FLEXIBLE CYLINDRICAL NEURAL PROBE WITH GRAPHENE ENHANCED CONDUCTIVE POLYMER FOR MULTI-MODE BCI APPLICATIONS

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ABSTRACT

This paper reports a novel flexible neural probe fabricated by cylindrical substrates lithography for Brain-Computer-Interface (BCI) applications. The electrode sites were patterned on the cylindrical surface to acquire high space selectivity and the micro channel was integrated to deliver drags or optical stimulation. The electrode sites of the probe were further modified with graphene enhanced conductive polymer composite to improve the interface performance for neural electrical recording and stimulation. The results show the composite coated probe has extreme high charge storage capacity (CSC), low impedance and good stability, which will become a promising BCI device.

INTRODUCTION

Neural probe is being investigated as a technique to diagnose and treat many diseases by electrophysiological recording and functional electrical/optical stimulation respectively. With the help of neural probe, researchers have already achieved clinical applications, such as relieving Parkinson's disease symptoms by deep brain stimulation (DBS) [1], restoring movement of paralyzed limb through functional electrical stimulation (FES) [2] and epileptic focus localization [3], respectively. Despite the fast growth in clinical applications, long-term stimulation and recording of the neural activity from a large number of closely located neurons are still challenge. Hence, chronically implantable neural probe must be developed to stimulate individual cells and monitor their response. A high density, high space selectivity and low impedance of electrode sites are demanded, while the size of the device must be minimized to decrease tissue reaction.

Recently, a lot of researches have focused on the application of cylindrical neural probes due to their high space selectivity and multi-functionality. However, most of these cylindrical neural probes were fabricated by plane lithography and subsequent crimping process which are time-consuming and unreliable [4, 5]. Therefore, this paper developed a novel fabrication method based on the unique cylindrical substrates lithography which has high pattern resolutions (±1μm alignment precision) and high reliability [6]. Using this system, a flexible neural probe with eight electrode sites distributed on the cylindrical surface of the probe was designed and fabricated. After that, we developed a novel electrochemical method to one-step electrodeposition PEDOT:PSS-rGO (PP-rGO) composite on the Pt electrode sites directly without any post reduction process. Compared to the previous synthesis method of PP-rGO, this work is more flexible, efficient, costless and eco-friendly [7-9]. After modification, the probe was found to have lower impedance and higher mechanical stability compared to the PEDOT-GO coated one based on our previous work [10].

MATERIALS AND METHODS

Neural probe fabrication

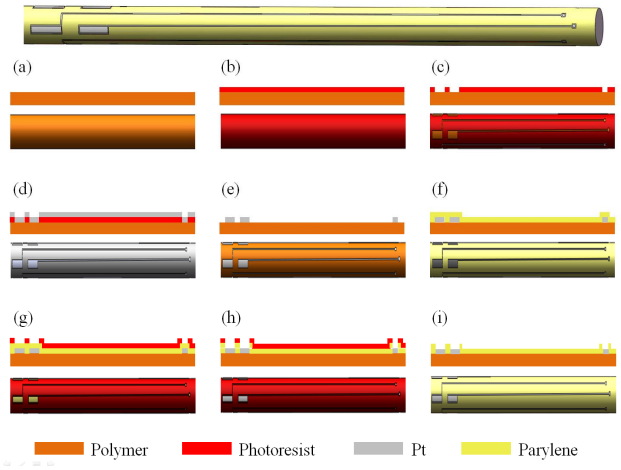

Figure 1: The fabrication processes of the neural probe based on cylindrical substrates lithography.

Figure 1 illustrates the fabrication process of the probe based on the cylindrical substrates lithography [6]. The lithography system mainly consists of four parts: a uniform illumination system, a reduced projection lithography system, a synchronized motion stage system, and a CCD multilayer alignment system. With this system, the photoresist can be sprayed and patterned directly on the cylindrical surface. As shown in Figure 1b, a layer of photoresist was sprayed onto the polyimide capillary (Furukawa Electric Co., Ltd) surface by a home-made

spray coating system firstly. Then the photoresist was patterned by programmable linear movements and angle rotations of mask and substrate simultaneously during the exposure (Figure 1c). After sputtering Pt layer and subsequent lift-off process (Figure 1(d, e)), a layer of parylene C was deposited as dielectric layer (Figure 1f). Then a second layer of photoresist was sprayed and patterned to expose the electrode sites and bonding pads (Figure 1(g, h)). The schematic diagram of the fabricated probe was shown in the top of Figure 1.

