Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-020-03025-5

RESEARCH PAPER

Highly selective and antifouling electrochemical biosensors for sensitive MicroRNA assaying based on conducting polymer polyaniline functionalized with zwitterionic peptide

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Received: 21 July 2020 / Revised: 26 September 2020 / Accepted: 23 October 2020

Abstract

Ultrasensitive and low-fouling microRNA electrochemical biosensors were successfully constructed by introducing thiol-terminated antifouling molecules (peptide sequence, polyethylene glycol, or mercapto alcohol) onto the surface of polyaniline-modified electrodes. For the three kinds of antifouling materials investigated, the newly designed and synthesized peptide exhibited superior antifouling ability to others, and it could effectively reduce the nonspecific adsorption of proteins and even prevent the fouling effect of serum. Compared with microRNA biosensors without antifouling capability, or those modified with polyethylene glycol or mercapto alcohol, the biosensor modified with the designed zwitterionic peptide showed the highest specificity for single-base mismatch, three-base mismatch, and completely complementary microRNAs. Most interestingly, the experimental results indicated that the introduction of antifouling molecules to the sensing interfaces did not significantly change the sensitivity of the biosensor. The strategy of constructing antifouling biosensors based on newly synthesized zwitterionic peptides and conducting polymers can be promisingly extended to the development of other electrochemical sensors and biosensors without encountering biofouling.

Keywords Antifouling · Zwitterionic peptide · Polyaniline · MicroRNA · Electrochemical biosensor

Introduction

The surface contamination from biomolecule and microorganism nonspecific adsorption is a widespread problem in numerous applications ranging from biosensors [1] to biomedical equipment and implants [2–4], and from food storage [5] to industrial and marine hulls [6]. For instance, nonspecific protein adsorption can reduce the property of surface-based diagnostic devices such as biosensors and tissue-engineered scaffolds and can result in adverse immunological responses [7]. To address this troublesome problem, an effective strategy is to introduce antifouling thin layer to material surfaces. At present, a few kinds of materials have been demonstrated for their antifouling performance in effectively suppressing nonspecific protein adsorption from complex media (e.g., serum, plasma), such as hydrophilic polyethylene glycol (PEG) [8], oligo(ethylene glycol) (OEG) [9], and zwitterionic poly(carboxybetaine methacrylate) [10]. Their electrical neutrality, hydrophilic nature, and steric hindrance are generally considered as the dominant reasons of resistance to nonspecific protein adsorption [11, 12]. However, PEG-based groups, which are the most widely used antifouling materials, are prone to oxidation in physiological conditions [13]; the terminal hydroxyl group of PEG can be oxidized to an aldehyde group, which may react with proteins [14]; and it is difficult to graft PEG chains to many substrates [15]. These drawbacks significantly limit their practical applications. Therefore, designing and fabricating new materials possessing ultralow fouling properties are highly desirable.

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Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-020-03025-5.

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Published online: 15 November 2020

More recently, synthetic and natural peptides (Pep) have served as a novel class of antifouling polymers with the advantages of biocompatibility, tunable structure, and facile synthesis and can conquer the biodegradability problems of PEG [16]. Natural peptides are made up of tandem amino acids of specific sequence with amide bonds, which emerge as zwitterions in a very broad range of pH [16]. Peptides have a strong ability to form hydrogen bonds because they are rich in the amine, carbonyl, and hydroxyl groups on the backbone and side chains. Furthermore, histidine (H), lysine (K), and arginine (R) are positively charged at physiological pH. Aspartic acid (D) and glutamic acid (E) are negatively charged under physiological conditions. They can be used for the design of zwitterionic antifouling peptides. It has been proved that hydrophilic, neutral peptide-based self-assembled monolayers (SAM) showed the ability to resist protein adsorption compared with the PEG SAM [16–19]. For instance, Huang group systematically investigated the effect of amphiphilic and zwitterionic structures on the suppression of nonspecific protein attachment onto peptide SAMs. And the results demonstrated that the zwitterionic peptide had relatively stronger antifouling property toward single protein and real complex media through stronger surface hydration of peptide SAMs than that of amphiphilic peptides [20]. Jiang group have compared the structure and function of the antifouling peptide EKEKEKE-PPPPC-Am with EKEKEKE-C-Am, and demonstrated that EKEKEKE-PPPPC-Am forms a secondary structure with ultralow fouling properties while EKEKEKE-C-Am has a random structure [17]. Our group has also synthesized a variety of the peptides, such as CPPPPEK2(EK)4(EK), EESKSESKSGGGGC,andCPPPPNQNQNQNQDHWRGWVA. All these peptides showed excellent antifouling properties in human blood serum and were used to construct biosensors with high sensing performance [21–23].

