Surface Modification of Neural Probes With Conducting Polymer Poly(hydroxymethylated-3,4-ethylenedioxythiophene) and Its Biocompatibility

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Abstract

A novel conducting polymer, poly(hydroxymethylated-3,4-ethylenedioxy-thiophene) (PEDOT-MeOH), was electrochemically deposited onto the electrodes of micromachined neural probes. Uniformly distributed film was obtained from aqueous solution when doped with polystyrenesulfonate. The surface morphology was rough and had good cellular adhesion. Impedance spectroscopy showed that the magnitude of coated electrode was lower than that of the bare gold over a range of frequencies from 10^0 to 10^5 Hz. Since the biocompatibility of the interface between the neural probes and brain tissue plays an important role when the probes are implanted in the central nervous system for long-term application, biomolecules were incorporated into the coating. Nonapeptide CDPGYIGSR was codeposited as the counterion in the conducting films. The surface morphology of the coating was fuzzy, providing many bioactive sites for interaction with neural cells. The magnitude of impedance was as low as 53 kΩ at the biologically relevant frequency of 1 kHz. An in vitro experiment demonstrated that the neuroblas-

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toma cells grew preferentially on the PEDOT-MeOH/CDPGYIGSR-coated electrode sites and spread beyond the electrode area.
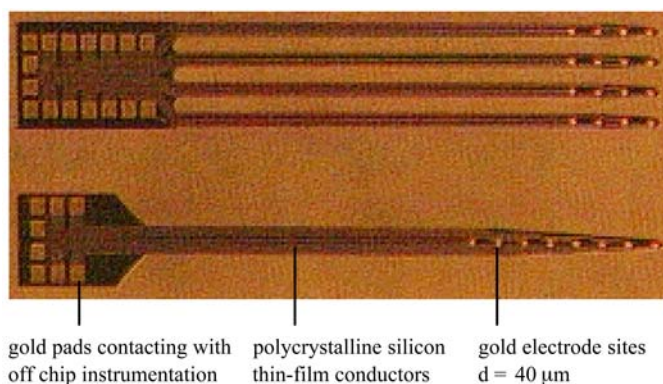
Index Entries: Conducting polymer; surface modification; micromachined neural probe; biocompatibility.

Introduction

Micromachined neural probes are multichannel electrode arrays. They have been fabricated with special techniques *(1,2)* to consist of micromachined silicon shanks that have gold or iridium sites on their surface (Scheme 1). These devices facilitate the functional stimulation and recording of impulses from the neurons in peripheral and central nervous systems *(3)*. However, when these devices are implanted into brain tissue, they need modification on the surface of the electrode sites in order to improve the signal transport at the electrode/tissue interface. Several aspects of the electrode/tissue interface need to be optimized for better performance. First, low impedance is important when an electrode serves in the transmission of stimulation pulses of neural signals. Second, the electrode sites are placed on the surface of the silicon-based probe and are smooth and flat or even concave as a result of the fabrication process. This apparently inhibits intimate contact between the electrode and tissue. A convex structure would be ideal for this purpose. Third, biocompatibility of the electrode material is critical for maintaining the functionality in long-term application.

Aqueous-compatible conducting polymers are polymers that are able to undergo electrochemical switching in aqueous media. Electron-rich conjugated polymers are especially useful for the formation of stable conducting polymer complexes that can be switched between their oxidized (doped) and charge-compensated (neutral) states rapidly over many redox cycles *(4)*. Of these polymers, polypyrrole (PPy) and poly(3,4-ethylene-dioxythiophene) (PEDOT) have been widely studied because of their ease of preparation, inherent high electrical conductivity, and controllable surface properties *(5–11)*.