Electrochemical modification and characterization

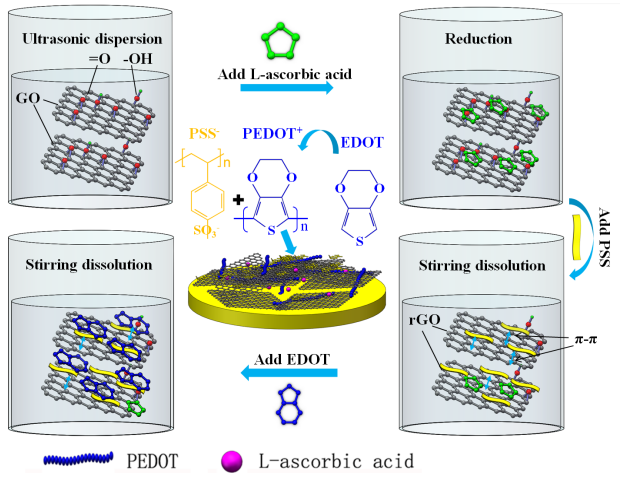

Figure 2: The synthesis flow diagram of the PP-rGO on Pt electrode site.

The fabrication schematic diagrams of the PP-rGO composite on microelectrode sites were shown in Figure 2. Firstly, GO dispersions (0.1mg/ml) were ultrasonic dispersed for 30 min. Then 1 mM L-Ascorbic (L-AA) was added and the pH of the solution was adjusted to 9-10 with 25% ammonia solution to promote the colloidal stability of the GO sheets through electrostatic repulsion. Secondly, GO dispersions were reduced by heating at 95 □ in oil-bath for 20 min to obtain the reduced graphene oxide (rGO) solution. The L-AA was adopted as reductant because it processes a mild reductive ability and nontoxic property. After reduction, 5 mg/ml PSS were added to the rGO solution to serve as counterions for the polymerization of EDOT. Meanwhile, the negative charged PSS adsorbed on rGO sheets by the non-covalent π-π interactions can also improve the stability of rGO sheets through electrostatic repulsion. Lastly, 0.01M EDOT was added to the above solution and electrochemical polymerization was conducted on microelectrode site by applying a constant current with deposition current density of 1 mA/cm^2. In this process, the EDOT monomer was polymerized to PEDOT$^+$ and then co-deposited on microelectrode site with PSS$^-$ covered rGO sheets by ionic bond interaction. The thickness of the as-deposited PP-rGO composite film can be controlled by the deposition time.

The electrochemical characterization of the prepared electrodes was conducted by measuring the electrochemical impedance spectrum (EIS) and cyclic voltammogram (CV) with Autolab (PGSTAT204, Switzerland) in phosphate buffered saline (PBS, pH 7.4). The electrochemical measures were conducted in three-electrode cell with the microelectrode used as working electrode, a saturated calomel electrode (SCE) as reference electrode and a Pt wire as the counter electrode. The EIS was measured at frequency ranging from 0.1 Hz to 100,000 Hz with input voltage amplitude of 0.01 V. The CV was scanned in the potential range between -0.6 V and 0.8 V at a scanning rate of 100 mV/s.

RESULTS AND DISCUSSION

Probe Design and Assemble

Figure 3(a-d) shows the photographs of the whole design of the flexible probe. The probe has an outer diameter of 330 μm and an inner diameter of 250 μm and the total length is 3.2 cm. There are eight electrode sites with a diameter of 30μm and a counter electrode site with a width of 200 μm distributed on the surface of the probe. Each four electrode sites aligned in a row with equal interval in the peripheral direction of the probe and the row distance is 230 μm. The distance between the counter electrode site and the first row electrode sites is 1000 μm. The bonding pads have a width of 900 μm and were bonded to varnished copper wire by conductive silver adhesives. Figure 3(c-i) show the assembled probe under bending, inserted into agarose, delivering drug and coated with PP-rGO. The bending test and the implantation test demonstrated the good flexibility and the sufficient insertion force during animal experiment. The drug delivering ability makes the probe suitable for chronic implantation by releasing anti-inflammatory agent or nerve growth factor (NGF). The hollow tubular structure of the probe also makes it possible for the application in the optogenetics by integrating optical fibers or LEDs.

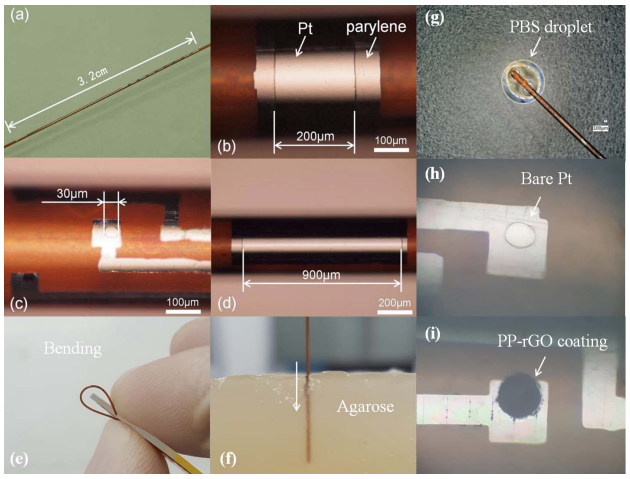

Figure 3: The photographs of (a-d) the fabricated neural probe (e) under bending, (f) inserted to agarose, (g) delivering drug and (i) coated with PP-rGO.

