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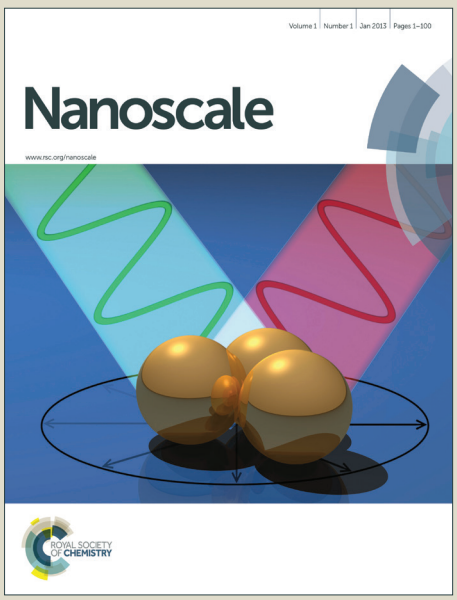

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ARTICLE

Covalent bonding of YIGSR and RGD to PEDOT/PSS/MWCNT-COOH composite material to improve neural interface

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Development of coating material for neural interface have been a pursuit to improve the electrical, mechanical and biological performances. For these goals, a bioactive coating was developed in this work featuring a poly(3, 4-ethylenedioxythiophene) (PEDOT)/carbon nanotube (CNT) composite and covalent bonded YIGSR and RGD. Its biological effect and electrical characteristic were assessed in vivo on microwire arrays (MWA). The coated electrodes exhibited significantly higher charge storage capacity (CSC) and lower electrochemical impedance at 1 kHz which are desired to improve the performance of stimulating and recording respectively. Acute neural recording experiments revealed that coated MWA possess higher signal/noise ratio capturing spikes undetected by uncoated electrodes. Moreover, coated MWA possessed more active sites and single units, and the noise floor of coated electrodes was lower than that of uncoated electrodes. There is little information in literature concerning the chronic performance of bioactively modified neural interface in vivo. Therefore in this work, chronic in vivo tests were conducted and PEDOT/PSS/MWCNT-polypeptide coated arrays exhibited excellent performance with the highest mean maximal amplitude from day 4 to day 12 during which acute response severely compromised the performance of electrodes. In a word, we developed a simple method of covalent bonding YIGSR and RGD to PEDOT/PSS/MWCNT-COOH composite improving both biocompatibility and electrical performance of neural interface. Our findings suggest that YIGSR and RGD modified PEDOT/PSS/MWCNT is a promising bioactivated composite coating for neural recording and stimulating.

1. Introduction

Neural interface is a bridge between nervous system and outside devices[1]. In order to improve the recording and stimulating ability of neural interface, it needs on the one hand to improve the electrochemical properties including the impedance, the charge transfer ability and the frequency response characteristic[2], and on the other hand, neural interface need to meet the requirements of chronic recording, which prefer small electrode size and biocompatible electrode materials with minimal inflammatory response[1]. Lowering of impedance makes neural interface more sensitive[3]. Decreasing of the size of an electrode raises its spatial resolution while reduces the inflammatory response[4]. In general, electrodes impedance are measured at 1 kHz, which is the typical frequency of neural biological activities[5]. Besides, high electrode impedance is associated with irreversible chemical reactions or heat accumulation at the neural-electrode interface, which can lead to electrode corrosion and seriously tissue response[6].

A lot of materials have been developed to improve the electrode-neural tissue interface[2, 4, 7, 8]. One of the most commonly used strategies is to coat microelectrodes with suitable materials. In recent years, poly(3,4-ethylenedioxythiophene) (PEDOT) has exhibited promising adhesion and biocompatibility among the known conducting polymer (CPs)[9], which are desired characteristics for implantable neural electrodes. However, the development of neural interface demands further improvement to the electrochemical property, the mechanical stability and the function of CPs. Carbon nanotube is another prominent material which has attracted much attention due to its intriguing physicochemical properties[5, 7, 10]. Carbon nanotubes can form rough and porous coatings on the neural electrodes which readily increase the surface area, improve the efficiency of charge transfer and contribute to the electrode biocompatibility[5]. However, the adhesion between sole CNT and substrate is generally poor[11, 12], and the biocompatibility of CNT is also influenced by the selection of different dispersants[13]. Studies have shown that CNT doped PEDOT as electrode coating material improves the biocompatibility of neuron–electrode interface[2, 8]. And due to the non-covalent bonding of MWCNT and PEDOT/PSS, there will be a nanolayer

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of PEDOT/PSS coated on the surface of CNTs, which is a key for an improved adhesion for PEDOT/PSS/MWCNT composites[14]. The biocompatibility of conducting polymer/carbon nanotube (CPs/CNT) composites can be further improved with bioactive materials. As a result, some studies has already been carried out to incorporate CPs or CNT with bioactive materials[15].

PEDOT has been doped with various bioactive materials including laminin (DCDPGYIGSR and DEDEDYFQRYLI), fibronectin (DCDPGYIGSR) and NGF yielding functional materials[15]. CNT can also be combined with biomolecule such as RGD peptide for improving the interaction of electrodes and neural cells[16]. Theoretically, bioactive peptides can be immobilized on CNT or CP surface by simple adsorption, entrapment or covalent bonding. But adsorbed or entrapped biomolecules are not stable because the surrounding extracellular matrix may displace it after implantation[15]. As a result, it is meaningful to covalently link these biomolecules to the coating surface. Recent studies showed that immobilized laminin-derived peptide GYIGSR (Gly-Tyr-Ile-Gly-Ser-Arg) can promote the adhesion, spreading and growth of PC12 cells and primary neurons on biomaterials, which required urgently by implanted electrodes[17]. And as is known to all that RGD is a most widely used polypeptide for facilitating adhesion of many kinds of cells[18]. So it's meaningful to use GYIGSR and RGD together improving neural interface.