PPy has been electrochemically deposited from aqueous solution onto the surface of the electrode sites with counterions of polystyrenesulfonate (PSS) *(12)* and biomolecules *(13)*, respectively. However, PPy has inherent weaknesses *(14–16)*: First, it has a poorly defined chemical structure in which not only is there α–α coupling, but also a significant amount of α–β coupling. The presence of these defects along the backbone induces structural disorder, limits the electrochemical response, and has been implicated as the primary site of polymer breakdown owing to overoxidation. Second, it is unstable to reduction by relatively weak but biologically relevant reducing agents such as dithiothreitol (DTT) and glutathione (GSH) *(4)*. This is obviously a disadvantage for chronic application in a living organism. Therefore, more stable conductive polymer matrices need to be developed to replace the relatively unstable PPy. PEDOT is a substituted derivative of polythiophene that carries a dioxyethylene bridging group

gold pads contacting with   polycrystalline silicon   gold electrode sites
off chip instrumentation   thin-film conductors   d = 40 μm

Scheme 1. Micromachined silicon-based neural probes. The top one is a four-shank probe and the bottom one is a one-shank probe. For this study, the probe was the bottom one and the electrode site area was 1250 μm^2.

across the 3- and 4-positions of the heterocycle that blocks the possibility of β–coupling and, consequently, has a structure in good order. It lacks the presence of undesired α–β coupling and β–β coupling often found in PPy within the polymer backbone. Oxygen attached to the β-position of the thiophene ring in PEDOT may support the delocalization of positive charges on the polymer backbone and consequently stabilize the system. Some scientists *(17)* compared the stability of PEDOT-coated film and PPy-coated film in water vapor and found that the surface resistance of PPy was increased from 1 kΩ to 10 MΩ, whereas there was virtually no influence on the resistance of the PEDOT-coated film. It was also reported that PEDOT was electroactive in aqueous solutions, exhibiting a stability superior to that of PPy, and that the combination of PEDOT/PSS resulted in a system with good film-forming properties and high conductivity *(18)*. However, one drawback is that the solubility of EDOT monomer in an aqueous solution is rather poor, and a concentration of 0.01 *M* is the limit for EDOT solubility in water. PEDOT doped with PSS could be electrochemically deposited onto electrodes with an anodic potential of 0.8 V *(19)*. However, deposition of PEDOT/biomolecules in water was unsuccessful. The working electrode potential was too high to get a stable film growth.

Capitalizing on the EDOT core, various research groups have synthesized a number of derivatives. It was found that the poor processibility (aqueous solubility) of EDOT can be improved by adding an appropriate pendant side group onto the backbone. Reynolds's research group synthesized EDOT with hydroxymethylate functional group (EDOT-MeOH) *(20)*; its chemical structure is shown in Scheme 2. EDOT is a colorless liquid, whereas EDOT-MeOH is a brown solid.

An advantage of the electrochemical synthesis of conducting polymers is that a number of other polyelectrolytes can be used as the macromolecular counterion. In the electrochemical deposition process, when a conducting polymer is in its oxidized state, it exists as a polycation with
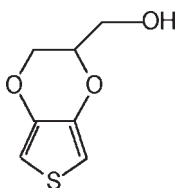
Scheme 2. Chemical structure of EDOT-MeOH.

delocalized positive charges along its conjugated backbone. Thus, counterions in solution are necessary in order to neutralize the positive charges. Polyelectrolyte having negative charges can be incorporated into the films via the following reaction:

$$nM + PE^- \xrightarrow{\text{Oxidized}} P^+ PE^-$$

in which *M* is the monomer, *P* is the corresponding polymer, and *PE* is the polyelectrolyte. Based on this mechanism, many negatively charged bioactive species have been patterned onto electrode sites together with conducting polymers *(21–25)*. These molecules would naturally be expected to influence the response of living tissues to which they would come in contact.

To improve the cellular adhesion of neural probes with brain tissue, biomolecules need to be incorporated into the conducting polymer. Nonapeptide CDPGYIGSR is a laminin fragment that has been proven to enhance cell attachment and migration and to promote neurite extension *(26,27)*.

In this paper, EDOT-MeOH was doped with PSS and nonapeptide CDPGYIGSR, respectively. The surface morphology, cellular adhesion of the coating surface, electrochemical properties, and biocompatibility of the coating will be studied and the mechanism will be discussed.

