Analytica Chimica Acta 1139 (2020) 155–163

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Glycyrrhiza polysaccharide doped the conducting polymer PEDOT hybrid-modified biosensors for the ultrasensitive detection of microRNA

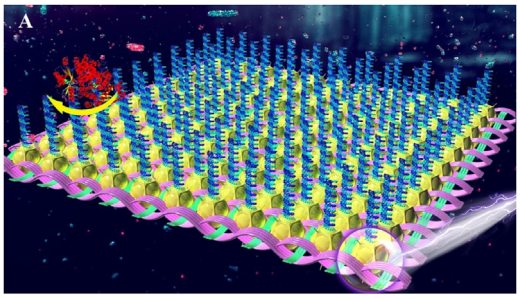
Hao Wang [a, 1], Haitao Lü [a, 1], Lili Yang [a], Zhiling Song [b], Ni Hui [a, *]

[a] College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao, PR China
[b] Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao, 266042, PR China

HIGHLIGHTS

- Conducting polymer PEDOT/GPS was prepared by a simple electrochemical way.
- The PEDOT/GPS demonstrated good conductivity and antifouling property.
- The low fouling, label-free biosensor based on PEDOT/GPS has been constructed for MicroRNA.
- The biosensor based on PEDOT/GPS/AuNPs has shown excellent sensing performances.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 August 2020
Received in revised form
19 September 2020
Accepted 29 September 2020
Available online 1 October 2020

Keywords:
Antifouling
Conducting polymer
PEDOT
Glycyrrhiza polysaccharide
Gold nanoparticles
microRNA biosensor

ABSTRACT

Biosensors have tremendous revolutionary potential for the precise location and rapid detection of biomarkers for human disease. However, the minimization of nonspecific protein adsorption interactions and surface contamination is critical for their application in complex media. We report herein, the antifouling interface was constructed by electrochemical copolymerization of poly (3,4-ethylenedioxythlophene) (PEDOT) and glycyrrhiza polysaccharide (GPS). Hydrophilic PEDOT/GPS can reduce the interference and nonspecific adsorption of biological protein macromolecules, which has been verified by electrochemical and fluorescent characterization. Gold nanoparticles (AuNPs) were subsequently modified onto PEDOT/GPS surface to attach biomacromolecules containing thiol groups. MicroRNA, the promising biomarker of a large numbers of genetic diseases, was used as the testing model. Due to the robust antifouling capability of PEDOT/GPS as well as high biocompatibility of GPS/AuNPs, the fabricated biosensor based on PEDOT/GPS/AuNPs demonstrated excellent sensing performance, such as a wide detection range (0.01 nM–10 nM), a low detection limit (300 fM) and high reproducibility, showing great potential of medical applications.

1. Introduction

In recent years, great progress has been made in the development of biosensors for human disease prevention detection, hormone quantitative analysis and drug serum level monitoring [1–3].

* Corresponding author.
E-mail address: huini1029@163.com (N. Hui).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.aca.2020.09.061
0003-2670/

However, due to the nonspecific adsorption of protein on the sensing surface and the accumulation of substrate fouling, the biosensor surface is gradually passivated, which seriously affects the performance of the biosensor [4]. In order to obtain the superior antifouling surfaces, enormous efforts have been made to excavate more antifouling materials. Polyethylene glycol (PEG) and oligo-ethylene glycol (OEG) have become the most widely used antifouling materials due to their surface hydrophilicity [5–9]. Zwitterionic polymers are considered to be more promising candidate materials for biomedical applications due to their easy functionalization and high biocompatibility [10,11]. However, PEG and OEG are easily oxidized in the presence of transition metal ions or oxygen in complex real media [12]. The synthesis of zwitterionic polymers is time-consuming and complex.

Polysaccharide (PS), as a promising alternative for antifouling material, has received much attention in biomedical fields such as biosensors, drug delivery and biological purification [13–15]. PS is an important biological macromolecule, which widely exists in animals, microbe and plants and plays an important physiological function [16]. PS is a kind of natural polymer linked by monosaccharide with glycosidic bond. The chemical composition of PS makes it highly hydrophilic and antifouling [17]. PS is non-conductive, and its application in the sensors can reduce the sensitivity. It is expected that the integration of PS with conducting polymers, such as poly (3,4-ethylenedloxythlophene) (PEDOT), may help to address this challenge.

Poly (3,4-ethylenedioxythiophene) (PEDOT) has the broad applications in electrochemical transistors, small bioelectrode coatings and various biosensors due to its characteristics (good conductivity, environmental stability, transparency and biocompatibility) [18–21]. In particular, PEDOT-based nanocomposites exhibit many excellent properties, such as large specific surface area, high mechanical properties, good biocompatibility and synergistic effect of functional groups [22–24]. PEDOT/PS composites combine the inherent biological activity, biocompatibility, biodegradability of PS with excellent electrical conductivity of conducting polymer [25,26]. For instance, dextran sulfonate, hyaluronic acid, chondroitin sulfate and heparin have been directly incorporated in PEDOT as the stabilizer and dopant [27,28]. PEDOT-dextran sulfate composite had no interference with L-929 cell growth in media and was absorbed into the PC12 cells, proving better biocompatibility [27]. PEDOT-hyaluronic acid composite was found to have good antifouling property. The biosensor based on PEDOT-hyaluronic acid was highly sensitive for the detection of carcinoembryonic antigen and capable of sensing in complex media without biofouling [28]. The antifouling interface of polysaccharides and conducting polymers has biocompatibility and excellent conductivity, so it has great application potential in biosensors.

The immobilization of bioprobes onto sensing interface can be achieved by gold nanoparticle linkages. Gold nanoparticles (AuNPs) are widely used in biosensors due to their good electrical conductivity, high catalytic properties, and good biocompatibility. AuNPs can immobilize biomolecules via gold-sulfur bonds [29] or gold-ammonia bonds [22,30]. The AuNPs-modified sensors have higher current response, which is conducive to the application of electrochemical biosensors. For instance, AuNPs/PEDOT/PEG supplied an ideal substrate for the attachment of alpha fetoprotein antibodies. The biosensor based on AuNPs/PEDOT/PEG has good antifouling performance and can detect alpha fetoprotein in 10% human serum samples, which has high clinical diagnostic potential [31].

In this work, we aim to combine the hydrophilic glycyrrhiza polysaccharide (GPS) with the conducting polymer PEDOT, thereby forming a new antifouling interface. The mechanism and process of the fabrication of the electrochemical sensor has been shown in Scheme 1. GPS with a negative charge can be used as a dopant to balance the positive charge of the PEDOT backbone. AuNPs were then bonded to PEDOT by gold-sulfur (provided by PEDOT) covalent bond (Scheme 1 B). The antifouling property of PEDOT/GPS surface was estimated through electrochemical impedance technique and confocal fluorescence experiment. The antifouling interface has demonstrated good electrochemical activity and antifouling performance, which provides an ideal substrate for the construction of new biosensors (Scheme 1 A). MicroRNA (miRNA) dysregulation is closely associated with a wide range of genetic diseases and can serve as promising biomarkers for many types of cancer [31]. MiRNA24 was used as the test model in this work. The combination of conductive PEDOT/GPS/AuNPs with DNA/RNA specific binding reaction was envisaged to support both antifouling property and high sensitivity for the electrochemical miRNA biosensor.