In the present study, we used a simple method, which has been discussed in previous works[19], to covalent bond YIGSR and RGD to PEDOT/PSS/MWCNT-COOH to give a coating composite with better biocompatibility and electrical performance on neural interface. The surface morphology of neural electrode coating composites was characterized by scanning electron microscope. In order to identify the covalent bonding of YIGSR and RGD on the composite film, a fluorescamine solution was utilized to react with amines of the two polypeptides and then observed it under a fluorescence microscope. Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and potential transient method were used to characterize the electrode–electrolyte interface of the modified electrodes.

While YIGSR and RGD have shown clear benefit for cell attachment and growth in vitro, there is little evidence in literature which supports their benefit to the chronic neural interface in vivo[15]. Therefore, in this work acute and chronic neural recording were conducted to compare the performances of the PEDOT/PSS, PEDOT/PSS/MWCNT, and PEDOT/PSS/MWCNT-polypeptide modified electrodes and uncoated electrodes. The noise amplitude, signal amplitude, percentage of active sites recorded single units and signal noise ratio (SNR) in vivo were analyzed. In addition, we measured the change of impedance over weeks in vivo reflecting the tissue response to MWA[18] which may help to predict chronic performance of MWA[20].

2. Materials and methods

2.1 Materials

3, 4-Ethylenedioxythiophene (EDOT) monomer (>98%) and poly(styrene sulfate) (PSS) were purchased from Yacoo Corp (Suzhou, China). GYIGSR and RGD peptide were synthesized by GL biochem Ltd. (Shanghai, China). Carboxyl (2wt%) modified multi-walled CNT with the length of 0.5–2μm and diameter of 10–20nm was a commercial product purchased from Chengdu Organic Chemicals Co. Ltd. (China). All other chemicals were of analytical grade. Deionized (DI) water was used in all experiments. All reagents were sterilized with autoclave if not being mentioned specially for in vitro experiments.

2.2 Preparation of electrodeposited substrates

16 nickel-cadmium microwires (California Fine Wire, Stablohm 675, d = 35.5μm) were arranged to a 4×4 array to form a MWA. Each electrode was about 5mm long and the electrode column spacing was 250μm and the row spacing 300μm, which was used as the substrate for the electrochemical deposition and measurements. Quartz rings (35mm×7mm×2mm in inner diameter×height×thickness) were glued to indium tin oxide (ITO) conducting glass plate (50mm×50mm×0.4mm in length×width×thickness; TOYOBO, Japan) by PDMS to form a homemade 2-wells culture plate as electrodeposited substrates for in vitro experiments. Prior to use, all of the substrates were ultrasonically cleaned successively in acetone and deionized water.

2.3 Electrochemical deposition process

The PEDOT/MWCNT film was polymerized electrochemically from an aqueous solution containing 2 mg/ml MWCNT, 0.1 wt% EDOT monomer and 0.2 wt% PSS in deionized water which was dispersed under ultrasonic irradiation for 1 h before use. We firstly electrodeposited Au onto the microwire electrodes with a current of $1x10^{-6}$A applied for 50s in galvanostatic mode to improve the adhesion between electrodes and CPs. Then PEDOT/MWCNT film was electrodeposited onto microwire electrodes with an isolated Ag/AgCl reference electrode and a large-area Pt counter electrode in galvanostatic mode. A current of $1x10^{-8}$A was applied for 50s on each electrode using electrochemical workstation (CHI 660D) and a current of $1x10^{-3A}$ was applied for 100s on ITO glass without Au electrodeposited on the surface, controlling the deposition voltage between 0.9V to 1.2V to maintain similar deposition properties for the MWA and ITO glass during deposition process.

2.4 Covalent bonding of YIGSR and RGD

The prepared PEDOT/MWCNT-COOH film was sterilized with autoclave and then immersed in a 0.1% (w/v) solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC=water soluble carbodiimide; 0.01g) in 0.1N MES buffer (2-(N-morpholino)-ethanesulfonicacid; 0.4 g in 10ml of MilliQ water; pH 3.5) for 2 h at 20°C. The samples were successively washed in sterile MES buffer and MilliQ water. The activated PEDOT/MWCNT-COOH film was immersed into a sterile PBS buffer containing 2mg/ml RGD and 2mg/ml YIGSR for 2 h at 20°C, then rinsed with sterile PBS and MilliQ water. The sample was air dried and stored in clean environment.

2.5 Characterization of polymer membranes

In order to demonstrate the presence of the covalently immobilized amino acids on the membrane surface, a

fluorescamine solution (1mg/ml in acetone) was mixed with 50mM borate buffer (pH 9.0) at a ratio of 10% (v/v) to react with surface amines. The composite samples were rinsed with deionized water and then incubated with the fluoresamine solution for 15 min. The samples were rinsed with methanol and then observed under a fluorescence microscope.