Cysteine (C), the amino acid, has a thiol side chain, which has generally been applied to stick peptides to gold (Au) surfaces [24]. However, the peptide applying cysteine as anchoring group was hard to attain the well-ordered arrangement of peptide structure that is dense enough to form antifouling layer onto Au surfaces [9]. In order to construct an immobilization matrix for the biomolecule conjugation, polyaniline (PANI) was polymerized onto the surface, and then antifouling materials containing thiol groups can be attached to the polymer PANI via covalent conjugation chemistry. PANI, as one of the most promising conducting polymers, has been extensively investigated as electrode material, due to its good conductivity, low-cost monomer, facile synthesis, tunable properties, and high environmental stability [25–27]. Especially, the electrochemical measurements of electroactive PANI can provide a novel detection strategy for biosensors [28]. In our previous reports, the redox current of PANI electrochemically deposited onto the electrode was used as the sensing signal for the detection of alpha-fetoprotein and carcinoembryonic antigen [29, 30].

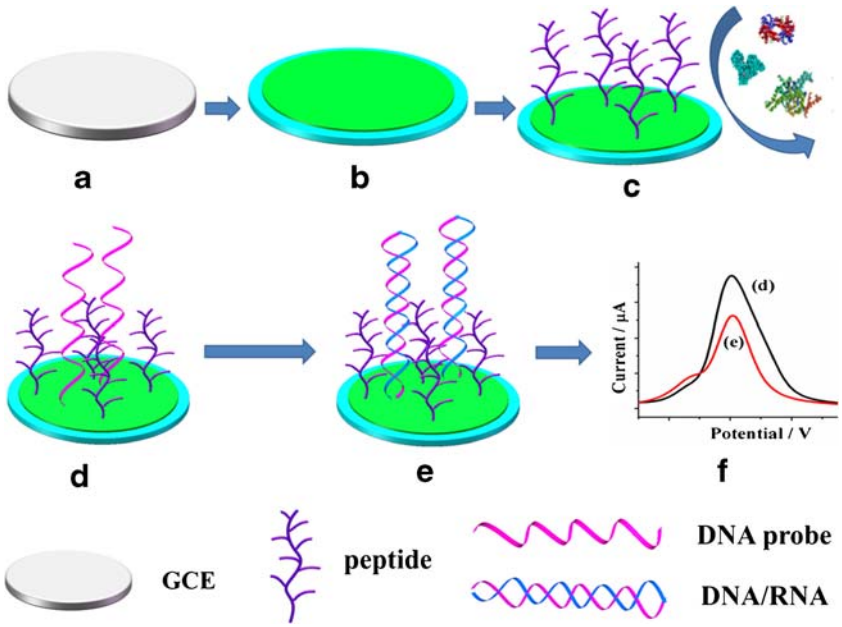
MicroRNAs (miRNAs) have emerged as a class of biomarkers for various diseases, including cancers, diabetes, neurological disorders, viral infections, and cardiovascular disease [31–35]. The accurate and sensitive detection of miRNAs is still challenging because they are very short in sequences (17–25 nucleotides), highly homologous (even single-base differences), and their concentrations are extremely low in actual samples (from aM to pM of miRNA in human serum) with severe interference of coexisting proteins [36]. The developed methods, such as Northern blotting [37], quantitative polymerase chain reaction (qPCR) [38], and microarray chips [39], are sensitive and specific for miRNA detection, but they suffer from a time-consuming multistep procedure and are highly susceptible to biofouling from the coexisting proteins in the complex samples [40].

Herein we designed a new zwitterionic peptide, which was hydrophilic and neutral in charge, and it can provide hydrogen bond acceptors/donator with a sequence of CTHNDRKQE. The peptide was covalently immobilized onto PANI surface via amino-thiol binding using a cross-linking agent of succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-1-carboxylate (SMCC). The antifouling performances of modified surfaces against single-protein solutions and complex media (human serum) were investigated. The designed peptide demonstrated unique antifouling capability, which is much stronger than that of the commonly used mercapto alcohol (MCH) and polyethylene glycol (PEG). More importantly, the developed peptide-based biosensor performances such as the detection sensitivity and the linear range are not negatively affected by the introduction of these antifouling molecules to the sensing interfaces (Scheme 1).