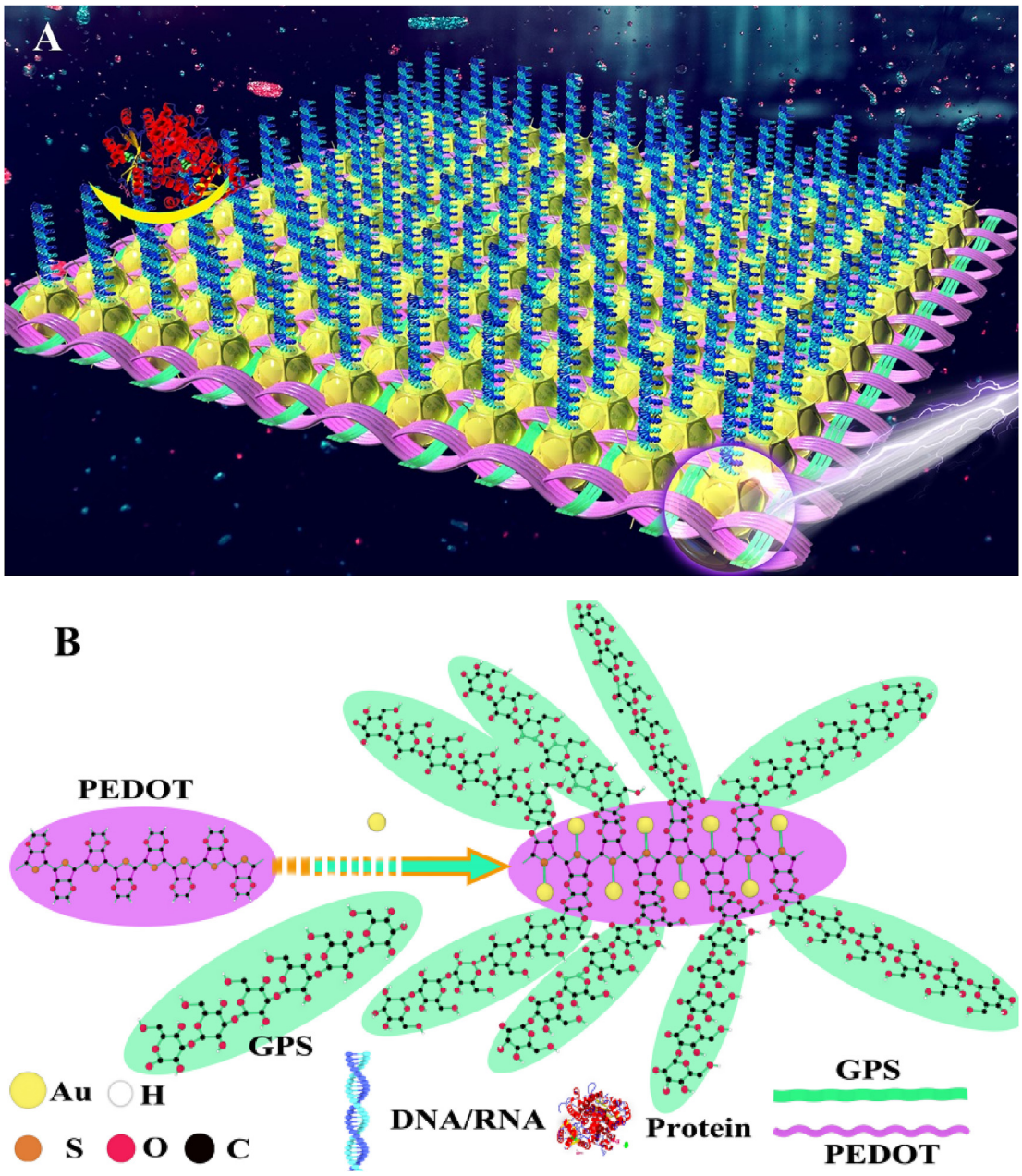
2. Experimental

2.1. Reagents

EDOT, K_3 [$Fe(CN_6]$, K_4 [$Fe(CN)_6]\cdot 3H_2O$, KCl, NaH_2PO_4, Na_2H-$PO_4\cdot 12H_2O$ and other chemicals were all purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Hemoglobin from bovine blood (Hb), bovine serum albumin (BSA), albumin human serum (HSA), lysozyme (LYS) and diethyl pyrocarbonate (DEPC)-treated water were purchased from Beijing Solibao Technology Co. Ltd. (Beijing, China). The base sequences of the DNA capture probe and miRNAs are as follows: DNA probe 5′—HS—C TGT TCC TGC TGA ACT GAG CCA-3′; miRNA24 (T) 5′-UGG CUC AGU UCA GCA GGA ACA G-3′; miRNA21 (M1) 5′-UAG CUU AUC AGA CUG AUG UUG A -3′; three-based mismatch miRNA24 (M2) 5′-UGG CUG AGU UGA GGA GGA ACA G-3′; single-based mismatch miRNA24 (M3) 5′-UGG CUC AGU UGA GCA GGA ACA G-3′. The extraction method of glycyrrhiza polysaccharide (GPS) and the preparation method of low-molecular-weight glycyrrhiza polysaccharide (LGPS) are shown in the supporting materials. In this experiment, DEPC water was used to dilute miRNA. PBS (0.2 M, pH 7.4) was used to dilute the protein. Other solutions used for experiments were prepared with pure water produced from a water purifying system (Milli-Q water system).

2.2. Instruments

The electrochemical impedance spectra (EIS) and cyclic voltammetry (CV) experiments were performed on a CHI660E electrochemical analyzer (Shanghai Chenhua Co. Ltd., China). EIS tests were carried out in phosphate buffered saline (PBS, 0.2 M, pH 7.4) containing 5.0 mM redox probe $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. EIS conditions were set as follows: frequency range from 1 to 100 000 Hz; the amplitude of the applied sine of 5 mV; the current potential of 0.20 V. CV conditions were set as follows: scanning range from −0.2 to 1.2 V; and scan rate of 0.10 V s^{-1}. The three-electrode system consisted of a modified glassy carbon electrode (GCE 3.0 mm diameter) as the working electrode, a saturated calomel electrode as the reference electrode (SCE, saturated KCl) and a counter electrode (Pt wire, 5 mm length, 1 mm diameter). The nanostructures and morphologies of the PEDOT/GPS and PEDOT/GPS/AuNPs nanocomposites were characterized by scanning electron microscopy (SEM) using JEOL JSM-7500F of Hitachi Corporation. Thermo-VG Scientific ESCALAB 250 spectrometer (Al monochromatic source, a voltage of 15 kV) was used to perform X-Ray photoelectron spectroscopy (XPS) analysis. TCS SP5 confocal laser microscope (Leica, Germany) was used to record the fluorescence images. DSA25 goniometer (KRÜSS GmbH, Germany) was used to carry out static water contact angle tests.