2.6 Scanning electron microscopy (SEM)

SEM was used to investigate the surface morphology and microstructure of PEDOT/PSS, PEDOT/PSS/MWCNT, PEDOT/PSS/MWCNT-polypeptide films on ITO glass and microwires. The samples were observed in Hitachi-S4800 FESEM with a typical voltage of 5 kV.

2.7 PC12 cell culture

Rat pheochromocytoma (PC12) cells as a neural cell model were cultured on the composite film on ITO conducting glass to investigate the neuronal adhesion. The culture medium was H-DMEM supplemented with 5% fetal bovine serum, 10% horse serum, 2mM L-glutamine, 100μg/ml streptomycin and 100units/ml penicillin in a 37°C incubator containing a humidified atmosphere comprising 5% CO_2 and 95% air. Prior to seeding the cells, the composite films were subsequently rinsed with sterile water followed by 0.01M PBS. After that, PC12 cells were seeded at a density of 1×10^4 cells per well on our homemade 2-wells culture plate. A PEDOT/PSS/MWCNT-polypeptide coated plate was served as the experimental group with a plain ITO plate, a PEDOT/PSS film coated plate and a PEDOT/PSS/MWCNT film coated plate serving as the three control groups. Half of cell culture medium was replaced every 2 days. 50ng/ml of NGF (Sigma, South San Francisco, CA, USA) was added on the second day to induce differentiation of PC12 cells. After 5 days in the presence of NGF, we observed the adhesion and neurite outgrowth of PC12 cells under microscope.

2.8 Electrochemical characterization

Electrochemical workstation was used to evaluate electrochemical characterization of electrode sites in vitro. A solution of 0.1M phosphate buffer solution (PBS, pH=7.2) at 25°C was used as an electrolyte in a three-electrode cell configuration. The electrochemical impedance spectroscopy (EIS) was measured through a 10mV RMS sine wave at a frequency range from 100kHz to 1Hz. Cyclic voltammetry (CV) was performed between potentials of −0.8V and 0.6V (vs. Ag/AgCl) with a scan rate of 500mV/s. Before each CV curve was recorded, the modified microelectrodes were immersed in PBS for 30 min and swept several cycles to insure that the coating material had reached a stable state. The cathodic charge storage capacity (CSCC) was calculated from the time integral of the cathodic current in a cyclic voltammogram over a set potential range. Current pulsing was performed with CHI instrument (Chenhua, China) that provided biphasic current pulses of desired amplitudes and precise width. All potential transient responses were measured in a three-electrode cell comprised with an Ag/AgCl electrode acting as a reference electrode and a large-area Pt electrode as a counter electrode.

2.9 Stability test

The electrochemical stability of the modified electrodes was evaluated by comparing their impedance increment at 1kHz after ultrasonic testing in GVS-6L ultrasonic cleaner(180W) for 5 min. We separately coated 8 sites with PEDOT/PSS films and 8 sites with PEDOT/PSS/MWCNT-polypeptide films in an MWA using the same electrodepositing condition, then conducted the ultrasonic testing experiment.

2.10 Implantation surgery

All adult male Sprague-Dawley rats weighting about 250g used for the implantation experiment were purchased from the Experimental Animal Center, Academy of Military Medical Science (Beijing, China). All experiments in the study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Chinese Academy of Military Medical Science, Beijing, China. After being anesthetized by intraabdominal injection with 2% sodium pentobarbital (30mg/kg), the rats were immobilized in a standard stereotaxic frame. After a midsagittal incision was made in the scalp, a 3.0mm×4.0mm rectangle hole was carefully made 4mm anterior to the Bergman and 2mm to the right of the midline with a portable rechargeable drill and four 1.5mm holes were made 2mm to the left of the midline to insert two stainless steel bone-screws into the skull. PBS was used to wash away the bone debris. The electrode connector was grounded to the bone-screw using a stainless steel wire. Electrode arrays were lowered into the V1 visual area of the rat brain and cemented with dental acrylic (Ortho-Jet, Lang Dental). A half-modified array (8 sites coated with PEDOT/PSS/MWCNT-polypeptide, 8 sites uncoated) was implanted to study the improvement of acute recording after modified. We also conducted chronic neural recording in four groups of rats which were respectively implanted by uncoated electrodes, PEDOT/PSS modified electrodes, PEDOT/PSS/MWCNT modified electrodes and PEDOT/PSS/MWCNT-polypeptide modified electrodes.

2.11 Electrophysiological recording

We performed acute recordings immediately after the electrode was implanted into brain with a 64-channel neural acquisition processor (Plexon, Dallas, TX, USA) and its preamplifier (Plexon, Dallas, TX, USA) was attached to the output connector of the MWA. Neural electrophysiological data for all the recording channels were sampled at 40kHz and bandpass filtered at 300-5000 Hz. Chronic neural recording was taken and in vivo impedance was measured every two days after implantation. Reference electrode and counter electrode were connected to the ground screw on the stainless steel bone-screws in the skull. The animals were anesthetized during chronic electrophysiological recording.

