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Ultrathin Cell Membrane-Mimic Phosphorylcholine Polymer Film Coating Enables Large Improvement for In Vivo Electrochemical Detection

Xiaomeng Liu,[b] Tongfang Xiao,[a] Fei Wu,[a] Mo-Yuan Shen,[c] Meining Zhang,[b]* Hsiao-hua Yu,[c]* and Lanqun Mao[a]*

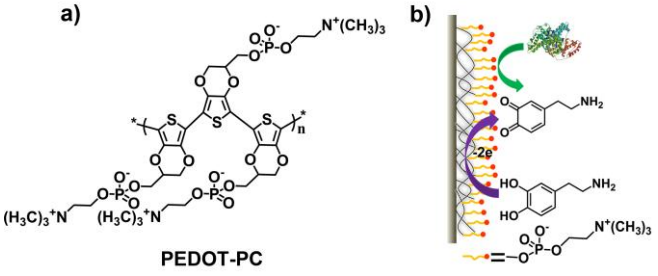
Abstract: Resisting biomolecules adsorption onto the surface of the brain-implanted microelectrode is a key issue for in vivo monitoring of neurochemicals. Here, we demonstrate for the first time an ultrathin cell membrane-mimic film of ethylenedioxythiophene tailored with zwitterionic phosphorylcholine (EDOT-PC) electropolymerized onto the surface of carbon fiber microelectrode (CFE) not only resists protein adsorption but also maintains the sensitivity and time response for in vivo monitoring of dopamine (DA). As a consequence, the as-prepared PEDOT-PC/CFEs could be used as a new reliable platform for tracking DA in vivo and would help understand the physiological and pathological functions of DA.

In vivo electrochemistry has been of great concern in both chemistry and neuroscience communities due to its capability to track the dynamics of neurochemicals with a high spatiotemporal resolution.[1] For in vivo electrochemical detection, a carbon fiber microelectrode (CFE) is generally implanted into the brain of animals and the electrode inevitably suffers from nonspecific adsorption of biomacromolecules (i.e., biofouling), proteins in particular, onto the surface.[2] Furthermore, biofouling often triggers foreign body responses, leading to the formation of a foreign body capsule surrounding the implanted electrode and thus isolating it from the tissue.[3] This hinders or completely prevents analyte from reaching the electrode and hence inactivates the implanted microsensor, leading to decreased sensitivity and prolonged response time for in vivo measurements.[4] To solve this problem, coating anti-biofouling films such as Nafion, base-hydrolyzed cellulose acetate (BCA), fibronectin, and polyethylenedioxythiophene (PEDOT)/Nafion, onto the microelectrode surface has proven to be one of the most effective strategies.[2c,5] Generally speaking, the anti-biofouling film should be biocompatible and easily coated onto the surface of the microelectrode. More importantly, it should not compromise the sensitivity of the microelectrode or the temporal resolution for in vivo measurements.

Towards the goal of minimizing the protein adsorption on the brain-implanted microelectrodes, we previously found that pre-treatment of CFEs with bovine serum albumin (BSA) can minimize further adsorption of proteins when the electrodes are implanted into the rat brain; however, this also causes significant sensitivity drop of CFEs to neurochemicals, including DA.[4b]

Herein, we report an antifouling film for in vivo tracking of neurochemical dynamics without leveling down the electrode performance by electropolymerizing ethylenedioxythiophene (EDOT) functionalized with cell membrane-mimic zwitterionic phosphocholine (PEDOT-PC) to form an ultrathin cell membrane-like film onto CFEs. Among all zwitterionic structural units, PC is one of the most ideal candidates resisting nonspecific protein adsorption, bacterial adhesion, and biofilm formation,[6] although some PC-polymers such as self-assembled long-chain aliphatic hydrocarbons-PC can passionate electrodes.[7] To solve this problem and confine PC-polymer onto the microelectrodes in a controllable manner, we choose the PC with a short 5-carbon chain, graft the short-chain PC onto EDOT to yield EDOT-PC, and form an ultrathin cell membrane-mimic film (i.e., PEDOT-PC) onto microelectrodes through an electropolymerization process, as shown in Scheme 1. As we found previously, PEDOT-PC film not only displays high resistance towards nonspecific binding of various proteins and nerve cells, but also exhibits excellent biocompatibility and stability in aqueous media.[8]

Another important reason for our choice of EDOT-PC is that EDOT is easily electropolymerized onto CFEs to form a well-confined polymer featuring fast electron transfer. Grafting PC onto EDOT leads to the formation of an insulating layer with EDOT-PC monomer through a self-controlled electropolymerization process. At the microelectrode modified with PEDOT-PC, neurochemicals like DA quickly penetrate the PC layer and efficiently exchange electrons at the PEDOT layer, avoiding the decrease in both sensitivity and response time. As



Scheme 1 (a) The structure of PEDOT-PC and (b) the schematic of the interface between PEDOT-PC/CFE and solution.