Experimental

Reagents and apparatus

PEG (2000 Da, Suzhou Nord Derivatives Pharm-Tech Co., Ltd) is 4-armed terminated with thiol groups. Human serum albumin (HSA), immunoglobulin G (IgG), hemoglobin (Hb), and bovine serum albumin (BSA) were purchased from Beijing Xinkezhongjing Biological Technology Co. Ltd. (Beijing, China). Aniline was bought from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). N-Hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), SMCC, and MCH were obtained from Aladdin Reagents (Shanghai, China). Peptide (CTHNDRKQE, pI 7.1, 1130.20 Da) was synthesized and purified from Bankpeptide biological technology Co., Ltd. (Hefei, China). Human serum samples were obtained from Qingdao Chengyang People's Hospital (Qingdao, China). Fluorescein isothiocyanate–labeled bovine serum albumin (FITC-BSA) and the related DNA (or



Scheme 1 Schematic diagram of the development process of antifouling miRNA biosensors. **a** Bare GCE. **b** PANI/GCE. **c** Pep/PANI/GCE. **d** DNA/Pep/PANI/GCE. **e** RNA/DNA/Pep/PANI/GCE. **f** The DPV response change of **d** and **e**

miRNA) sequences were purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Capture DNA probe (C1), target miRNA24 (complementary) (T2), one-base mismatched miRNA24 (M1), three-base mismatched miRNA24 (M2), and non-complementary miRNA24 (M3) sequences are as follows:

Capture DNA probe (C1): 5′ SH-GAT TTT CTT CCT TTT GTT C 3′;

Target RNA24 (T2): 5′ GAA CAA AAG GAA GAA AAT C 3′;

One-base mismatched RNA24 (M1): 5′ GAA CAA AAC GAA GAA AAT C 3′;

Three-base mismatched RNA24 (M2): 5′ CAA CAA AAG CAA CAA AAT C 3′;

Non-complementary RNA24 (M3): 5’ CCT TGT TGG ACT CCC TTC T 3′.

Other reagents were of analytical grade. All solutions used for experiments were prepared with pure water produced from a water purifying system (Milli-Q water system).

X-Ray photoelectron spectroscopy (XPS) measurements were accomplished using a Thermo-VG Scientific ESCALAB 250 spectrometer with a Al monochromatic source at a voltage of 15 kV. Static water contact angle measurements (CA) were performed using a JC2000 goniometer (Shanghai Zhongchen Instrument Co., China). CHI660E Electrochemical Workstation (Shanghai CH Instrument Co., China) was employed to carry out electrochemical measurements. The modified glassy carbon electrode (GCE, diameter 3.0 mm) was applied as the working electrode, and a Ag/AgCl electrode and a platinum wire were used as the reference and auxiliary electrodes, respectively.

The preparation of the antifouling molecule-modified PANI

PANI thin film was polymerized onto the glassy carbon electrode (GCE) surface by CV from − 0.4 to 0.8 V for 30 cycles, in a solution containing 0.02 M aniline and 1.0 M $HClO_4$. The obtained PANI/GCE was incubated in 2.0 mM sulfo-SMCC solution for 1.0 h at room temperature. Subsequently, the modified electrode was incubated in a phosphate-buffered saline (PBS, 0.2 M, pH 7.4) containing different antifouling material (1.0×10^{-4} M Pep, 1.0 mM MCH, or 5.0×10^{-4} M PEG, respectively) for 3 h to immobilize the antifouling molecules onto the PANI surface via covalent bonds [41]. Finally, the electrodes modified with antifouling molecules (Pep/PANI/GCE, PEG/PANI/GCE, and MCH/PANI/GCE) were equilibrated in PBS (0.2 M, pH 7.4) for 12 h at room temperature in order to remove unbound antifouling materials.

Characterization of the antifouling property

To evaluate the antifouling property of various antifouling material-modified PANI surfaces, four different protein solutions (1.0 mg mL^{-1}) and complex biological media (human serum with different dilutions) were employed. Differential pulse voltammetry (DPV) technique was utilized to assess the nonspecific protein attachment on the modified electrode surfaces (including PANI/GCE, MCH/PANI/GCE, PEG/PANI/GCE, and Pep/PANI/GCE), and the DPV performing parameters were set as follows: potential increment, 4.0 mV; amplitude, 50 mV; pulse period, 0.5 s. The DPV signals of biosensors before and after immersion (different single-

protein solution or serum for 1.0 h, and followed by washing with PBS and water) were taken in PBS (0.2 M, pH 5.7), and the signal changes of the modified electrode were compared.