3. Results and discussion

3.1 Identification of polypeptide

To identify if the polypeptide had been modified on the composite, we used fluorescamine to react with PEDOT/PSS/MWCNT-polypeptide film, then observed under a fluorescent microscope. As shown in Fig.1A, PEDOT/PSS film surface are smooth in an appearance of uniform blue. After adding MWCNT to deposition solution, the composite film surface was full of black dots (Fig.1B). There were a lot of florescent specks on the film after being modified with RGD

and YIGSR peptide, which indicate that RGD and YIGSR peptide successfully covalent bonded to PEDOT/ PSS/MWCNT film (Fig.1C). Moreover, the distribution of fluorescent specks on PEDOT/ PSS/MWCNT-polypeptide film and black dots on PEDOT/PSS/MWCNT film were similar but not identical (Fig.1B, C). The main reason of this phenomenon was that some of MWCNT were wrapped by PEDOT/PSS apart from the MWCNT inlaid in the PEDOT/PSS surface that could bind polypeptide, which would be further indicated in SEM results[14, 21, 22]. Moreover, some florescent specks without enough polypeptide binding were too small to see.

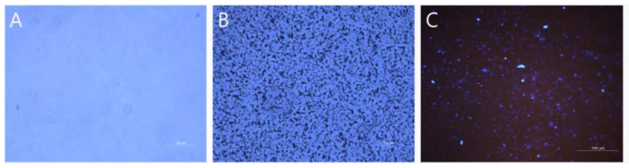

Fig.1 The conjugation of PEDOT/PSS film (A), PEDOT/PSS/MWCNT-polypeptide film (B) and PEDOT/PSS/MWCNT-polypeptide film with fluorescamine (C) on the substrate of ITO glass plate. Bar=100μm

3.2 Morphology

The electrical properties of neural electrode coating composites correlate with the surface morphology[23]. The surface morphology of the PEDOT/PSS, PEDOT/PSS/MWCNT and PEDOT/PSS/MWCNT-polypeptide films prepared on microelectrodes are shown in Fig.2. We can find that the geometrical surface area of electrodes increased distinctly after MWCNT being added to the electrochemical deposition solution, which benefits the electrical properties a lot (Fig.2G, Fig.3C). Moreover, we further observed that the MWCNT was surrounded by some materials after being modified by polypeptide, which might influence its electrical properties and we will discuss it in the section of electrochemical impedances (Fig.2H). The similar characteristic of surface morphology on ITO glass was indicated in Fig.3. Comparing the results of surface morphology of PEDOT/PSS/MWCNT film and PEDOT/PSS/MWCNT-polypeptide film on ITO glass and microwire, we could find that the exposed MWCNT stretched out of the surface farther after being modified by polypeptide, which formed a more stereo nanostructure that could promote the attachment of neural cells and create an intimate cell-electrode interface[18, 20].

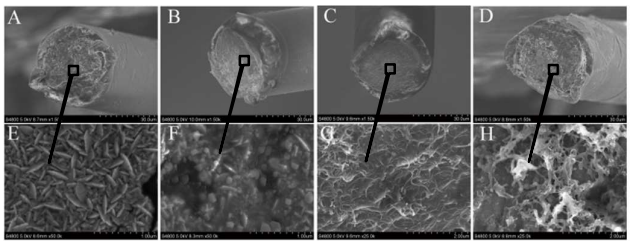

Fig.2. SEM images of different coating layer on microwire electrodes: Au (A,E), PEDOT/PSS (B, F), PEDOT/PSS/MWCNT (C, G) and PEDOT/PSS/MWCNT-polypeptide (D, H) composite film.

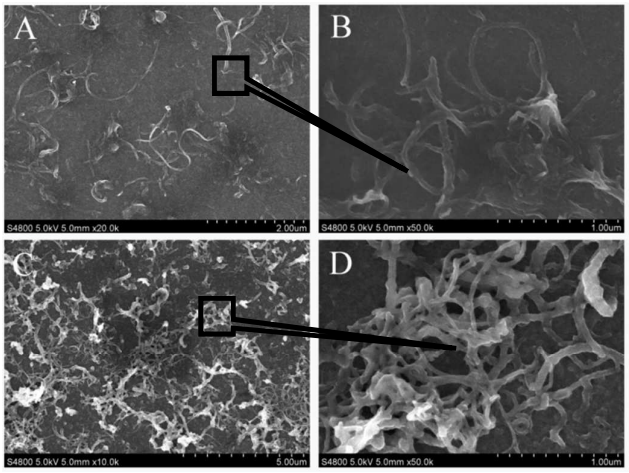

Fig.3. SEM images of different coated layer on ITO glasses: PEDOT/PSS/MWCNT (A, B) and PEDOT/PSS/MWCNT-polypeptide (C, D) composite film.

3.3 Electrochemical impedance

The impedance at 1 kHz is a commonly utilized parameter reflecting the performance of neural interface. PEDOT/PSS, PEDOT/PSS/MWCNT and PEDOT/PSS/MWCNT-polypeptide modified and bare nickel-cadmium microwire electrodes were tested in a solution of 0.1M phosphate buffer solution (PBS, pH=7.2) at 25°C for assessing the electrochemical characteristics at their interfaces. As shown in Fig.4, the 1KHz impedance of the PEDOT/PSS, PEDOT/PSS/MWCNT and PEDOT/PSS/MWCNT-polypeptide modified microelectrodes dropped from about 600kΩ to 40kΩ , 10kΩ and 15kΩ , which is ~90%, 98% and ~97% lower compared to that of the unmodified nickel-cadmium microwire electrodes respectively. The cause of the slightly increased impedance after polypeptide being modified might be that MWCNT was surrounded by some materials as SEM result showed (Fig2F, 3D).