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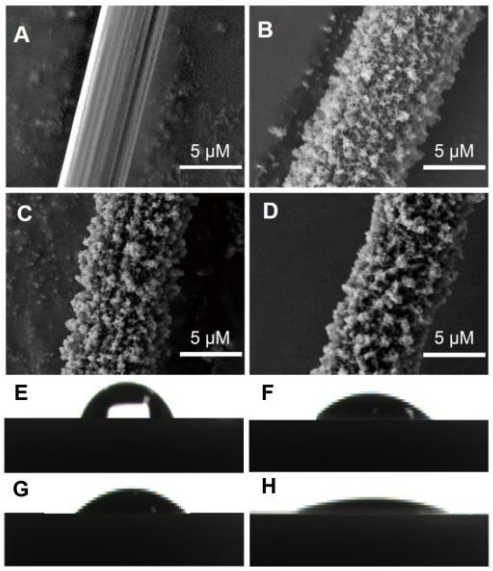

Figure 1. Scanning electron microscopy (SEM) images of (A) bare CFE, (B) PEDOT/CFE, (C) PEDOT-OH/CFE, and (D) PEDOT-PC/CFE. The contact angles of (E) GC, (F) PEDOT/GC, (G) PEDOT-OH/GC and (H) PEDOT-PC/GC.

a consequence, the ultrathin cell membrane-like PEDOT-PC film is expected to greatly improve the sensing performance of the electrodes for in vivo electrochemical detection.

Compared to EDOT and EDOT-OH monomers, EDOT-PC is difficult to be electrochemically polymerized onto bare CFEs due to the steric hindrance and hydrophilic nature of the big tail of PC group. To solve this problem, EDOT-OH was firstly electropolymerized onto the surface of CFE to form PEDOT-OH/CFE, on which EDOT-PC was then electropolymerized to form PEDOT-PC/EDOT-OH/CFE (denoted as PEDOT-PC/CFE hereafter). As shown in Figure S1, electropolymerization of EDOT-PC at the surface of PEDOT-OH was a self-controlled process (Figure S1C), which was different from those of EDOT (Figure S1A) and EDOT-OH (Figure S1B). For instance, the electropolymerizing current of EDOT-PC was much smaller (ca. 30 nA at 1.3 V) than those of EDOT (i.e., 0.8 μA at 1.3 V) and EDOT-OH (i.e., 0.3 μA at 1.3 V), and the current decreased with continuous potential cycling and almost reached the background level at the 5th cycle. Atomic force microscopy (AFM) images show that the further formation of PEDOT-PC onto the PEDOT-OH did not significantly increase the film thickness (Figure S2), suggesting the formation of an ultrathin film with PEDOT-PC on the surface of PEDOT-OH. To avoid big charging current resulted from the thick conducting PEDOT-OH film, EDOT or EDOT-OH was later electropolymerized for only one cycle onto CFEs. SEM images of PEDOT/CFE (Figure 1B), PEDOT-OH/CFE (Figure 1C), and PEDOT-PC/CFE (Figure 1D) demonstrate a full coverage of CFEs by PEDOT, PEDOT-OH or PEDOT-PC film. Contact angle measurements show that the surface of PEDOT-PC was more hydrophilic (i.e., 17°, Figure 1H) than those of PEDOT (i.e., 43°, Figure 1F), PEDOT-OH (i.e. 32°, Figure 1G), and bare glassy carbon (GC) substrate (i.e., 75°, Figure 1E), which might improve its resistance to protein adsorption.[9] Moreover, the short 5-carbon chains of PC units and the good conductivity of EDOT units well facilitate both the mass transport through the PEDOT-PC ultrathin film and the electron transfer between neurochemicals and CFE, as illustrated below.

In order to investigate the antifouling ability of the PEDOT-PC/CFEs against proteins, the electrodes were immersed into a solution of fluorescein isothiocyanate (FITC)-labeled BSA (40 mg mL^{-1}) for 2 h before water rinsing. For comparison, bare CFE and PEDOT-OH/CFE were also treated with FITC-BSA under the same condition. As shown in Figure 2, the amounts of BSA adsorbed on surfaces of PEDOT/CFE (B and B') and PEDOT-OH/CFE (C and C') were much less than that of the bare CFE (A and A'). Increased surface hydrophilicity appeared to disfavor nonspecific protein adsorption, which was further proved by the absence of FITC-BSA on the surface of the PEDOT-PC/CFE. The strong anti-fouling property of PEDOT-PC/CFE can thus be ascribed to the highly hydrophilic surface and cell membrane-mimic structure of the PEDOT-PC film.