Fabrication of different biosensors for miRNA24

To investigate the effect of different antifouling materials on the prepared biosensors, four groups of biosensors were prepared based on the PANI/GCE, which were all incubated in 2 mM cross-linker sulfo-SMCC solution for 1 h. The first group of electrodes were incubated in 200 μL PBS containing 1.0×10^{-6} M DNA for 3 h to allow the covalent immobilization of DNA probes to the PANI, and the obtained biosensors were defined as the DNA/PANI/GCE. The second group of electrodes were incubated in 200 μL PBS containing 1.0×10^{-6} M DNA and 1.0×10^{-4} M Pep for 3 h to obtain the DNA/Pep/PANI/GCE. The third group of electrodes were incubated in 200 μL PBS containing 1.0×10^{-6} M DNA and 5.0×10^{-4} M PEG for 3 h to get the DNA/PEG/PANI/GCE. The fourth group of electrodes were incubated in 200 μL PBS containing 1.0×10^{-6} M DNA and 1.0 mM MCH for 3 h to acquire the DNA/MCH/PANI/GCE.

Sensing of miRNA24 with four different biosensors

For the target hybridization reaction and electrochemical assay, different biosensors were incubated in PBS (0.2 M, pH 7.4) containing various concentrations of miRNA24 (T2) for 1 h at ambient room temperature, followed by rinsing with PBS and water, respectively. The PANI electrochemical signals of the biosensor before and after the hybridization reaction were recorded by DPV in PBS (0.2 M, pH 5.7) over the potential range of −0.2 to 0.6 V. PBS of pH 5.7 was chosen as the detection solution for experiments in this study. PBS of pH 5.7 can not only keep the PANI considerably electroactive but also guarantee the occurrence of the hybridization reaction [29, 42]. In this media, the biosensors can achieve the highest current response. To test the specificity of the assay, one-base mismatch (M1), three-base mismatch (M2), and non-complementary (M3) miRNA sequences were utilized as controls.

Signal suppression (SS%) was calculated using the following equation [43, 44]:

$$SS\% = (I_0 - I)/I_0 \times 100\%$$

where I_0 is the DNA biosensor current in the absence of the miRNA targets and I is the current in the presence of the miRNA targets.

Results and discussion

The fabrication and characterization of the antifouling surfaces

The electrochemical property of the modified electrodes was investigated by CV to conform the successive immobilization of PANI and antifouling materials (MCH, PEG, and Pep). The CV curves of PANI/GCE at PBS (pH 5.7) demonstrate that a couple of well-defined redox peaks appear at about 0.2 V and 0.3 V, which is in accordance with previous reports [45, 46]. The redox peaks are ascribed to the transition of PANI from the leucoemeraldine form to the emeraldine form [47], showing that PANI has been electrodeposited onto the GCE surface successively and retained good conductivity. As shown in Fig. 1, the typical peak of PANI changed significantly after the immobilization of MCH and PEG, while the change of PANI peak was relatively small after Pep was fixed onto PANI. For instance, the oxidation potential shifted positively and the peak current decreased at the same time. The apparent change may be due to the fact that MCH, PEG, and Pep are of poor conductivity.

To further prove the successful attachment of Pep and the mobilization of DNA probes to the PANI surface, XPS analysis was utilized to characterize the changes in surface elements. As shown in Fig. 2a, the PANI-modified ITO surfaces displayed Cl 2p, C 1s, N 1s, In 3d, and O 1s peaks, where the C 1s and N 1s peaks were ascribed to PANI. Clearly, after the covalent binding of peptide onto the PANI, typical S 2p peak appeared, which originated from the Pep of the Pep/PANI surface (curve B), proving that the PANI surface was covered with Pep. Subsequently, after both DNA probes (C1) and synthesized Pep were attached to the PANI surface, the specific peak of P 2p from DNA probes and S 2p from peptides appeared (curve C), along with an obvious decrease of C 1s peak. These results proved the successful immobilization of DNA capture probes and the Pep together on the PANI surface.