Morphology and Structure Characterization

The SEM pictures shown in Figure 4 illustrate the PP-rGO has a porous wrinkle structure with large effective surface area which is beneficial to charge transfer and storage. As shown in Figure 4b, there are many pin holes located between the PP-rGO sheets and the mean holes size is about 1 μm. These pin holes can serve as charge transfer channels which can increase the charge transfer rate. The

PP-rGO sheets are wrinkled and tangled with each other to form a 3D structure with high effective surface area and good mechanical stability. The 3D microscope pictures show the PP-rGO attached to the cylindrical surface tightly and the mean thickness is about 2 um. TEM picture shows PEDOT:PSS particles with dark color were covered on the surface of rGO sheets which further confirms the π-π interactions between the rGO sheets and PSS. The π-π interactions may play a key role in the rGO depositon process.

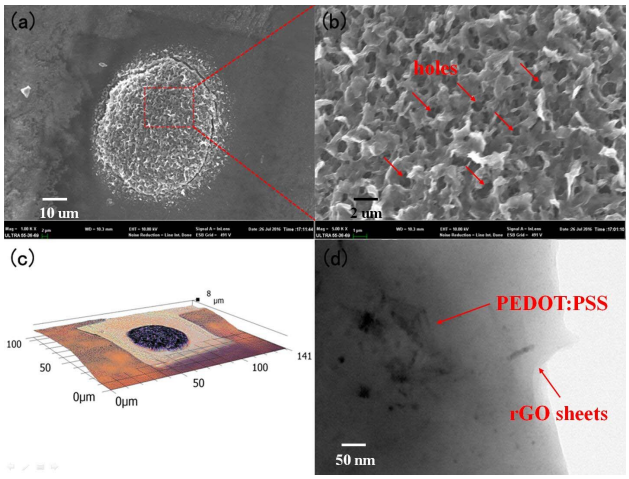

Figure 4: The SEM pictures at (a) 1000×, (b) 5000× magnification,(c) the 3D microscopy pictures at lateral view and (d) the TEM picture of the as-deposited PEDOT:PSS-rGO on Pt electrode site.

Electrochemical Characterization

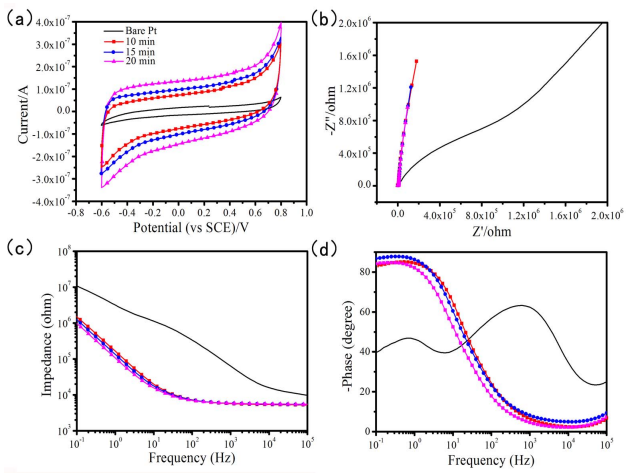

Figure 5: The cyclic voltammetry curves and electro-chemical impedance spectroscopy of PEDOT:PSS-rGO coated electrodes with different deposition time.

Figure 5 shows the EIS and CV curves of the bare Pt electrode and the PP-rGO modified electrode. The EIS and CV properties of the electrodes are determining factors influencing the performance of functional electrical stimulation and quality of electrophysiological signal recording. The CSC is essentially a measure of the total amount of charge available for a stimulation pulse. Therefore, the high CSC values always mean good change injection capacity of the electrode for neural stimulation. As shown in Figure 5a, the CSC values calculated from the time integral of the cathodic current show continuous increase with the deposition time of PP-rGO. The CSC value increased from 7.1 mC/cm^2 of bare Pt electrode to 85.6 mC/cm^2 of PP-rGO coated electrode with a deposition time of 20 min. The CSC values increased almost 12 times compared to bare Pt electrode. The high CSC value of the PP-rGO coated electrode mainly benefit by the large effective surface area as shown in Figure 4. Meanwhile, the corresponding impedance decreases from 253 kΩ of bare Pt electrode to 6.9 kΩ of PP-rGO coated electrode at 1 kHz. The impedance of the electrode decreased almost two magnitudes after PP-rGO coating indicates the excellent electrochemical performance of the PP-rGO composite. The phase plots show that the frequency angles are approximately 0° at high frequencies and 90° at low frequencies after PP-rGO deposition, which indicates that the PP-rGO acts as resistive material and capacitive material, respectively. Compared to the bare Pt electrode, the modified electrode has a lower phase shift at 1 kHz. In Nyquist plots, the PP-rGO coated electrodes show steeper gradient line at low frequencies than bare Pt electrode which represents the ion diffusion controlled electrochemical process. In other words, the charge transfer rate of the bare Pt electrode has increased after PP-rGO coating.

