber and flexible electrode. It combines the advantages of flexible electrodes and light stimulation, and solves the problem of difficult implantation of flexible electrodes. The front end of the optical fiber is tapered by wet etching to reduce the damage of the implantation. The optical fiber is corroded and then stripped of the coating layer to obtain the smooth surface, and it garners a better consistency. Flexible recording electrode is prepared by MEMS technology. An alteration of photolithographic steps from three to two ensures the electrode quality when simplifying the process. Multi-recording sites increase the recordable range of the optrode. The weight of the assembled optrode is only 0.3 g, and the cross-sectional area of the implanted portion is 0.09 mm^2, which alleviates the electrode implantation damage and enables long-term recording in animals. The electrode impedance is 300 kΩ at 1 kHz, and this lower impedance can effectively reduce the noise. The optrode records a clear signal of neurons under the stimulation of blue light in vivo testing, with the effective signal-to-noise ratio reaching 6:1. The above experimental results can comprehensively prove the practicality of the optrode in optogenetics. We will optimize the long-term reliability of this optrode to make it commercially viable in future.

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