In order to characterize the hydrophilicity, the static water contact angles of PANI, Pep/PANI, and DNA/Pep/PANI-modified surfaces were examined. As shown in Fig. S1 and Table S1 (see Electronic Supplementary Material, ESM), the modification of PANI thin film onto the ITO surface resulted in a smaller contact angle (61.5°) than that of the bare ITO (75.5°), which was largely ascribed to the influence of the amine groups of PANI [29]. The Pep/PANI and DNA/Pep/PANI-coated ITO surfaces exhibited much smaller contact angles (21.4° and 12.5°, respectively), indicating excellent hydrophilicity. The strong hydrophilicity of the Pep-modified surface, in addition to its charge neutrality related with its amino groups (NH_2) and carboxyl groups (COOH) in sequence, makes the DNA/Pep/PANI-modified surface capable of preventing nonspecific protein adsorption.

The antifouling ability of the newly designed peptide

In order to assess the antifouling ability of the PANI, MCH/PANI, PEG/PANI, and Pep/PANI against proteins, DPV responses of these modified electrodes were measured in PBS (0.2 M, pH 5.7) before and after immersing in various protein solutions (1.0 mg mL^{-1}), such as BSA, IgG, Hb, and HSA. PBS (0.2 M, pH 7.4) was used to dilute the protein. HSA (pI = 4.7–4.9), BSA (pI = 4.9), Hb (pI = 7.07), and IgG (pI = 8.6) have different isoelectric point (pI). In PBS (pH 7.4), HSA and BSA are negatively charged; IgG are positively charged; Hb carries little charge. As displayed in Fig. 3a, the DPV signals of only PANI-modified electrode significantly changed after the immersion in single-protein solution, along with significant peak current decrease and peak potential variation. For instance, the peak potential of the PANI-modified electrodes changed by about 53.7% of its origin value, because the HSA adsorption hindered the electrochemical reaction. PANI/GCE has stronger adsorption capacity for BSA and HSA (both with negative charge), but much less for IgG (with positive charge) and Hb (with little charge). According to the conductive mechanism of conductive polymers, the conducting polymer molecules are oxidized to produce cationic-free radicals, and a partially filled valence band is obtained. The Π electrons of conducting polymer move freely on the conjugated chain [48, 49]. Therefore, the conductive polymer PANI has a strong adsorption capacity for HSA and BSA with negative charge. When MCH, PEG, and Pep were coated onto the PANI surface, the DPV response changes of the MCH/PANI, PEG/PANI, and Pep/PANI were much less than that of the bare PANI. In particular, the response of Pep/PANI remained almost constant after immersing in different protein solutions. Therefore, the MCH/PANI, PEG/PANI, and Pep/PANI were

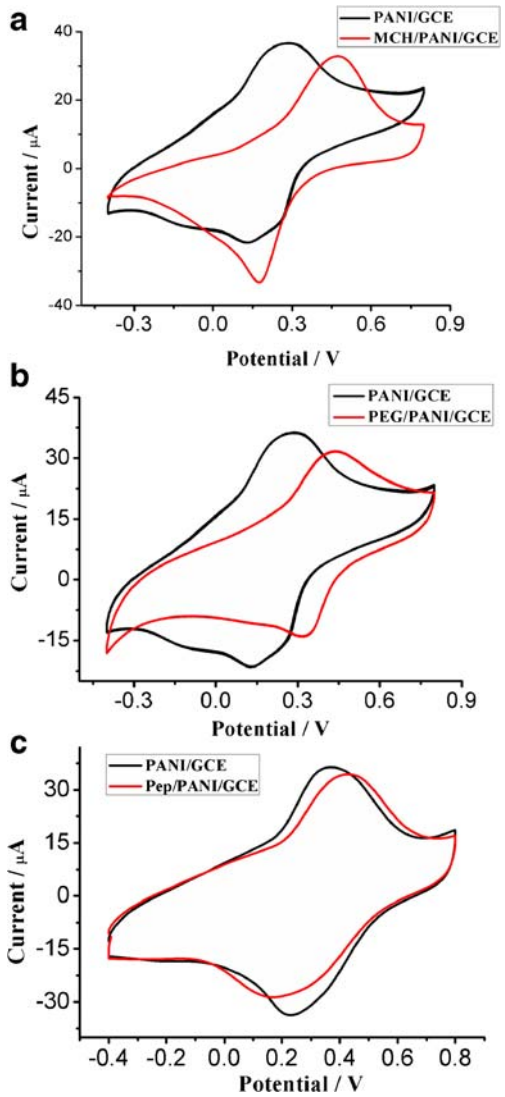


Fig. 1 CV curves of the modified electrodes measured in PBS (0.2 M, pH 5.7) with the scan rate of 0.1 V s^{-1}. Black lines represent PANI/GCE; red lines correspond to the MCH/PANI/GCE (**a**), PEG/PANI/GCE (**b**), and Pep/PANI/GCE (**c**)