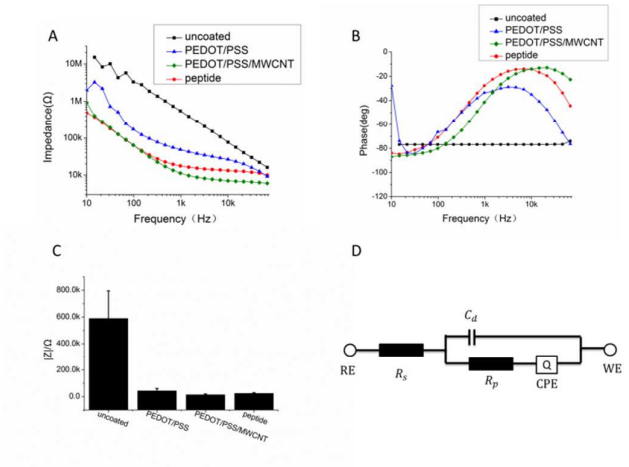

Fig.4. Electrochemical impedance of modified and unmodified electrodes. The electrochemical impedance spectroscopy (EIS) of

electrodes with different interfaces materials: impedance (A), phase(B), impedance of different interfaces materials at 1kHz (C) and proposed equivalent circuit model for electrochemically deposited electrodes (D).

In order to draw further useful information from the EIS results, an equivalent circuit model were used as shown in Fig. 4D, which has been discussed in previous work[5]. The results obtained from fitting the equivalent circuit model were shown in table 1. The circuit elements are: solution resistance (R_s), coating capacitance (C_d),pore resistance (R_p), double layer interface impedance (Z_{CPE}). Due to the complex solution condition and the rough electrode surface, constant phase element (CPE) was used as the equivalence element which is presented as Q to represent double layer capacitance. The CPE is defined through the following equation:

$$Z_{CPE} = Z_Q = \frac{1}{q(jw)^n} \quad (1)$$

The parameter indicates the capacitance value of CPE as n approaches 1. When n changed from 0 to 1, CPE changed from a pure resistance to a pure capacitance. Parameter n reveals the properties of electrode-electrolyte interface, which is relevant to the actual surface area, the distribution of the reaction rates, the non-uniform current distribution and so on.

The experimental results have been fitted to the model to calculate the parameter values using ZSimpWin software (Table 1).

As shown in table 1, solution resistance (R_s) decreased after the adding of MWCNT to the deposition solution, which indicated the increase of efficient surface. The pore resistance (R_p) of the PEDOT/ PSS microelectrode was reduced dramatically after MWCNT added to the composite, which meant that MWCNT improved porosity of the coating materials, meanwhile promoted the transfer of ions within the polymer film. Coating capacitance (C_c) and CPE-q correlated with the variation in the CSCC, So CSCC increased after adding MWCNT and decreased a little after modified by polypeptide which is in agreement of the result from cyclic voltammetry curve. R_s decreased after modified with polypeptide, which indicated that polypeptide might create more electrochemically active surface area as SEM images shows it formed a more stereo nanostructure, and R_p increased which can probably be attributed to that polypeptide might change the surface ploy porous structure of PEDOT/PSS/MWCNT film. Furthermore, the CPE-n values of these deposited microelectrodes are close to 1, suggesting that all of the neural interaces are close to the ideal capacitance.

Table 1. Fitting data obtained by the equivalent circuit model shown in Fig. 4

Parameters	uncoated	PEDOT/PSS	PEDOT/PSS/MWCNT	peptide
Z_{1kHz} (kΩ)	589.4	42.9	14.2	23.8
R_S (Ω/cm^2)	1.355×10^{-13}	0.007786	4.009×10^{-7}	2.089×10^{-7}
C_d (F/cm^2)	_	2.922×10^{-5}	13.53	2.073
R_P (Ω/cm^2)	_	0.3543	3.153×10^{-7}	1.415×10^{-6}
CPE-q ($S s^n/cm^2$)	13	0.001183	365.2	341.3
CPE-n ($0<n<1$)	0.8402	0.9495	0.9478	0.9554

3.4 Cyclic voltammetry (CV)

CV was used to explore the capacity of charge transfer density for PEDOT that were deposited with the same charge density. The capacity of charge transfer observably increased using PEDOT/PSS/MWCNT, as could be seen in Fig.5. The integration of I(t) within the cycled region gave CSCC of composite film. After electrochemical deposition, CSCC increased from 0.227 mC/cm^2 to 3.99 mC/cm^2, 8.6061mC/cm^2 and 7.5032 mC/cm^2 for the PEDOT/PSS, PEDOT/PSS/MWCNT and PEDOT/PSS/MWCNT-polypeptide modified microelectrodes, respectively.

As the CSCC represented the total amount of electroactive material of the electrode, the increased CSCC implied that MWCNT promoted the deposition of CPs, and then enhanced the capacitance performance.

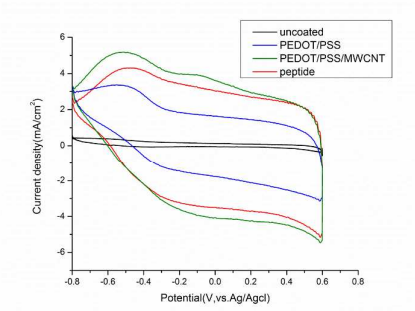

Fig.5. Cyclic voltammetry of different neural interface materials.