Materials and Methods

Chemicals

EDOT-MeOH monomer was provided by Prof. John R. Reynolds from the University of Florida. PSS was purchased from Aldrich. Nonapeptide CDPGYIGSR was synthesized by the peptide synthesis core of the University of Michigan. All other routine chemical reagents were obtained from Aldrich. Deionized water was used in the experiments.

Electrochemical Polymerization

PEDOT-MeOH/PSS and PEDOT-MeOH/CDPGYIGSR were deposited galvanostatically on the gold electrode sites of the neural probe, respectively. The diameter of each gold electrode was 40 μm. The current supply was a Gamry PC4/FAS1T Femtostat Potentiostat/Galvanostat/ZRA.

The current density was 0.5 mA/cm^2. The electrochemical apparatus was a 3-mL three-electrode cell. Individual electrodes, a platinum wire soldered at the bottom of the cell, and a saturated calomel electrode acted as the working electrode, the counterelectrode, and the reference electrode, respectively. The monomer solution was purged with N$_2$ for 5 min before electrochemical deposition.

Apparatus

A Philips Field Emission Gun XL30 scanning electron microscope (SEM) was used to study the surface morphology of the coating with a voltage of 5 kV and spot size of 3. A thin film of gold/palladium was sputtered onto the probe using a Hummer-600 sputtering system to help image the organic film.

Atomic force microscopy (AFM) images were obtained with a Digital Instruments (Santa Barbara, CA) Nanoscope IIIa in contact mode. A probe was glued onto a 13-mm steel puck and placed on the headstage of the atomic force microscope. Data were collected using a contact mode tip with a radius of 40 nm. The scan sizes were 10×10 µm^2.

A gold-plated silicon wafer (1×1 cm^2) and a similar wafer coated with PEDOT-MeOH/PSS conducting polymer were sterilized under ultraviolet (UV) light. The wafers were then attached to the bottom of a tissue culture dish (Becton-Dickinson) with 2% agarose. Under standard cell culture techniques, neuroblastoma cells (SHSY5Y) were washed with phosphate-buffered saline and suspended in Dulbecco's modified Eagle's medium (DMEM) (DMEM, 10% fetal bovine serum, 1% penicillin/streptomycin). From this preparation, 5 µL was micropipetted directly onto the silicon wafers, which were then incubated (37°C, 5% CO$_2$) for 30 min to allow initial cell attachment on the wafers. After incubation, a Petri dish was filled with 2 mL of DMEM and incubated for 4 d. Cell selection was based on its representative features related to isolation from other cells, and the cell's relative flatness. AFM images were then taken, starting with a small scan area (20×20 µm^2), and gradually progressing to larger images (40×40 µm^2).

The optical microscope used for imaging the conductive polymer coating was an Optiphot 2-POL (Nikon), with a Diagnostic Instruments SPOT RT digital camera. Images were acquired on an Apple MacIntosh G3 computer.

The instrument for measuring the impedance was a Gamry PC4/FAS1T Femtostat Potentiostat/Galvanostat/ZRA used in electrochemical deposition. The system was operated under computer control using a EIS software. A 0.1 M phosphate buffer solution (pH 7.0) was used as the electrolyte in a three-electrode cell. Impedance was measured at 1 kHz and over a range of 10^0–10^5 Hz.

In Vitro Experiment

For the in vitro experiment, before electrochemical deposition the probes were subjected to a base cleaning procedure. After deposition of
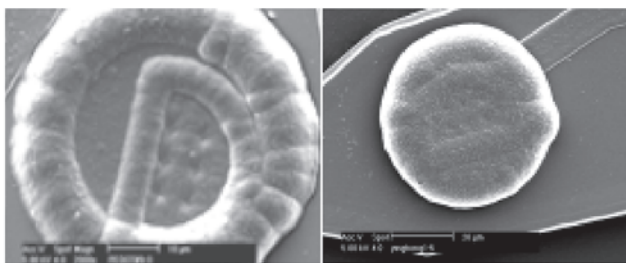
Fig. 1. Morphology comparison of (left) PEDOT/PSS and (right) PEDOT-MeOH/ PSS coatings. The two films were electrodeposited with the same current density and for the same time.