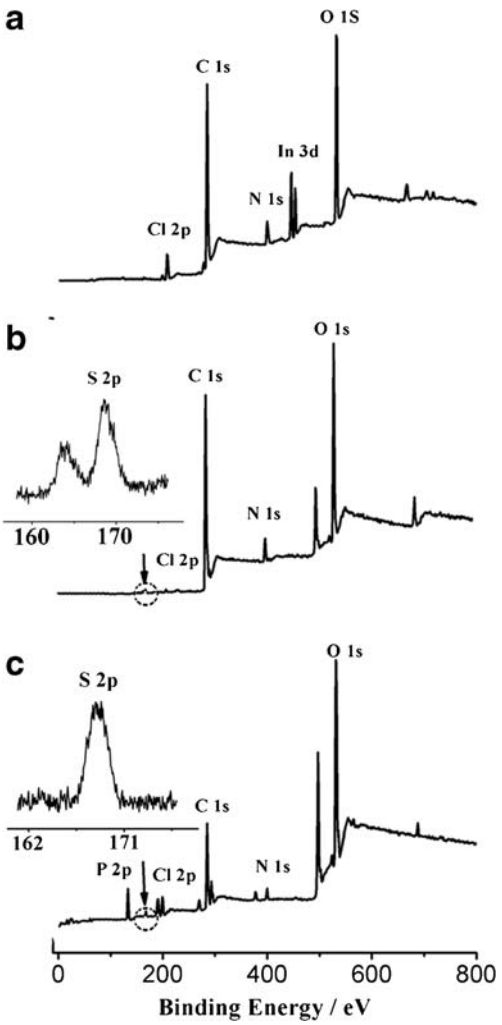


Fig. 2 X-ray photoelectron spectra of **a** PANI, **b** Pep/PANI, and **c** DNA/Pep/PANI-modified indium tin oxide (ITO) surfaces