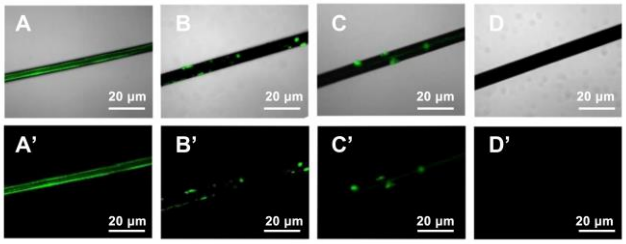

Figure 2. Confocal fluorescent images of FITC-BSA treated electrodes. (A, A') bare CFE, (B, B') PEDOT/CFE, (C, C') PEDOT-OH/CFE, (D, D') PEDOT-PC/CFE in merged (upper) and dark (lower) fields.

We next investigated electrochemical activity of the PEDOT-PC/CFEs toward DA. As displayed in Figure 3A, at the PEDOT-OH/CFE, the charging current (i.e., 50 nA, red curve) increased to almost 25 times of that of bare CFE (i.e., 2 nA, black curve), due to improved conductivity by the PEDOT-OH film. The same phenomenon was also observed at the PEDOT/CFE (Figure S3) When the PEDOT-PC film was electropolymerized onto the surface of PEDOT-OH/CFE, the charging current of the electrode was dramatically decreased to 2.2 nA because of the insulating nature of the PC layer. Nevertheless, the faradic current response of DA at the PEDOT-PC/CFE (Figure 3B, red curve) remained almost the same as that at bare CFE (Figure 3B, black curve). In addition, cyclic voltammetric (CV) results revealed that the $|E_{3/4}\text{-}E_{1/4}|$ values at the PEDOT-PC/CFE (38 mV, Figure 3B) and bare CFE (39 mV, Figure 3B) were almost unchanged at different scan rates up to 200 mV s^{-1} (Figure S4), which was indicative of similar interfacial electron transfer rates of DA at both electrodes. These results demonstrate that the coating of PEDOT-PC membrane does not affect the electrode sensitivity toward DA. This is a step forward from electrode coatings by conventional antifouling membranes or BSA compromising current responses of DA.[4b,7a] Furthermore, the current recorded at the PEDOT-PC/CFE was much stable as compared to that at bare CFE (Figure 3C and Figure S5),

suggesting that PEDOT did not adsorb the product of DA oxidation, which was frequently observed at carbon electrodes.[10]

To further study the antifouling property of PEDOT-PC/CFE against proteins, we performed in vitro electrochemical studies with BSA, a model protein commonly used in antifouling researches.[2c,4b,5c] As a starting point, we continuously recorded the amperometric response of the PEDOT-PC/CFE toward DA followed by addition of BSA into solution. As shown in Figure 3D, the addition of BSA induced quick decreases in amperometric current responses recorded at bare CFE (ca. 60%, blank curve), PEDOT/CFE (ca. 52%, blue curve), and PEDOT-OH/CFE (ca. 40%, green curve). In contrast, at the PEDOT-PC/CFE, the current only decreased by ca. 8% upon the addition of BSA (red curve). This result reveals that the presence of protein did not remarkably influence the performance of the PEDOT-PC/CFE in DA sensing, because nonspecific protein adsorption was efficiently reduced as illustrated by the "clean" surface of the PEDOT-PC film out of a proteinaceous solution (Figure 2D and 2D'). This property, along with the good electrochemical activity for the oxidation of DA and biocompatibility of the PEDOT-PC film, well enables the PEDOT-PC/CFE for in vivo monitoring of DA with a high reliability.