3.5 Stability test

The stability of the composites was characterized by the variation in impedance at 1kHz after ultrasonic testing. As can be seen from Fig.6, the impedance at 1kHz of PEDOT/PSS/MWCNT coated microwires increases 5.0% while impedance of PEDOT/PSS/MWCNT-polypeptide coated microwires increases 5.9% after ultrasonic testing, which indicated that the coating materials may slightly peel off from the edge of electrode sites, the increment of the two coating materials have no significant difference (p=0.53286, two sample t test, n=8, data pooled from one array). The result showed that both coating composites can perform good adhesion ability on microelectrode.

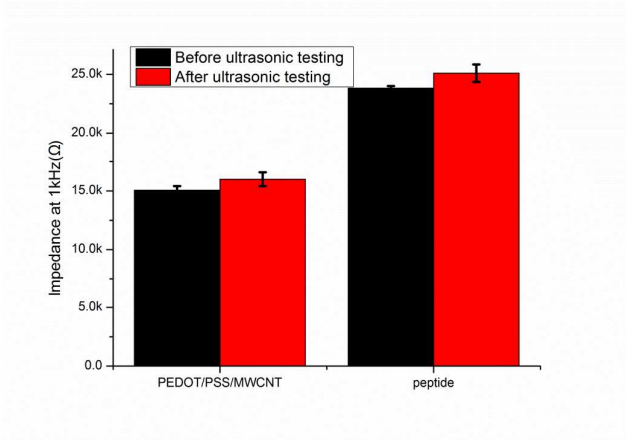

Fig.6. The change of impedance at 1kHz after ultrasonic testing.

3.6 Stimulation

The preferred neural electrode should have a higher charge injection capability, which would generate lower electrode voltage at a given pulse current and is safer for neuronal stimulation[24]. To simulate the stimulation conditions of neural stimulation applications, a symmetric charge balance biphasic current pulses with 1ms pulse width at an interval of 10ms were applied to the microelectrodes in PBS solution to induce voltage response (voltage excursion) of an electrode which was a direct indicator of its charge injection capability. As shown in Fig.7, under the same stimulation conditions, the coated electrode exhibited a much lower voltage excursion than the uncoated electrode. The results indicated that the coated electrodes could deliver higher charge densities without generating high voltages that might harm surrounding tissues.

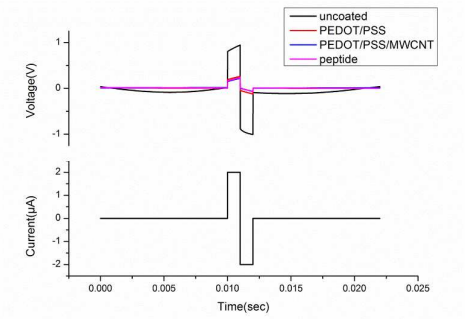

Fig.7. The symmetric charge balance biphasic current pulse (bottom) and voltage responses of coated and uncoated electrodes (top).

3.7 PC12 cell culture

PC12 cells were used for the study of neuronal development, which can be induced to differentiate toward sympathetic neurons in the presence of nerve growth factor (NGF). The state of PC12 cells cultured on the ITO substrate, PEDOT/PSS, PEDOT/PSS/MWCNT and PEDOT/PSS/MWCNT-polypeptide ITO substrates in the presence of 50 mg/ml NGF on the fifth day are shown in Fig.8. The neurite extensions induced by the NGF were observed and the cells grew well on all of the surfaces. However, the cells number on the plain ITO was significantly less than that on the coated ITO substrate, suggesting that these three coated substrates are more favorable to cell attachment and growth.

Neurite length was quantitatively assessed with NeuronJ[25] to indicate the morphological differentiation in different groups. Individual neurites in all images were traced and measured using NeuronJ. Length measurements were converted into microns. As shown in Fig.9, the average neurites length on the PEDOT/PSS/MWCNT coated substrate was significantly longer than the other three substrates. This result indicated that the PEDOT/PSS/MWCNT coated substrates are more favorable to neurite growth.

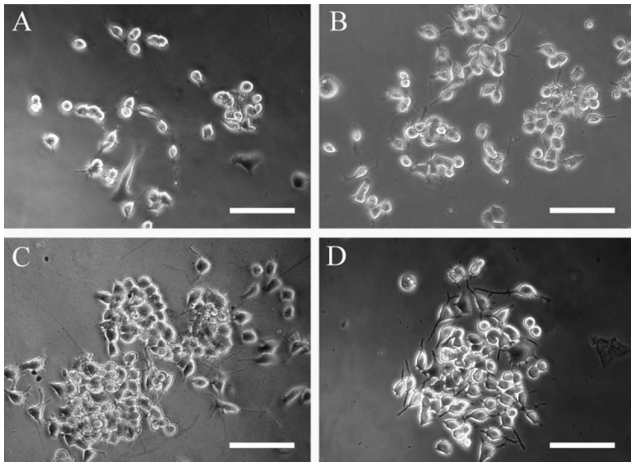

Fig.8. Adhesion and neurite growth of PC12 cells on different substrates: ITO (A), PEDOT/PSS (B), PEDOT/PSS/MWCNT (C), and PEDOT/PSS/MWCNT-polypeptide (D). Bar=100μm

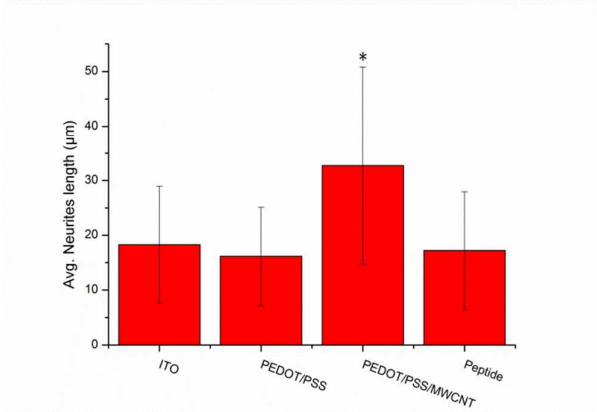

Fig.9. The comparison of average neurite length of PC12 cells on different substrates. * indicate One-way ANOVA and Students't test, p<0.05.