Material Stability Characterization

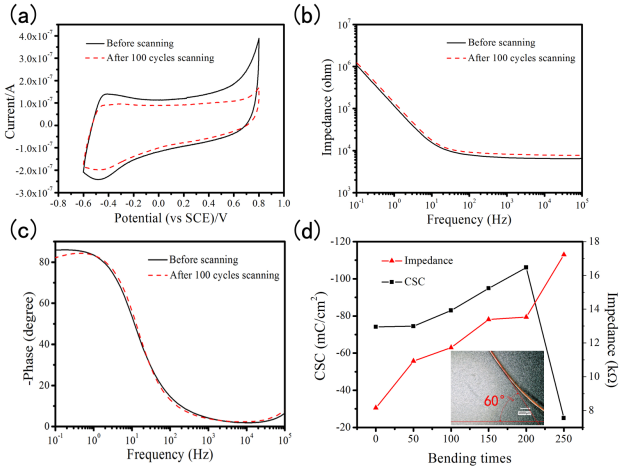

Figure 6: The (a) CV, (b) impedance and (c) phase plots of PEDOT:PSS-rGO coated electrodes before (solid line) and after (dashed line) 100 cycles CV scanning, the scanning rate is 0.1 V/s. (d) The CSC and impedance variation after repeatedly bending to the angle of 60° for 50, 100, 150, 200 and 250 times.

In order to test the mechanical stability of the PP-rGO coating on Pt electrode sites during implantation and stimulation, CV scanning test and probe bending test were conducted and the corresponding CSC and EIS were monitored subsequently. As shown in Figure 6(a, b), the calculated CSC values of the PP-rGO coated electrode shows only 9% decrease after 100 cycles scanning between -0.6 V and 0.8 V in PBS solution at a scanning rate of 100

mV/s. The corresponding impedance increased from 6.8 kΩ to 8.2 kΩ with only 20% increment. The phase also shows little change in all frequency range. The CV scanning results illustrated the good electrochemical stability of the PP-rGO coating during electrophysiological stimulation. After CV scanning test, the cylindrical probe was bended into 60° repeatedly and the CSC and impedance were recorded every 50 times as shown in Figure 6d. The results show slight increase of CSC and impedance during the first 200 times bending which implies good mechanical stability. The increase of CSC from 74.1 mC/cm^2 to 106.8 mC/cm^2 in the first 200 times bending may because of the improvement of effective surface area caused by material stretch during the bending and the impedance increase from 8.2 kΩ to 13.5 kΩ may because of the partial divorce of PP-rGO from the electrode sites. After another 50 times bending, sharp increase of impedance and decrease of CSC imply the exfoliation of the PP-rGO coating from the electrode site. As the actual bending angle will not reach to 60° during implantation, the results still can be considered to be very stable.

CONCLUSION

In summary, we fabricated an implantable multi-mold flexible neural probe by the unique cylindrical substrate lithography to obtain high space selectivity, high density and multi-functionalities. The fabricated probe integrated recording and stimulation electrodes with micro channel for drug delivery and can be used in optogenetics by integrating LED or optical fibers in the channel. The minimized dimension and excellent flexibility of the probe can reduce tissue reaction during implantation and the integrated micro channel can facilitate neurons viability by relieve anti-inflammatory agent or NGF. The neural probes were further modified with PP-rGO composite by a novel one-step electrodeposition method to improve the interface performance. Compared to the previous synthesis method of PP-rGO, this work is more flexible, efficient, costless and eco-friendly. After modification, the morphology and microstructure of PP-rGO were characterized with SEM and TEM. The SEM pictures show a porous wrinkle structure with large effective surface area which is beneficial to charge transfer and storage. The TEM picture shows PEDOT:PSS particles were covered on the surface of rGO sheets which confirms the π-π interactions between the rGO sheets and PSS. The CV and EIS results demonstrate the PP-rGO coated electrode has high CSC, low impedance and good stability. The developed implantable multi-mode flexible neural probe provided new opportunity to research on BCI applications.

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