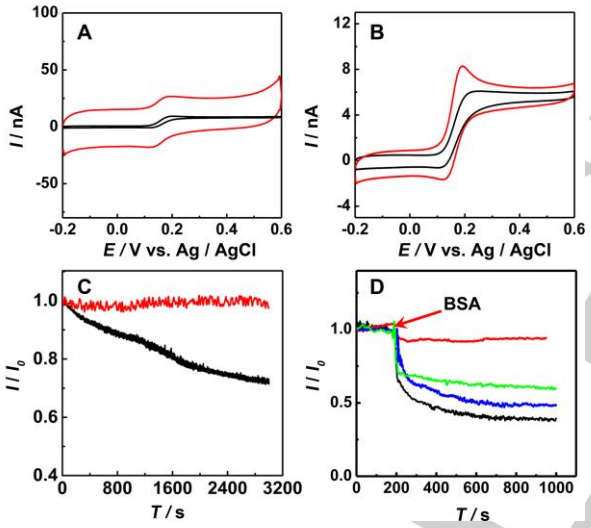

Figure 3. Typical cyclic voltammograms (CVs) obtained at (A) PEDOT-OH/CFE (red curve), (B) PEDOT-PC/CFE (red curve), and bare CFE (black curves in A and B) in aCSF containing 20 μM DA. Scan rate was 50 mV·s^{-1}. (C) Amperometric current response towards 20 μM DA recorded with CFE (black) and PEDOT-PC/CFE (red) at +0.20 V. I_0 and I were the current values at starting time and given time, respectively. (D) Amperometric current response towards 20 μM DA recorded with CFE (black), PEDOT/CFE (blue), PEDOT-OH/CFE (green), and PEDOT-PC/CFE (red) at +0.20 V upon the addition 10 mg mL^{-1} BSA as indicated in the figure. I_0 and I were current values at starting time and given time, respectively.

The antifouling property of the PEDOT-PC/CFEs was also evaluated in vivo. To do this, the electrodes were implanted into the live brains of rats for several hours (typically 2 h) before in vitro assessment of their amperometric responses toward DA. As observed previously, adsorption of proteins onto CFEs during in vivo implantation essentially led to significant sensitivity loss, which could be as high as 70%, because the analytes were hindered from reaching the electrode surfaces.[2c,4b,5c] This would definitely influence the accuracy of in vivo measurement. Interestingly, as seen in Figure 4A and Figure S6A, the current response toward DA was almost maintained at the PEDOT-PC/CFE after in vivo implantation of the electrode. The ratio of the sensitivity by post-calibration (S_{post}) to that by pre-calibration (S_{pre}) was calculated to be 0.92 ± 0.07 for the PEDOT-PC/CFE (Figure 4B), demonstrating a high resistance to nonspecific adsorption of proteins in the cerebrospinal fluid and greatly improved reliability for in vivo measurements. For comparison, the ratios were 0.43 ± 0.04 and 0.52 ± 0.05 for the PEDOT/CFE (Figure S6B, and S6D) and the PEDOT-OH/CFE and PEDOT-OH/CFE (Figure S6C and S6E), respectively, suggesting large decreases in sensitivities after in vivo implantation without the additional PC layer.

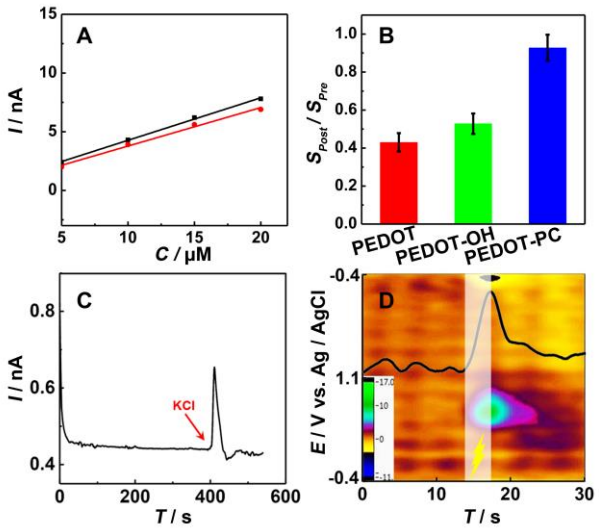

Figure 4. (A) Pre- and post-calibration curves obtained with the PEDOT-PC/CFE upon successive additions of DA (each addition, 5 μM) in aCSF before (black curve) and after (red curve) in vivo implantation of the electrode in the striatum of rat brain for 2 h. (B) The ratios of sensitivities by post-calibration of the PEDOT/CFE (red column), PEDOT-OH/CFE (green column) and PEDOT-PC /CFE (blue column) to that by pre-calibration in aCSF after in vivo electrode implantation for 2 h. (C) Amperometric response recorded with PEDOT-PC/CFE in the striatum by locally injecting KCl to evoke DA release. (D) In vivo FSCV recorded with the PEDOT-PC/CFE by stimulating medial forebrain bundle (MFB) (white shadow, 3 seconds at 60 Hz, ± 250 μA, 2 ms per phase). Current versus time trace (black line) was extracted from the colour plot at the peak oxidation potential (ca. +0.50 V) for DA. Scan rate, 400 V s^{-1}.