Scheme 1. (A) Demonstration of antifouling property of the constructed GCE/PEDOT/GPS/AuNPs/DNA/RNA. (B) The formation mechanism of PEDOT/GPS/AuNPs nanocomposites.

2.3. Synthesis of PEDOT/GPS interface

Before use, GCEs were polished to a mirror using alumina (0.3 and 0.05 μm) slurries. After polishing, it was rinsed with doubly distilled water, and then ultrasonicated in ethanol and pure water for 3 min successively. The PEDOT/GPS was electrodeposited onto the GCE using a polymerization solution of 5 ml (containing 2.0 mg l^{-1} GPS and 0.02 M EDOT monomer) by CV for 30 cycles. The scanning potential was set from −0.2 to 1.5 V at a scan rate of 100 mV s^{-1}. Meanwhile, the electrodeposition of AuNPs onto GPS/PEDOT nanocomposite was also performed using CV for 8 cycles in a solution containing 1.0 mM $HAuCl_4$. The electrodeposition potential was set from −1.5 to 0.5 V with a scan rate of 100 mV s^{-1}.

2.4. Characterization of antifouling ability

To evaluate the antifouling properties of the PEDOT/GPS interface, different protein samples were used to test the nonspecific adsorption by EIS technique, including single protein solution (1.0 mg ml^{-1}) and real biological samples (porcine serum). At the same time, fluorescence imaging technology was used to show the antifouling property of the interface more intuitively. All interfaces were immersed in 0.1 mg ml^{-1} FITC-BSA solution for 0.5 h at room temperature and washed three times in turn with PBS and pure water. Finally, the images of the three interfaces were recorded under a confocal fluorescence microscope.

2.5. Immobilization of capture probes

The biosensor was constructed as illustrated in Scheme 1. In brief, electrodes modified with PEDOT/GPS/AuNPs nanocomposite were incubated in 1.0 μM thiol-functionalized DNA probes solution (prepared with 0.2 M PBS, pH 7.4) for 90 min at room temperature. In this process, DNA probes terminal thiol groups were covalently attached with AuNPs. To prevent nonspecific adsorption on the AuNPs surface, GCE/PEDOT/GPS/AuNPs/DNA was incubated in 1 mg mL^{-1} BSA solution for 30 min to block nonspecific binding sites before detection. GCE/PEDOT/GPS/AuNPs/DNA was obtained successfully.

2.6. Sensing with the constructed biosensor

EIS measurements were performed to test the specific binding reaction between DNA and miRNA at GCE surfaces in a solution containing 0.1 M KCl and equimolar 5 mM $[Fe(CN)_6]^{4-/3-}$ at the frequency range from 1 to 100 000 Hz. The amplitude of the applied sine was set as 5 mV with the current potential of 0.22 V. The constructed biosensor was incubated in different concentration of miRNA24 solution in PBS (0.2 M, pH 7.4) for 45 min at room temperature. To test the specificity of the method, M1, M2, M3 sequences were utilized as controls.

3. Results and discussion

3.1. The formation and characterization of PEDOT/GPS

The voltage range of PEDOT/GPS deposition, the deposition cycles of CV, and the quality of GPS were optimized in details, as shown in Fig. S1. The deposition voltage range was selected as -0.5–1.5 V. The deposition cycles of CV was chosen to be 15 cycles. At these conditions, the EIS value of the modified electrode was the minimum, which can effectively improve the sensitivity of the sensor. The optimum dopant quality of GPS was selected as 2.0 mg l^{-1} and PEDOT/GPS composite has the uniform morphology.

The PEDOT/GPS and PEDOT/GPS/AuNPs composites were electrodeposited on GCE by cyclic voltammetry under the optimized conditions, and their microstructure and surface morphology were evaluated by SEM. As shown in Fig. 1 (A and C), the prepared PEDOT/GPS nanocomposite manifested a rough and porous morphology, which is composed of many nano-microspheres to form a prominent porous network. Micellar anionic in the GPS solution played a guiding role in the growth of PEDOT, proving successful electrochemical deposition of the PEDOT/GPS nanocomposite [28]. In Fig. 1 (B and D), it can be seen that PEDOT/GPS/AuNPs antifouling interface was significantly smoother and more tightly covered after AuNPs were modified. Therefore, the porous nanocomposite increases the specific surface area and supply large area for the immobilization of DNA probes.

XPS analysis was used to evaluate the successful preparation of PEDOT/GPS and PEDOT/GPS/AuNPs composites as well as the successful attachment of DNA capture probes. The PEDOT/GPS showed strong signals at 164, 286, and 532 eV (curve a, Fig. 2), which are typical characteristic peaks of S (2p), C (1s), and O (1s). After AuNPs deposition onto PEDOT/GPS, an Au (4f) characteristic peak at 84 eV appears newly (curve b). The new appearance of P (2p) and the increase of N (1s) (curve c) confirms the successful immobilization of the DNA probes onto the PEDOT/GPS/AuNPs composites.

Moreover, the successful synthesis of PEDOT/GPS composites was also verified by FTIR technique. Fig. 2B showed the FTIR spectra