PEDOT-MeOH/CDPGYIGSR, the probes were rinsed with deionized water and then soaked in it for 48 h. Finally, the probes were separated from the PC-board and attached to Petri dishes with silicone elastomer glue (Nusil, MED4011). After curing, the Petri dishes were sterilized under UV light for 48 h. SHSY5Y cells suspended in DMEM were prepared with standard cell culture techniques. Approximately 1000 cells were placed by micropipet equidistant from the probes, and the dishes were incubated for 2 h. After cell adhesion was confirmed by inverted microscope, 2 mL of DMEM was added, and the dishes were incubated for 3 d. Micrographs were obtained by fixing the cells with paraformaldehyde. A Zeiss Axiophot-2 fluorescence microscope (Carl Zeiss, Thornwood, NY) was used to image cells on the neural probe. The images were obtained using an Optronics RGB-cooled digital camera.

Results and Discussion

EDOT-MeOH was mixed with 0.1 M PSS in water and electrochemical polymerization was performed. A thin film of PEDOT-MeOH/PSS could be easily obtained on the gold electrode sites while the concentration of EDOT-MeOH was only 0.02 M. The SEM image in Fig. 1A shows that an adherent conducting polymer coating was grown on the gold electrode site. A significant phenomenon is that it did not present the characteristics of the growth of PEDOT/PSS film shown in Fig. 1A: maximum growth of the film occurred at the edge of the electrode. This indicated that in the electrodeposition of EDOT-MeOH/PSS there was even current distribution on the whole electrode site. By electrodeposition of the EDOT-MeOH/ PSS system, more uniform coating can be prepared. This will benefit signal transportation over the whole electrode.

To characterize the surface topography, AFM was performed on a PEDOT-MeOH/PSS-coated electrode site. From Fig. 2 it can be seen that the surface of the PEDOT-MeOH/PSS film appeared to consist of round globular particles and became very rough. The roughness largely increased the surface area of the electrode and would evidently provide stronger adhesion to brain tissue. This conclusion is demonstrated by an AFM test
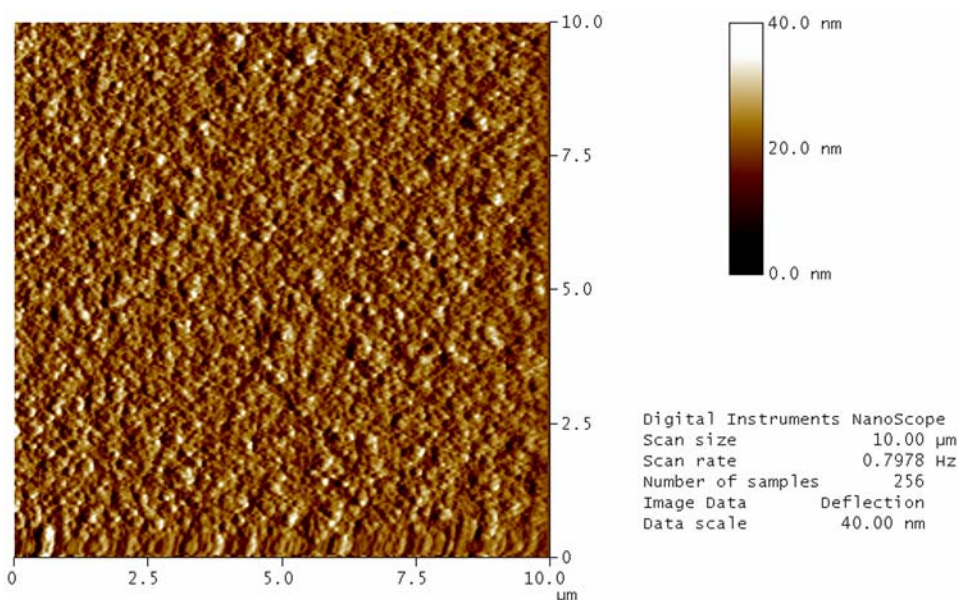
Fig. 2. AFM image of PEDOT-MeOH/PSS coating.