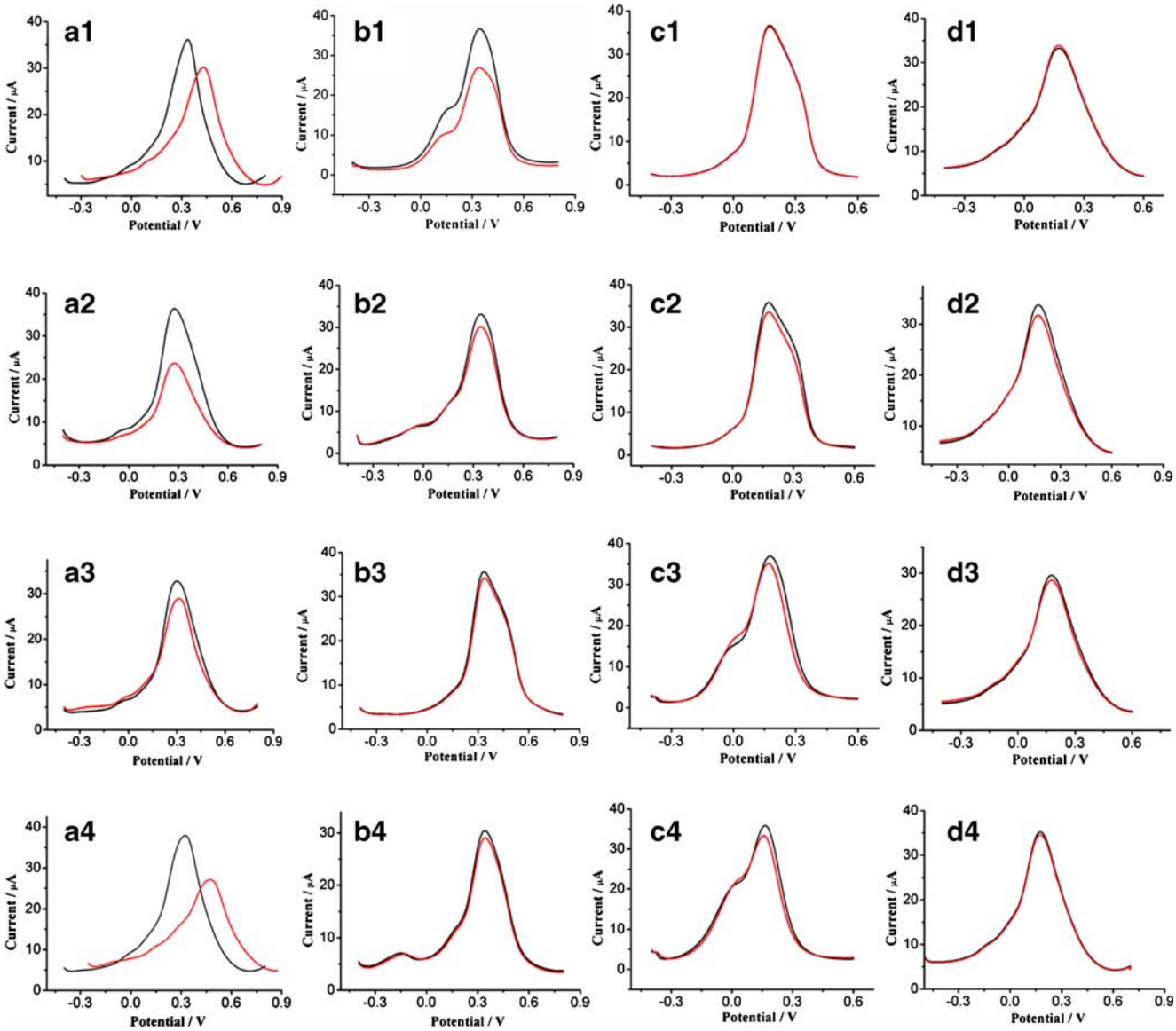


Fig. 3 DPV responses of the PANI (**a**), MCH/PANI (**b**), PEG/PANI (**c**), and Pep/PANI (**d**)-modified electrodes before (black line) and after (red line) incubation in different protein solutions (1: BSA; 2: IgG; 3: Hb; 4: HSA; 1.0 mg mL^{-1} each) in PBS (0.2 M, pH 5.7)

proved to be able to suppress nonspecific adsorptions of various single proteins.

MCH/PANI/GCE has a greater adsorption capacity for BSA than PEG/PANI/GCE and Pep/PANI/GCE. The following two reasons can be explained: first, the hydrophilicity of MCH/PANI interface (36.5°) is lower than that of PEG/PANI (25.2°) and PEP/PANI (21.4°) (ESM Fig. S1 and Table S1), so its ability to resist nonspecific adsorption of protein is weaker than that of PEG/PANI and PEP/PANI. Second, although BSA and HSA have certain similarity (structure and isoelectric point, etc.), but the adsorption capacity of BSA and HSA onto surfaces is still different. The experiments proved that MCH/PANI interface can adsorb more BSA.

The antifouling properties of the MCH/PANI, PEG/PANI, and Pep/PANI were investigated in detail in real complex media, as shown in Fig. S2 (see ESM). Along with the increase of the serum concentrations, the signal value of the MCH/PANI-modified electrode decreased, and after incubation in 10% human serum, it reduced to about 82.0% of its original value, suggesting that the MCH/PANI is not able to resist fouling in complex media. In contrast, after incubation in different concentrations of human serum, the current signals of the Pep/PANI-modified electrodes varied very slightly. The results demonstrated that the Pep/PANI has the most promising antifouling ability amongst all the other tested materials. The unique antifouling capability of the Pep/PANI material was further characterized with fluorescence using FITC-BSA, and it was found that there was almost no fluorescent-labeled BSA adsorption on the Pep/PANI interface (Fig. 4 and ESM Figs. S3–S5).