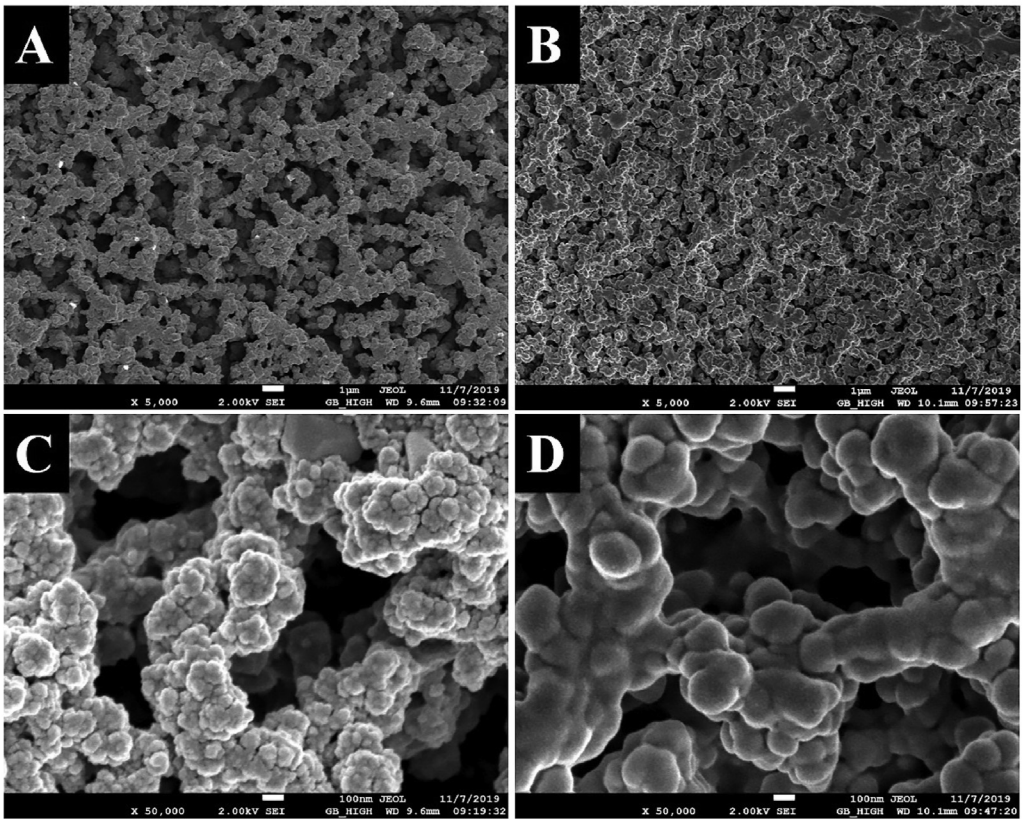


Fig. 1. SEM images of electrodes modified with PEDOT/GPS (A and C) and PEDOT/GPS/AuNPs (B and D) interfaces (low and high magnification images, respectively).

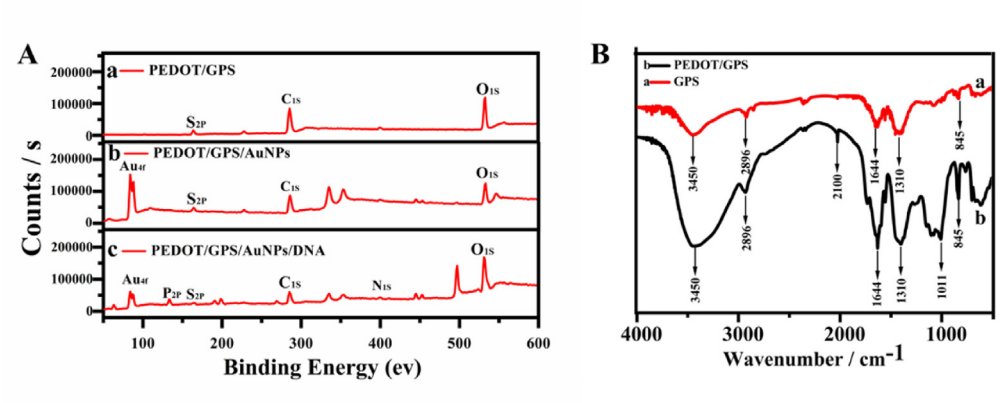


Fig. 2. (A) XPS spectra of PEDOT/GPS (curve a), PEDOT/GPS/AuNPs (curve b), and PEDOT/GPS/AuNPs/DNA (curve c). (B) FTIR spectrum of GPS (curve a) and PEDOT/GPS (curve b).

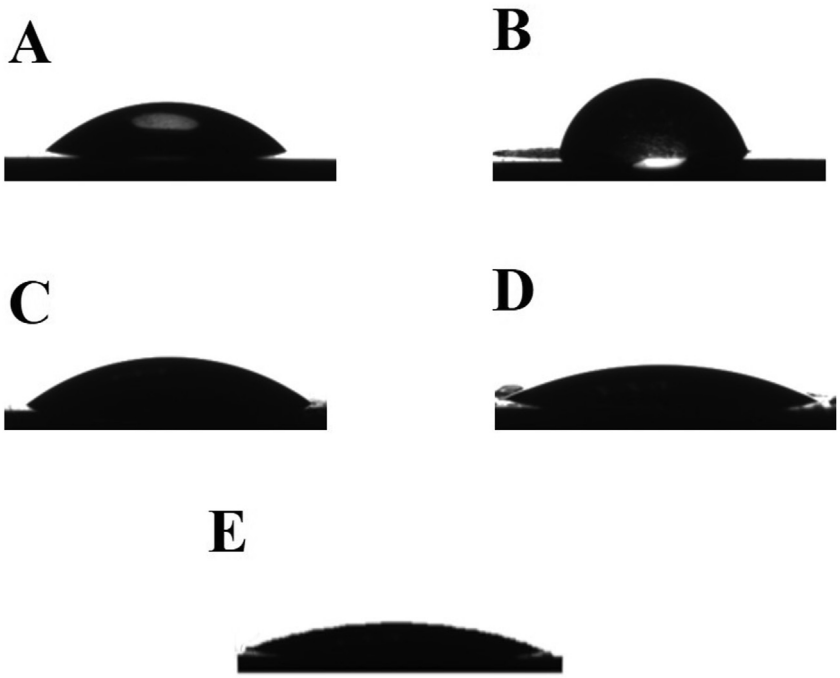


Fig. 3. Static water contact angles of bare electrode (A), GCE/PEDOT/$LiCO_4$ (B), GCE/PEDOT/GPS (C), GCE/PEDOT/GPS/AuNPs (D), and GCE/PEDOT/GPS/AuNPs/DNA (E) surfaces.

of (a) GPS and (b) PEDOT/GPS. Both GPS and PEDOT/GPS showed absorption bands at 3450 cm^{-1} (-OH tensile vibration), 2896 cm^{-1} (C—H surface bending vibration), 1644 and 1310 cm^{-1} (C═O tensile vibration) and 845 cm^{-1} (absorption peak of α-pyranose) [32] These characteristics are attributed to GPS. Expectedly, for the FTIR spectrum of PEDOT/GPS, new bands appeared at 1011 and 2100 cm^{-1}, which corresponded to C—S—C tensile vibration of PEDOT [33]. In a word, these results demonstrated that PEDOT/GPS composites have been successfully synthesized.

As shown in Fig. 3, static water contact angles of PEDOT/GPS (38.8°) PEDOT/GPS/AuNPs (26.7°) and PEDOT/GPS/AuNPs/DNA (17.8°) were much lower than that of bare electrode (45.1°) and PEDOT/$LiCO_4$ (82.6°). It is worth noting that when GPS and AuNPs were incorporated into PEDOT, the hydrophilicity of the PEDOT/GPS and PEDOT/GPS/AuNPs composites increased significantly. The enhanced hydrophilicity indicated that the prepared material had good potential of antifouling property and the PEDOT/GPS/AuNPs/DNA composite was successful prepared [34].