as well. Two cells were cultured on two wafers, respectively. One was on the bare gold wafer, and the other was on the PEDOT-MeOH/PSS-coated wafer. Qualitatively, the AFM image was easier to obtain on the PEDOT-MeOH/PSS-coated wafer, rather than uncoated gold. This is most likely the result of cell preference for a rougher textured surface. Figure 3 shows representative deflection images of cell on gold (Fig. 3A) and on PEDOT-MeOH/PSS coating (Fig. 3B). The cell on the surface of the PEDOT-MeOH/PSS coating appeared to be very flat, indicating that it adhered well to the coated surface. In general, cells on polymer coating revealed a complex texture, probably representing the actin cortex of the cytoskeleton. However, cells on the gold surface lacked these internal structures and, instead, demonstrated a smoother topography. The cell on the gold surface appeared to be bulgy, which indicated that it did not have good compatibility with the bare gold substrate. The presence of prominent cytoskeletal features could relate to the larger surface area of adhesion, flattening the cell, and allowing features to protrude under the film.

The contact mode tip was dragged with the same force on the two cells, respectively. A thin edge of the cell on the bare gold wafer indicated that the cell moved slightly on the gold surface with the force, whereas the cell on the PEDOT-MeOH/PSS-coated surface did not move. The rough surface anchored the cell tightly on the substrate.