3.8 Acute neural recording

In order to investigate what kind of recording quality is improved by this low impedance and nanostructured rough

coating composite, a 16-channel microelectrodes half modified with PEDOT/PSS/MWCNT-polypeptide was implanted into rat V1 to acquire and analyze neural signals. Firstly, we compared the best channel recorded stable single unit of coated electrode and uncoated electrode in terms of signal power spectrum, noise floor (V_n), signal amplitude (V_s) and signal to noise ratio ($10\log_{10}(\frac{V_s^2}{V_n^2})$). As shown in Fig.10A, after being coated, the SNR and signal amplitude of electrodes increased from 6dB and 62μV to 16dB and 179μV respectively and the noise floor decreased from about 30μV to 20μV. As shown in Fig.10B, the power of this coated electrode was higher than the uncoated electrode at every frequency from 1–2000Hz, which indicated the increased information content of the coated electrode acquired data. The result was similar to Edward W. Keefer's work[20]. Furthermore, another 12 arrays with 16-channel microelectrodes (6 arrays each group) were implanted to investigate the difference of acute recording between PEDOT/PSS/MWCNT-polypeptide coated and uncoated electrodes. The results in Fig.10C showed that the mean noise floor of coated arrays decreased from 26.7±4.6μV to 18.8±3.6μV compared to uncoated arrays (p=0.00741, two sample t test, n=6, data pooled from all electrodes). The mean signal amplitude of all single unit changed slightly for the threshold 37 μV was set to detect spikes which was in agreement with previous research[4]. But the maximal peak to peak signal amplitude of each coated array was significantly higher (260.3±47.7μV versus 371.0±45.8μV, two sample t test, n=6, p=0.00173), the total active channels and total recorded single units of coated arrays are 52 and 77 respectively which are more than uncoated arrays with 26 active channels and 46 single units. Besides, the lowest signal amplitude of a discriminable single unit recorded by coated electrode is 50μV which was 13μV lower than the uncoated electrodes, and suggested that coated electrodes could capture spikes from neurons or axons that could not be detected by the uncoated electrodes.

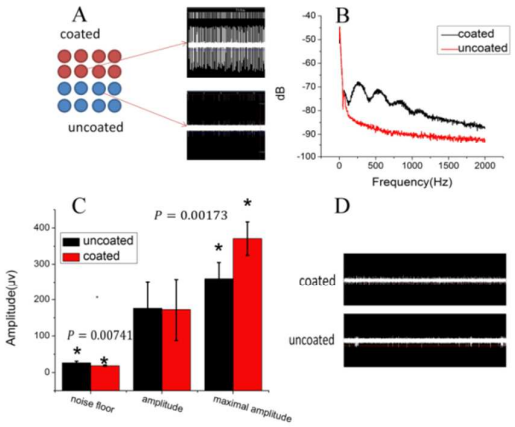

Fig.10. Acute neural recording experiment. (A) A schematic diagram of our home-made electrode(left) and best signals of coated and uncoated electrodes in an array (right); (B) Power spectrum calculated from 60 s of the two neural recording signal in A, respectively; (C) The noise amplitude, signal amplitude and maximal amplitude of coated and uncoated arrays; (D) The lowest signal amplitude of a discriminable single unit recorded by coated and uncoated electrodes.

3.9 Chronic neural recording

To investigate the chronic performance of the coated materials, we separately implanted PEDOT/PSS, PEDOT/PSS/MWCNT, PEDOT/PSS/MWCNT-polypeptide coated electrodes and uncoated electrodes into rat's V1 visual cortex. As shown in Fig.11, both PEDOT/PSS and PEDOT/PSS/MWCNT-polypeptide coated arrays exhibit excellent performances that active electrodes and recorded stable single units were obviously more than the other two kinds of electrode at all days. The results suggested that the neuron density around these two kinds of electrode was higher than the other two groups.

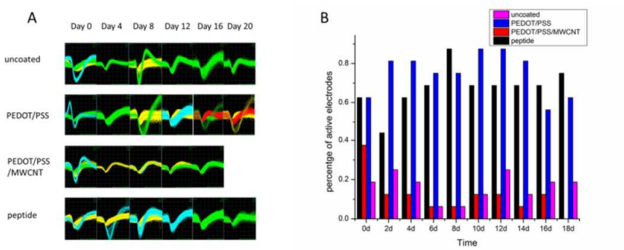

Fig.11. The change of signal waveforms of maximum amplitude with time. Representative signal waveforms of coated and uncoated arrays at different time (We could not get a stable single unit on PEDOT/PSS/MWCNT coated array at day 20) (A) and the change of percentage of active electrodes on arrays with time (B).