3.2. Assessment of antifouling property of PEDOT/GPS、PEDOT/LGPS and PEDOT/$LiCO_4$ surfaces

To verify the antifouling ability of the nanocomposite interface, PEDOT/GPS, PEDOT/LGPS and PEDOT/$LiCO_4$ were separately deposited onto the electrode surface by cyclic voltammetry under the same conditions. The nonspecific adsorption of single protein (Hb, BSA, HSA, LYS) was characterized by EIS, and the results are shown in Fig. 4 and Fig. S2. HSA (pI = 4.7–4.9), BSA (pI = 4.9), Hb (pI = 7.07) and LYS (pI = 6.99) have different isoelectric point (pI). In PBS (pH 7.4), HSA and BSA are negatively charged; Hb and LYS carries little charge. In a typical experiment, the modified electrodes were immersed in the different protein for 30 min. Fig. 4 shows that the impedance values change significantly for PEDOT/$LiCO_4$ and PEDOT/LGPS before and after soaking the protein, while that of PEDOT/GPS hardly change under the same conditions. This is because a large amount of protein adsorbed onto the surfaces of PEDOT/$LiCO_4$ and PEDOT/LGPS, and these protein molecules prevent electron transfer of these surfaces. These results proved that

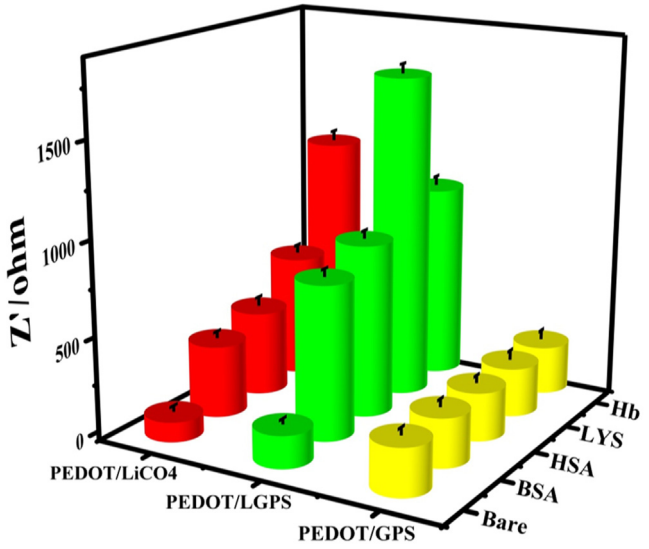


Fig. 4. EIS responses of GCE/PEDOT/$LiCO_4$, GCE/PEDOT/LGPS and GCE/PEDOT/GPS before and after incubation in different protein solutions (1: BSA; 2: HSA; 3: Hb; 4: LYS; 10^{-6} g mL^{-1} of each protein solution). Error bars here represented the standard deviations across three repeated measurements.

PEDOT/$LiCO_4$ and PEDOT/LGPS have no antifouling property while PEDOT/GPS exhibits excellent resistance to single protein adsorption.

To further test the antifouling property of these interfaces, different concentrates of porcine serum were used to test nonspecific adsorption in the complex media. As shown in Fig. S3, the EIS values on the PEDOT/GPS surface has no evident change while those of EDOT/LGPS and PEDOT/$LiCO_4$ surface increase significantly after immersing in porcine serum, demonstrating that the PEDOT/GPS interface had better antifouling performance in real media. PEDOT/GPS composite is a new antifouling material with good biocompatibility and electrochemical activity, which has revolutionary potential in the clinical application of biosensors.

PEDOT is considered as one of the most commercial conducting polymers at present. In particular, the surface of PEDOT/GPS composite is much smoother and closer, and its antifouling performance is superior to other materials. Moreover, the antifouling ability of GCE, PEDOT/$LiCO_4$ and PEDOT/GPS modified surfaces were further investigated using FITC-BSA (Fig. 5), where almost no FITC-BSA was detected at the PEDOT/GPS interface. All these results showed that the PEDOT/GPS interface had excellent antifouling ability in nonspecific protein adsorption in both single protein solution and real complex media. The excellent antifouling ability of PEDOT/GPS is mainly attributed to GPS. Polysaccharides are a promising material for nonfouling surfaces because they are highly hydrophilic and have the ability to store water [35,36]. Then the surface of PEDOT/GPS can be used to produce inert coatings.

3.3. Electrochemical characteristics of PEDOT/GPS based biosensor

Both CV and EIS techniques were utilized to characterize the construction process of the biosensor. The charge transfer resistance (Rct) is equivalent to the semicircle diameter. Fig. S4A showed the EIS plots of the bare GCE, GCE/PEDOT/GPS, GCE/PEDOT/GPS/AuNPs, GCE/PEDOT/GPS/AuNPs/DNA, and GCE/PEDOT/GPS/AuNPs/DNA/RNA. Obviously, compared to the Rct value of bare GCE, the Rct of GCE/PEDOT/GPS was much smaller, proving that PEDOT/GPS is an acceptable conductive material. The Rct value increased after the DNA probed were immobilized by the Au—S bond, because the negatively charged phosphate backbone of the DNA probes repels $[Fe(CN)_6]^{3-/4-}$ (the negative probe) and inhibits electron transfer [5]. Subsequently, with miRNA specific binding to the electrode surface, the Rct value of PEDOT/GEC/AuNPs/DNA/RNA also became large. In order to further characterize the modified electrode by CV, after the bioprobes were attached onto the modified electrodes, the peak current value was significantly reduced (Fig. S4B). These experimental results were consistent with those of EIS. It can also be confirmed that the DNA probes and miRNA were successfully attached onto the modified electrodes.

3.4. Analytical performances of the biosensor

As shown in Fig. S5A, the Rct value of the modified electrode increased with the increase of the incubation time from 60 min to 90 min. After the DNA probes incubation time is longer than 90 min, the Rct value did not increase with time because the biological probes reached saturation. The results showed that the optimal incubation time of DNA probes was 90 min. With the increase of incubation time, the Rct value of the biosensor continuously increased, and reached the maximum value at 2.0 h, indicating that the incubation time of miRNA24 was 2.0 h (Fig. S5B).

The biosensor performance of PEDOT/GPS/AuNPs/DNA on target miRNA24 related sequences was evaluated by CV and EIS test in PBS containing 5.0 mM redox probe $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl under the optimized conditions. As shown in Fig. 6A, the concentration of miRNA24 was investigated from 10^{-14} M to 10^{-9} M. The peak current (I_p) of the biosensor is unchanged when the concentration of miRNA24 was larger than 10^{-9} M. As shown in Fig. 6B, the peak current changes ($\Delta I_P/I_0$) changed linearly with the logarithm of miRNA24 concentration in the range from 10^{-14} M to 10^{-9} M, with the limit of detection (LOD) of $1.07X10^{-14}$ M (Please refer to the supplementary materials for calculation method). A regression equation of $y = 0.0498\ x+0.7295$ ($R^2 = 0.9941$) was obtained, where y represents the $\Delta I_P/I_0$ value, and x corresponds to the logarithmic concentration of miRNA24 (Fig. 6B). Additionally, as shown in Fig. S6, the Rct changes ($\Delta R/R_0$) changes were also linear with the logarithm of miRNA24 concentration.