Impedance spectroscopy (IS) is an attractive method to study the electrical behavior of coated and uncoated neural prosthetic devices. It involves measuring the electrode impedance over a spectrum of frequencies. Not only can it determine the magnitude of the resistive and
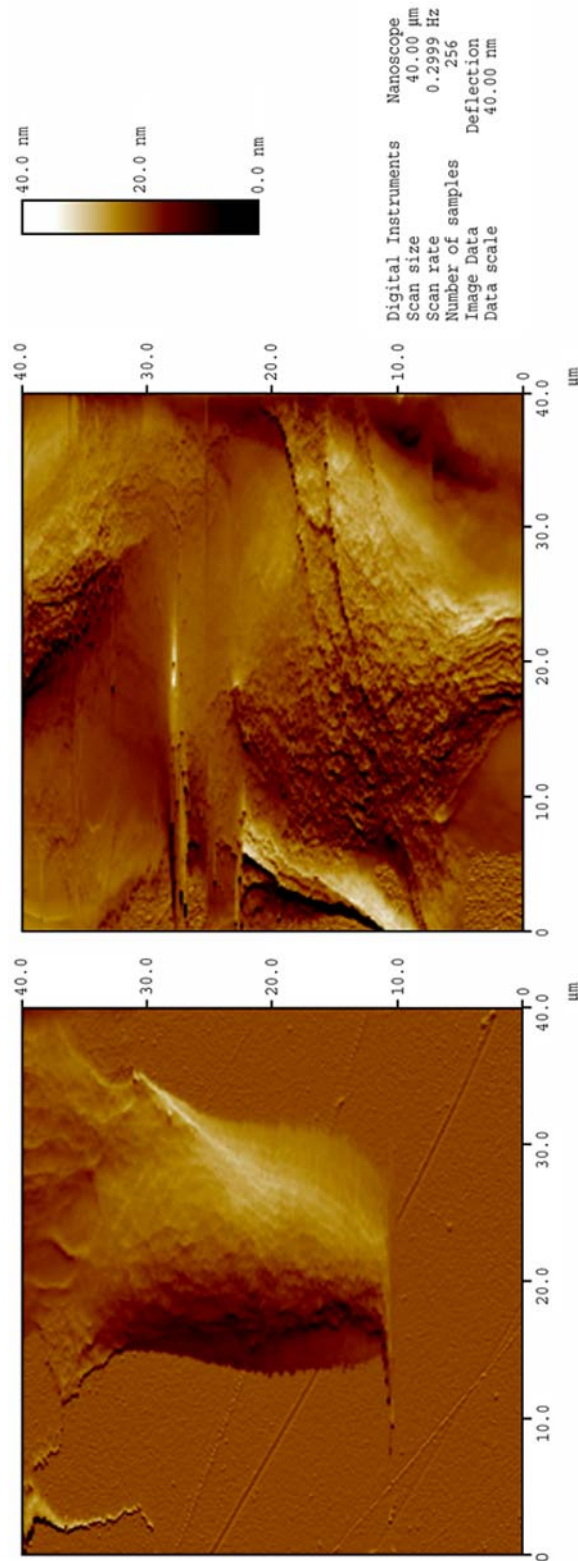
Fig. 3. AFM images of cells cultured on (left) bare gold wafer and (right) PEDOT-MeOH/PSS-coated wafer.
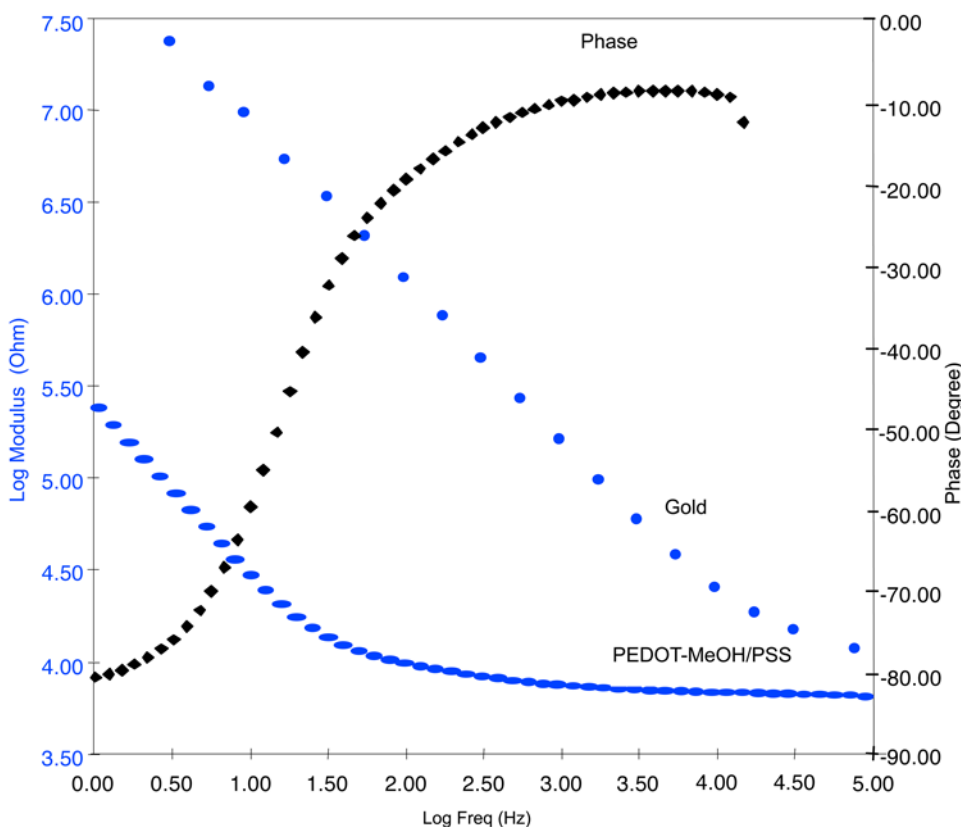
Fig. 4. IS of PEDOT-MeOH/PSS-coated electrode.

capacitive response, but it can also examine their performance over a wide range of frequencies. In our study, the impedance was measured over a range of frequencies from 10^0 to 10^5 Hz. Magnitude and phase information, which characterizes the impedance, was measured directly. By using these data, one can obtain qualitative and quantitative information about the electrical properties of the coated and uncoated electrodes. One kilohertz is the frequency characteristic of neural biologic activity; therefore, impedance at this point is frequently used as a standard to evaluate a neural electrode *(28)*.