To further examine the biocompatibility of coating materials with polypeptide, we analysed the mean maximal amplitude of in vivo recorded chronic signals over time as Fig.12 and Fig.13 shows. The mean maximal amplitude is the mean value of maximal signal amplitude at every electrode in an array. The coating materials with polypeptide has an increasing mean maximal amplitude compared to the other three kinds of electrodes from day 2 to day 6, and the mean maximal amplitude of peptide modified electrodes is the highest among all the arrays during day 4 to day 12 when acute response impacted electrodes seriously, these phenomenon probably attribute to its better biocompatibility promoted cells creating a more intimate cell-electrode interface.

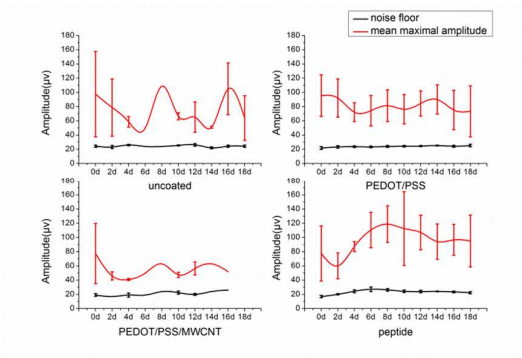

Fig.12. The change of the noise floor and mean maximal amplitude of in vivo recorded chronic signals over time.

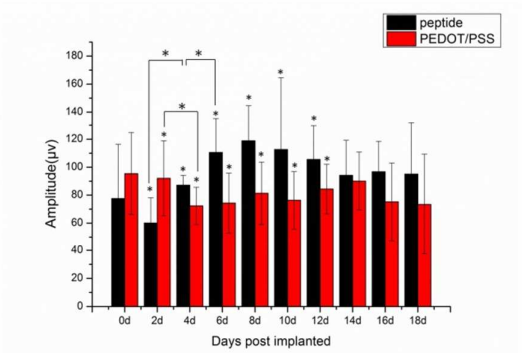

Fig.13. The comparison of mean maximal amplitude between polypeptide coating material and PEDOT/PSS coating material. Error bars indicate s.e.m.; * indicate two sample t test, unequal variance statistical significance, p<0.05.

It was reported that the change of in vivo impedance mainly ascribed to the variation of matrix surrounding the electrode. When the density of cells immediately adjacent to the electrode surface increased, the extracellular space decreased and thus cause the increase of in vivo impedance[26].

As shown in Fig.14, the PEDOT/PSS/MWCNT-polypeptide coated arrays having the biggest change of in vivo impedance during the first week compared to the four kinds of array, in consideration of its increasing active electrode number and mean maximal amplitude, that also suggest there were more neurons around the interface of the array. The PEDOT/PSS coated array had a good neural recording performance from the beginning, and the high in vivo impedance at day 0 also indicates that the cell density around the interface is high. But the active electrodes decreased from day 2 to day 8, and the mean max amplitude decreased from day 2 to day 4, which suggested the cell density decreased during the first week, and this result probably explained why the in vivo impedance of PEDOT/PSS electrodes changed a little during the first week. And for the four kinds of electrodes, the impedance curve reached its peak in about one week after implantation. Combining knowledge of electrode materials with histological analysis, a predictive model of electrode failure could be built in the future.

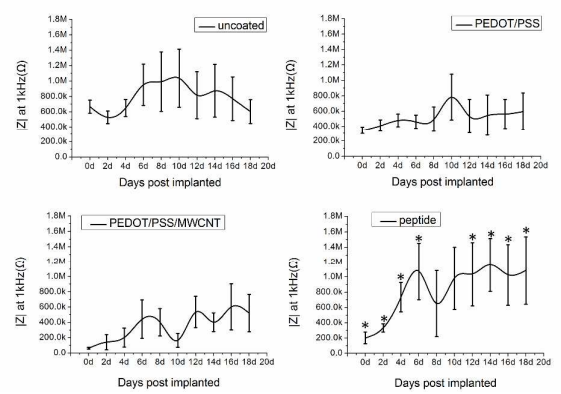

Fig.14. The change of impedance in vivo over time. Error bars indicate s.e.m.; * indicate two sample t test between polypeptide coating material and PEDOT/PSS coating material, unequal variance statistical significance, p<0.05.

4. Conclusion

In this work, bioactive PEDOT/PSS/MWCNT-polypeptide composite film was coated on home-made MWA by electrodeposition. The adding of MWCNT contributed to the raise of CSC and charge injection capability. Although the bonding of polypeptide slightly raised the in vitro impedance, it proved beneficial to the chronic performance and biocompatibility of neural interface in vivo. PEDOT/PSS/MWCNT-polypeptide coated MWA in the chronic recording from V1 area of rat's visual cortex exhibited improvements in multiple parameters, i.e. active electrode number, mean maximal amplitude and in vivo impedance. To our knowledge this is a first comprehensive report on the chronic effect of PEDOT/PSS/MWCNT-polypeptide composite neural interface in vivo. This chronic data is also valuable to the understanding of tissue response to the bioactive neural interface and helpful to predict the curve of electrode failure in vivo. In summary, PEDOT/PSS/MWCNT-polypeptide composite provided an excellent option to modify neural interface. This work demonstrated the great potential of using CPs/CNT architecture and covalent bonded bioactive molecules to improve neural interface.

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