In order to test the feasibility of the fabricated biosensor to complex biological samples, spiking experiments were performed by adding specified concentration of miRNA24 into 3% (V/V) human serum (Fig. 6C). As shown in Fig. 6D, the peak current changes ($\Delta I_P/I_0$) changed linearly with the logarithm of miRNA24 concentration in the range from 10^{-14} M to 10^{-9} M, with the limit of detection (LOD) of $3.45X10^{-14}$ M. A regression equation of $y = 0.0413x+0.6371$ ($R^2 = 0.9987$) was obtained, which proved the potential of this biosensor in practical application.

3.5. Selectivity, stability and reproducibility of the sensing system

To estimate the selectivity of the sensing surface, the Rct changes of the constructed biosensors were tested in different solutions containing M1, M2, M3 and the target miRNA24, respectively. As shown in Fig. S7A, the prepared biosensor showed good selectivity towards its target miRNA24, and the biosensors showed much smaller Rct changes towards the base mismatch miRNA24. Therefore, the fabricated miRNA biosensor has a good selectivity towards its target miRNA24, which is due to the antifouling property of the PEDOT/GPS and the strong specific binding of DNA/miRNA.

To assess the long-term stability of the biosensor, the prepared biosensor was immersed in BSA (0.1 M) solution and its EIS was measured every 24 h for 7 consecutive days. As can be seen from Fig. S7B, the EIS profile demonstrates no large changed, proving

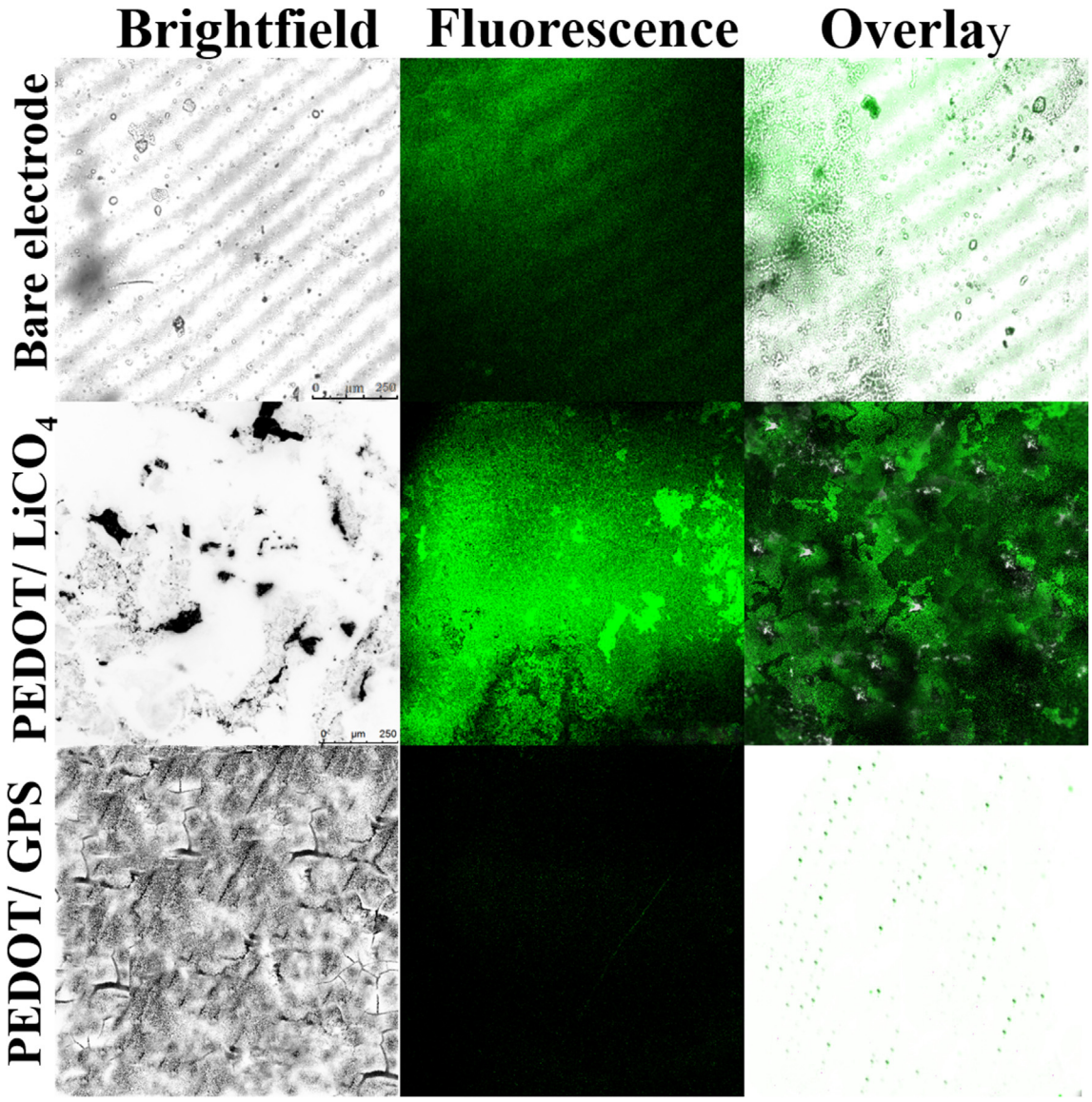


Fig. 5. The fluorescence microscopy images of bare GCE, GCE/PEDOT/$LiCO_4$ and GCE/PEDOT/GPS after incubation in 0.1 mg mL^{-1} FITC-BSA solution for 0.5 h.