Figure 4 presents the results of the IS of the PEDOT-MeOH/PSS-coated electrode. The magnitude of the bare gold electrode over this range of frequencies was put in the same spectrum as the control. It could be seen that the magnitude of PEDOT-MeOH/PSS film decreased not only in the lower frequency range from 10^4 to 10^0 Hz, but also in the higher range from 10^4 to 10^5 Hz. The magnitude of the PEDOT-MeOH/PSS-coated electrode at 1 kHz was only 10 kΩ, where the deposition charge was 28 μC. It was almost two orders lower than that of the bare gold electrode (600 kΩ). From the phase plot, one can see that the phase angle for the PEDOT-MeOH/PSS-coated electrode at 1 kHz was 10°, whereas the phase angle of bare gold was
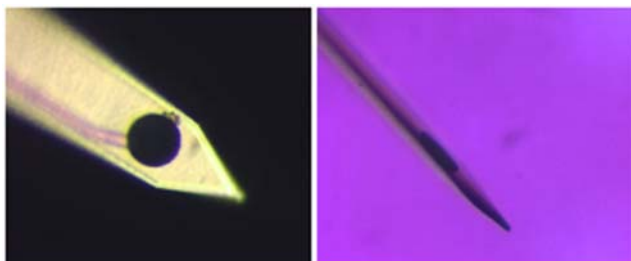
Fig. 5. Optical images of PEDOT-MeOH/peptide-coated electrode showing (left) top view and (right) side view from which convexity of coating can be observed.
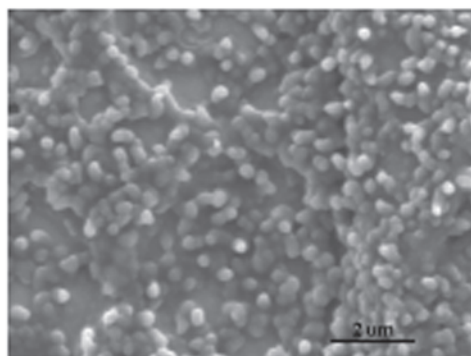

Fig. 6. SEM image of PEDOT-MeOH/peptide coated on electrode site of neural probe.

about 80°. This indicates that the coated electrode was more resistive and the uncoated was close to pure capacitor. The conducting polymer facilitated the neural signal transport from the ionically conductive brain tissue to the electronically conductive metal electrodes.

To fulfill our goal of establishing a stable and intimate connection between neurons and the electrode in vivo, nonapeptide CDPGYIGSR was incorporated into the conducting polymer onto the electrode site to enhance neuron-binding ability to the electrodes. One drawback of the EDOT/peptide system is that with the poor solubility of EDOT in water attempts to electrochemically deposit EDOT/peptide in water was unsuccessful. Water was preferred as the electrochemical polymerization medium because organic solvent is toxic to living organisms. On the other hand, biomolecules can keep their bioactivity in aqueous medium. EDOT-MeOH has better water solubility than EDOT with a hydroxymethylate functional group along its backbone. It becomes a promising monomer to prepare coatings in water with biomolecules incorporated. Nevertheless, although PEDOT-MeOH/PSS coatings could easily be obtained with a concentration of 0.02 M EDOT-MeOH, the same conditions could not be applied to EDOT-MeOH/peptide. No films were grown at this concentration.
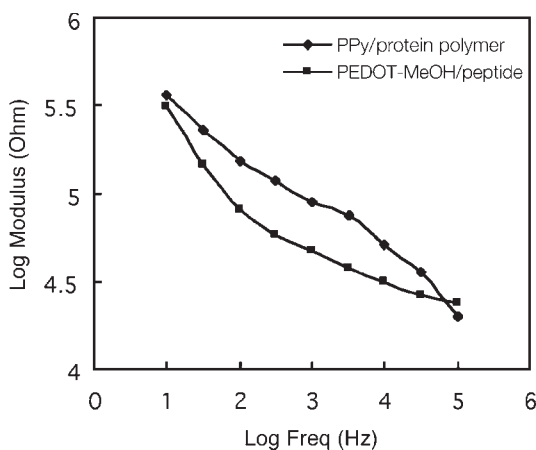
Fig. 7. IS of PPy/protein polymer and PEDOT-MeOH/peptide.