We compared the surface morphology of PEDOT/CFE, PEDOT-OH/CFE and PEDOT-PC/CFE before and after implantation into the brain tissue. After a 2 h implantation in the rat brain, the surfaces of nanostructured PEDOT (Figure S7A) and PEDOT-OH (Figure S7B) had large aggregates of biomacromolecules, the amount of which was obviously smaller on the surface of the PEDOT-PC/CFE (Figure S7C). Reduced accumulation of biomacromolecules was most likely due to lessened adsorption of proteins onto the electrode surface. It could thus explain much smaller decrease in the sensitivity of

the PEDOT-PC/CFE after in vivo operation (Figure 4B, blue column).

Although some coating films have been reported, our use of PEDOT-PC to coat CFEs for in vivo analysis is remarkable in terms of the large improvement in the analytical properties of the method. For example, Nafion, base-hydrolyzed cellulose acetate (BCA), fibronectin, and PEDOT/Nafion, and BSA only realized anti-fouling at some extent and compromise the sensitivity of bare electrodes in sensing monoamines (i.e., ca.70% for BSA, ca. 20% for fibronectin, and ca. 15% for BCA).[2c,4b] Although PEDOT/Nafion showed high anti-fouling property to proteins, the thickness of film should be carefully controlled to maintain desired sensitivity.[5a,5c]

To validate the PEDOT-PC/CFE for monitoring DA release in the brain, we used both amperometry and fast-scan cyclic voltammetry (FSCV) to track DA in vivo. Figure 4C shows the typical amperometric response obtained at the PEDOT-PC/CFE implanted in the rat striatum when the animal was stimulated by local injection of 70 mM KCl. Consistent with the previous reports,[11] KCl stimulation induced a rapid increase in the current due to the release of DA. We also used FSCV to monitor DA release with the PEDOT-PC/CFE in the rat NAc by bipolar stimulating in rat MFB (Figure 4D) and evaluated DA release according to pre-calibration (Figure S8). We can see the stimulated release of DA peak pattern and kinetics recorded by FSCV, which were almost the same as that reported previously.[12] Taken together, these results demonstrate that the PEDOT-PC/CFE could monitor the release of DA in vivo as normal CFEs without time lap,[12] but with less protein adsorption as described above. Moreover, the excellent properties of the PEDOT-PC could also be used to establish in vivo electrochemical methods for monitoring of other molecules such as oxygen with high reliability (Figure S9).

In summary, we have developed an in vivo electrochemical method with CFEs coated with a cell membrane-mimic film, PEDOT-PC. The PEDOT-PC coating not only efficiently prevents the protein adsorption but also well maintains the sensitivity and temporal resolution toward DA. These unique properties successfully endow the in vivo measurement of neurochemicals with high reliability. This strategy is believed to advance the further development of a new and facile platform for in vivo measurement of neurochemicals in the brain.

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Keywords: In vivo electrochemistry • Antifouling • Electropolymerization • Zwitterionic phosphocholine • Dopamine

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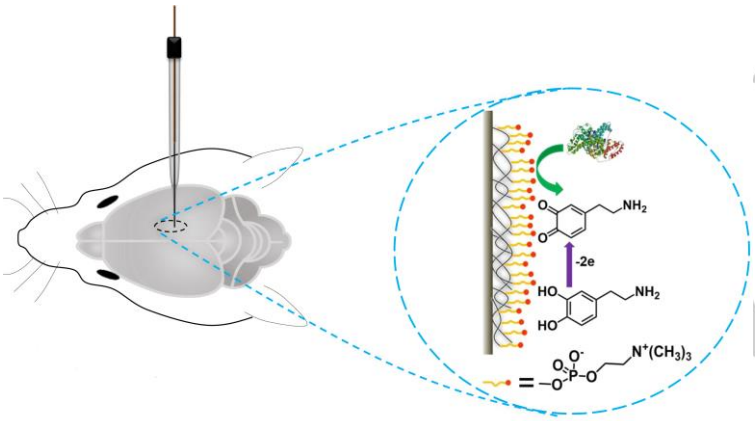

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Ultrathin cell membrane-mimic film of ethylenedioxythiophene tailored with zwitterionic phosphorylcholine electropolymerized onto the surface of carbon fiber microelectrode (CFE) was found for the first time not only to resist protein adsorption but also to maintain the sensitivity and temporal resolution for in vivo monitoring of dopamine (DA). The as-prepared PEDOT-PC/CFEs could be used as a new reliable platform for tracking DA in vivo and would help understand the physiological and pathological functions of DA.