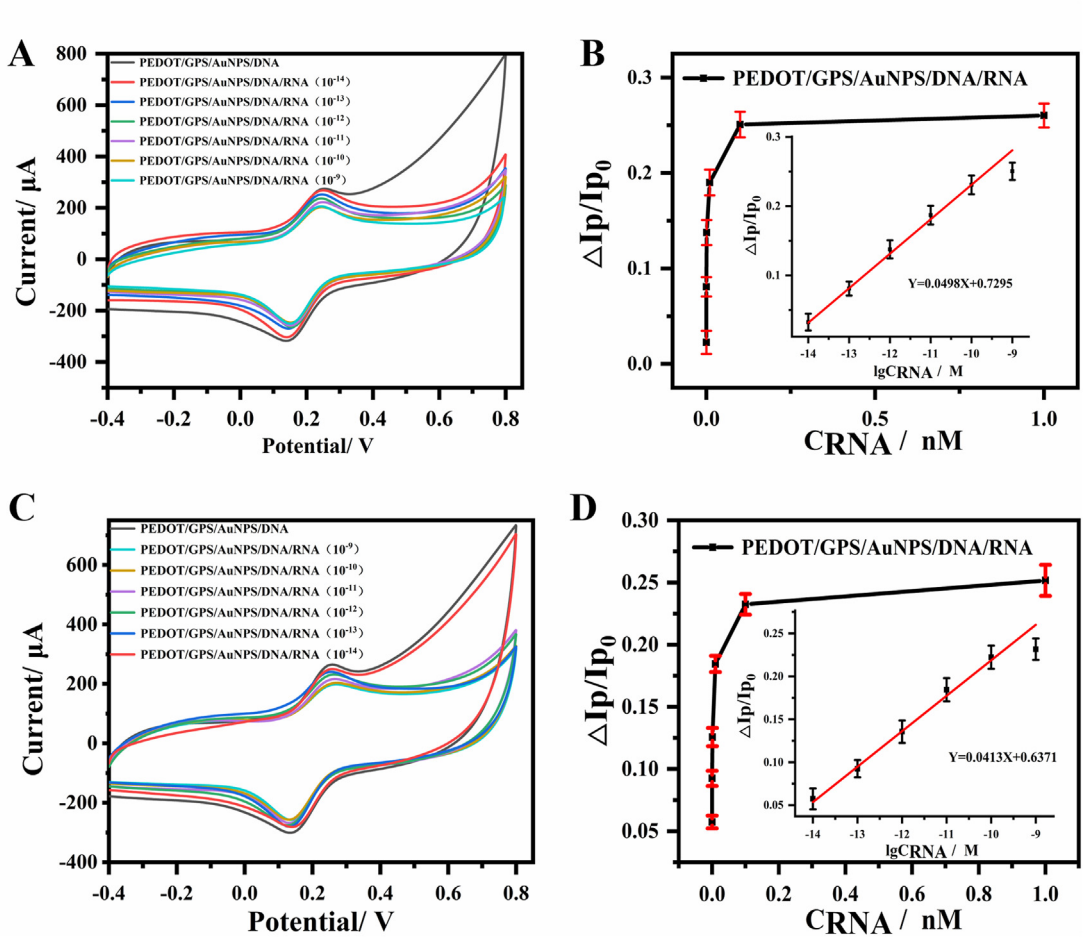


Fig. 6. CV response of the biosensor towards different miRNA24 concentration in PBS (A), or in 3% (V/V) human serum (C), peak current changes ($\Delta I_p/I_0$) of the biosensor as a function of miRNA24 concentration (B and D) (miRNA24 at concentrations from 10^{-14} M to 10^{-9} M).

that the biosensor has excellent antifouling ability and acceptable long-term stability.

For the inter-sensor and intra-sensor reproducibility tests, seven biosensors independently prepared were used to detect target miRNA24 (0.10 nM), and the relative standard deviation (RSD) was 2.46%. The same biosensor was used to detect the same miRNA24 (0.10 nM) repetitively for seven times, and the RSD was 1.15%. These experimental results proved a good reproducibility of the biosensor.

4. Conclusion

A biocompatible PEDOT/GPS nanocomposite with a rough and porous morphology, large electrochemical active surface area, and good long-term stability was successfully prepared by the one-step copolymerization of PEDOT and GPS. A sensitive and low fouling miRNA biosensor was constructed through the attachment of AuNPs and DNA probes in sequence. Owing to the excellent antifouling ability of PEDOT/GPS and the excellent biocompatibility of AuNPs, GCE/PEDOT/GPS/AuNPs/DNA shows strong binding affinity toward miRNA24, and the biosensor exhibits high stability and sensitivity, and the low detection limit. More importantly, the biosensor was capable of detecting miRNA24 in 3% (V/V) serum samples, proving a very promising advance for clinical diagnostic application. Therefore, this strategy has a bright avenue for the preparation of versatile electrochemical biosensors with antifouling property and high sensitivity based on copolymerized PEDOT/GPS.

CRediT authorship contribution statement

Hao Wang: Writing - original draft, Conceptualization, Methodology, Software. **Haitao Lü:** Investigation, Conceptualization, Methodology, Software. **Lili Yang:** Writing - review & editing. **Zhiling Song:** Methodology, Software. **Ni Hui:** Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (21705088), the National Key Technology R&D Program of China (2017YFD0501500), Shandong Key Laboratory of Biochemical Analysis (SKLBA2008), Shandong Province Agricultural Application Technology Innovation Project (SD2019NJ001-2), and the Qingdao Agricultural University High-level Talent Project (663/1117025).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.09.061.