Fig. 8. Two neural probes cultured with human neuroblastoma cells: (left) uncoated; (right) PEDOT-MeOH/CDPGYIGSR coated.

EDOT-MeOH at a concentration of 0.1 M was mixed with 6 mg/mL of CDPGYIGSR. Electrodeposition was performed at a current density of 0.5 mA/cm². In the process, the working electrode potential was 0.8–1.0 V and kept stable. This presented an obvious comparison with the EDOT/peptide system. The potential for the latter was >1.7 V *(19)*, which could cause overoxidation of the monomer easily. Figure 5 provides optical microscopic images of the coated electrode site. As shown, a stable and uniform coating that is blue black was obtained. The area of a site can be increased beyond the underlying gold site (Fig. 5A). Another advantage is that the site becomes convex in side view, which puts the side in more intimate contact with the surrounding tissue. This convexity may be especially important for chronic application.

Figure 6 is an SEM image of a PEDOT-MeOH/CDPGYIGSR-coated electrode. The coating had a rough and fuzzy surface morphology. The fuzzy morphology will largely increase the real interfacial area between the electrode site and the biologic environment and provide more bioactive sites to the neurons in the brain tissue.

Impedance is an important factor when an electrode serves in the transmission of stimulation pulses of neural signals. At 1 kHz, the lowest

magnitude of the PEDOT-MeOH/CDPGYIGSR-coated electrode was 53 kΩ and the phase angle was 16°. The coating also established a bridge between the ionically conductive brain tissue and the electronically conductive metal electrodes.

Figure 7 provides an IS comparison of PEDOT-MeOH/peptide and PPy/protein. The magnitude of PEDOT-MeOH/peptide coating was lower than that of PPy/protein film over a wide range of frequency. It indicated that the former conducting polymer film had a better electrical property than the latter one.

To investigate the response of cells to the coated and bare gold electrode sites, neural probes with PEDOT-MeOH/CDPGYIGSR-coated sites and uncoated probes were cultured with human neuroblastoma cells; in vitro results are presented in Fig. 8. The neuroblastoma cells selectively attached on the coated sites (Fig. 8B), whereas the bare gold electrode showed no attraction to cells (Fig. 8A). The cells showed a preference to grow on the biospecies-containing electrode and spread beyond the electrode site. Thus, the cells between two sites linked together and formed a network. Statistical results of the influence of the surface modification on the morphology and activity of the cells will be investigated in the future.

With the technology of electrochemical deposition of conducting polymer/bioactive molecule blends, biospecies with different functionality can be patterned onto the surface of the electrodes. The biospecies in the coating can improve the biocompatibility of the neural probes with the brain tissue. Thus, cells are selectively grown on the coated electrode sites. This would evidently help improve the communication between these cells and the recording or stimulating system.

Conclusion

Conducting polymer PEDOT-MeOH, of which the monomer EDOT-MeOH has better water solubility than the parent EDOT, was electrochemically deposited onto the electrode sites of neural probes. The PEDOT-MeOH/PSS film had a rough surface morphology and improved cellular adhesion greatly. The impedance magnitude of the PEDOT-MeOH/PSS-coated electrode was lower than that of the uncoated one over a wide range of frequencies from 10^0 to 10^5 Hz. The magnitude was 10 kΩ at a biologic relevant frequency of 1 kHz. Moreover, a convex and fuzzy morphology of a coating containing bioactive molecules was obtained, which seemed to be better than a flat surface for establishing an intimate interfacial contact with brain tissue. At 1 kHz the magnitude was as low as 53 kΩ. The electrical property of PEDOT-MeOH/CDPGYIGSR was better than that of PPy/biomolecules previously studied. Preferred cell attachment was found in an in vitro experiment. The cells were selectively seeded onto the biospecies-containing electrode whereas no cells grew on the bare gold sites. Future work will involve in vivo experiments to test the ability of the electrodes to record electrical signals from brain tissue.

Acknowledgments

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