References

[1] S. Nambiar, J.T. Yeow, Conductive polymer-based sensors for biomedical applications, Biosens. Bioelectron. 26 (2011) 1825–1832.
[2] D. Svirskis, J. Travas-Sejdic, A. Rodgers, S. Garg, Electrochemically controlled drug delivery based on intrinsically conducting polymers, J. Contr. Release 146 (2010) 6–15.
[3] P. Zarras, N. Anderson, C. Webber, D.J. Irvin, J.A. Irvin, A. Guenthner, et al., Progress in using conductive polymers as corrosion-inhibiting coatings, Radiat. Phys. Chem. 68 (2003) 387–394.
[4] X.C.R. Erik Holmlin, Robert G. Chapman, Shuichi Takayama, George M. Whitesides, Zwitterionic sams that resist nonspecific adsorption of protein from aqueous buffer, Langmuir 17 (2001) 2841–2850.
[5] N. Hui, X. Sun, S. Niu, X. Luo, PEGylated polyaniline nanofibers: antifouling and conducting biomaterial for electrochemical DNA sensing, ACS Appl. Mater. Interfaces 9 (2017) 2914–2923.
[6] J. Ladd, A.D. Taylor, M. Piliarik, J. Homola, S. Jiang, Label-free detection of cancer biomarker candidates using surface plasmon resonance imaging, Anal. Bioanal. Chem. 393 (2009) 1157–1163.
[7] C. Leng, H.C. Hung, S. Sun, D. Wang, Y. Li, S. Jiang, et al., Probing the surface hydration of nonfouling zwitterionic and PEG materials in contact with proteins, ACS Appl. Mater. Interfaces 7 (2015) 16881–16888.
[8] N. Liu, J. Song, Y. Lu, J.J. Davis, F. Gao, X. Luo, Electrochemical aptasensor for ultralow fouling cancer cell quantification in complex biological media based on designed branched peptides, Anal. Chem. 91 (2019) 8334–8340.
[9] S. Lowe, N.M. O'Brien-Simpson, L.A. Connal, Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates, Polym. Chem. 6 (2015) 198–212.
[10] H.S. Sundaram, X. Han, A.K. Nowinski, N.D. Brault, Y. Li, J.R. Ella-Menye, et al., Achieving one-step surface coating of highly hydrophilic poly(carboxybetaine methacrylate) polymers on hydrophobic and hydrophilic surfaces, Adv. Mater. Interfaces 1 (2014) 1–8.
[11] X. Luo, J.J. Davis, Electrical biosensors and the label free detection of protein disease biomarkers, Chem. Soc. Rev. 42 (2013) 5944–5962.
[12] K. Yoshimoto, T. Hirase, J. Madsen, S.P. Armes, Y. Nagasaki, Non-fouling character of poly[2-(methacryloyloxy)ethyl phosphorylcholine]-modified gold surfaces fabricated by the 'grafting to' method: comparison of its protein resistance with poly(ethylene glycol)-modified gold surfaces, Macromol. Rapid Commun. 30 (2009) 2136–2140.
[13] R. Jelinek, S. Kolusheva, Carbohydrate biosensors, Chem. Rev. 104 (2004) 5987–6016.
[14] S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, J. Contr. Release 100 (2004) 5–28.
[15] B. Lu, H. Yuk, S. Lin, N. Jian, K. Qu, J. Xu, et al., Pure PEDOT:PSS hydrogels, Nat. Commun. 10 (2019) 1043.
[16] Y. Qi, M. Yang, W. Xu, S. He, Y. Men, Natural polysaccharides-modified graphene oxide for adsorption of organic dyes from aqueous solutions, J. Colloid Interface Sci. 486 (2017) 84–96.
[17] S. Bauer, M.P. Arpa-Sancet, J.A. Finlay, M.E. Callow, J.A. Callow, A. Rosenhahn, Adhesion of marine fouling organisms on hydrophilic and amphiphilic polysaccharides, Langmuir 29 (2013) 4039–4047.
[18] D.T. Simon, E.O. Gabrielsson, K. Tybrandt, M. Berggren, Organic bioelectronics: bridging the signaling gap between biology and technology, Chem. Rev. 116 (2016) 13009–13041.
[19] X. Strakosas, B. Wei, D.C. Martin, R.M. Owens, Biofunctionalization of polydioxythiophene derivatives for biomedical applications, J. Mater. Chem. B. 4 (2016) 4952–4968.
[20] J. Rivnay, R.M. Owens, G.G. Malliaras, The rise of organic bioelectronics, Chem. Mater. 26 (2013) 679–685.
[21] K.M.P. Anna Herland, Vanessa Lundin, Mats Fahlman, Magnus Berggren, a.A.I. T. Edwin, W.H. Jager, Angew. Chem. Int. Ed. 50 (2011) 12529–12533.
[22] S.P. Kausik Mukhopadhyay, V.P. Vinod, Ashavani Kumar, R.V.C. Mala Rao, Murali Sastry, Gold nanoparticles assembled on amine-functionalized Na-Y zeolite: a biocompatible surface for enzyme immobilization, Langmuir 19 (2003) 3858–3863.
[23] H.Y. Du, X.X. Liu, Z. Ren, P.P. Liu, Capacitance characteristic of PEDOT electrodeposited on different substrates, J. Solid State Electrochem. 22 (2018) 3947–3954.
[24] K. Sun, S. Zhang, P. Li, Y. Xia, X. Zhang, D. Du, et al., Review on application of PEDOTs and PEDOT:PSS in energy conversion and storage devices, J. Mater. Sci. Mater. Electron. 26 (2015) 4438–4462.
[25] J. Isaksson, P. Kjall, D. Nilsson, N.D. Robinson, M. Berggren, A. Richter-Dahlfors, Electronic control of Ca^{2+} signalling in neuronal cells using an organic electronic ion pump, Nat. Mater. 6 (2007) 673–679.
[26] L. Liu, M. Li, M. Yu, M. Shen, Q. Wang, Y. Yu, et al., Natural polysaccharides exhibit anti-tumor activity by targeting gut microbiota, Int. J. Biol. Macromol. 121 (2019) 743–751.
[27] D.G. Harman, R. Gorkin, L. Stevens, B. Thompson, G.G. Wallace, Poly(3,4-ethylenedioxythiophene): dextran sulfate (PEDOT: DS) - a highly processable conductive organic biopolymer, Acta Biomater. 14 (2015) 33–42.
[28] W. Wang, M. Cui, Z. Song, X. Luo, An antifouling electrochemical immunosensor for carcinoembryonic antigen based on hyaluronic acid doped conducting polymer PEDOT, RSC Adv. 6 (2016) 88411–88416.
[29] J. Ahn, Y. Choi, A.R. Lee, J.H. Lee, J.H. Jung, A duplex DNA-gold nanoparticle probe composed as a colorimetric biosensor for sequence-specific DNA-binding proteins, Analyst 141 (2016) 2040–2045.
[30] S.K.S. Shiv Shankar, Hemant K. Daima, P.R. Selvakannan, Jayant M. Khire, V.B. Suresh, K. Bhargava, Charge-switchable gold nanoparticles for enhanced enzymatic thermostability, Phys. Chem. Chem. Phys. 17 (2015) 21517–21524.
[31] M. Cui, Z. Song, Y. Wu, B. Guo, X. Fan, X. Luo, A highly sensitive biosensor for tumor maker alpha fetoprotein based on poly(ethylene glycol) doped conducting polymer PEDOT, Biosens. Bioelectron. 79 (2016) 736–741.
[32] S. Wang, L. Zhao, Q. Li, C. Liu, J. Han, L. Zhu, et al., Rheological properties and chain conformation of soy hull water-soluble polysaccharide fractions obtained by gradient alcohol precipitation, Food Hydrocolloids 91 (2019) 34–39.
[33] H. Wang, H. Chen, L. Li, Y. Wang, L. Su, W. Bian, et al., High responsivity and high rejection ratio of self-powered solar-blind ultraviolet photodetector based on PEDOT:PSS/beta-Ga_2O_3 organic/inorganic p-n junction, J. Phys. Chem. Lett. 10 (2019) 6850–6856.
[34] C. Jiang, G. Wang, R. Hein, N. Liu, X. Luo, J.J. Davis, Antifouling strategies for selective in vitro and in vivo sensing, Chem. Rev. 120 (2020) 3852–3889.
[35] S.L. McArthur, K.M. McLean, P. Kingshott, H.A.W.S. John, R.C. Chatelier, H.J. Griesser, Effect of polysaccharide structure on protein adsorption, Colloids Surf., B 17 (2000) 37–48.
[36] X.Y. Cao, S.L. Conlan, W. Wagner, A.D. Ho, A.S. Clare, J.A. Callow, M.E. Callow, M. Grunze, A. Rosenhahn, Resistance of polysaccharide coatings to Proteins,Hematopoietic cells, and marine organisms, Biomacromolecules 10 (2009) 907–915.