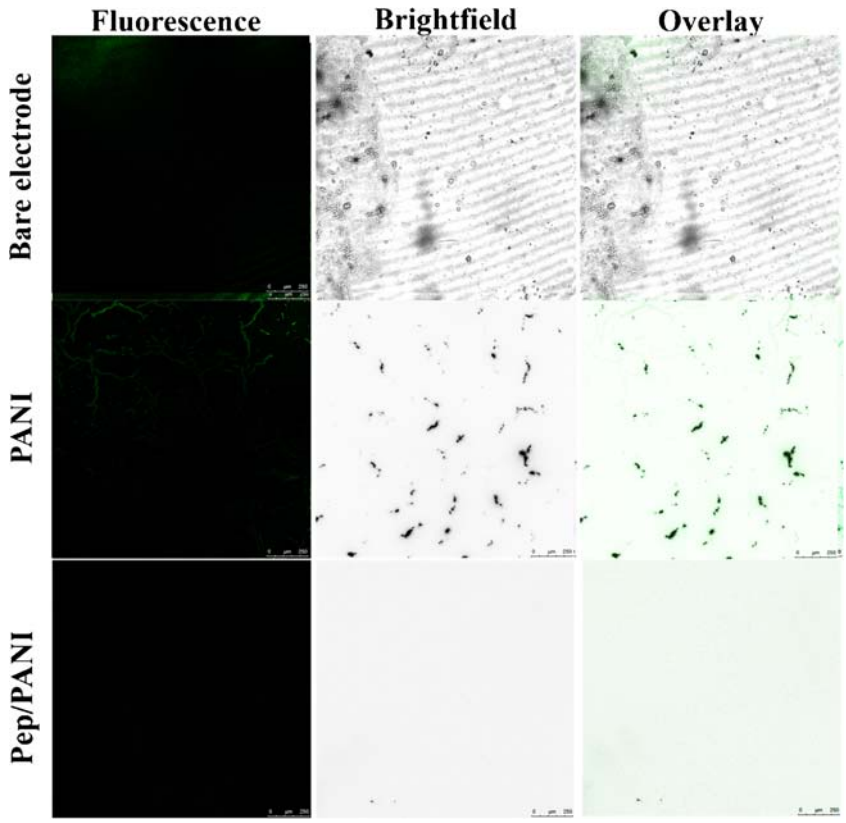


Fig. 4 The fluorescence microscopy images of the bare GCE, PANI/GCE, and Pep/PANI/GCE after immersing in FITC-BSA solution (0.1 mg mL^{-1}) for 0.5 h

Performances of the biosensor for miRNA assay

Optimization of assaying conditions

To maximize the sensitivity and efficiency of the biosensor, the conditions for DNA probe concentration, immobilization time for DNA probes, and peptide concentrations were optimized in detail. The concentration of DNA probe demonstrated great effect on the signal suppression (SS%) before and after miRNA24 hybridization, and the SS% reached a maximum at the concentration of 1.0 μM DNA probe (ESM Fig. S6). Similarly, the immobilization time for DNA probes was selected as 1 h (ESM Fig. S7), and 1.0×10^{-4} mol L^{-1} peptide as antifouling material was selected as the optimized concentration in the following experiments (ESM Fig. S8).

The specificity of the biosensor

One-base mismatched miRNA24 (M1), three-base mismatched miRNA24 (M2), and non-complementary miRNA24 (M3) were detected to investigate the specificity of developed biosensor. As shown in Fig. 5, four kinds of biosensors showed much smaller SS% toward M1, M2, and M3 than that of the same concentration of T2. It is obviously shown that the specificity of DNA/PANI was very poor in comparison to the others that were modified with antifouling agents. More interestingly, the DNA/Pep/PANI/GCE demonstrated the most excellent specificity. The specificity factor (SS%(T2)/SS%(M)) was calculated and shown in Table S2 (see ESM), and clearly, the DNA/Pep/PANI/GCE showed the largest specificity factor. The

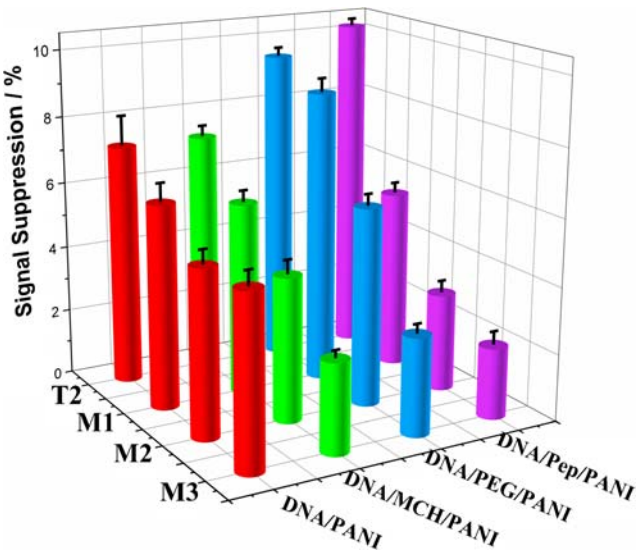


Fig. 5 Signal suppression of four kinds of biosensors to various miRNAs (T2, M1, M2, and M3). The concentrations of miRNAs are all 1.0×10^{-11} mol L^{-1}. Error bars here represented the standard deviations across three repeated measurements

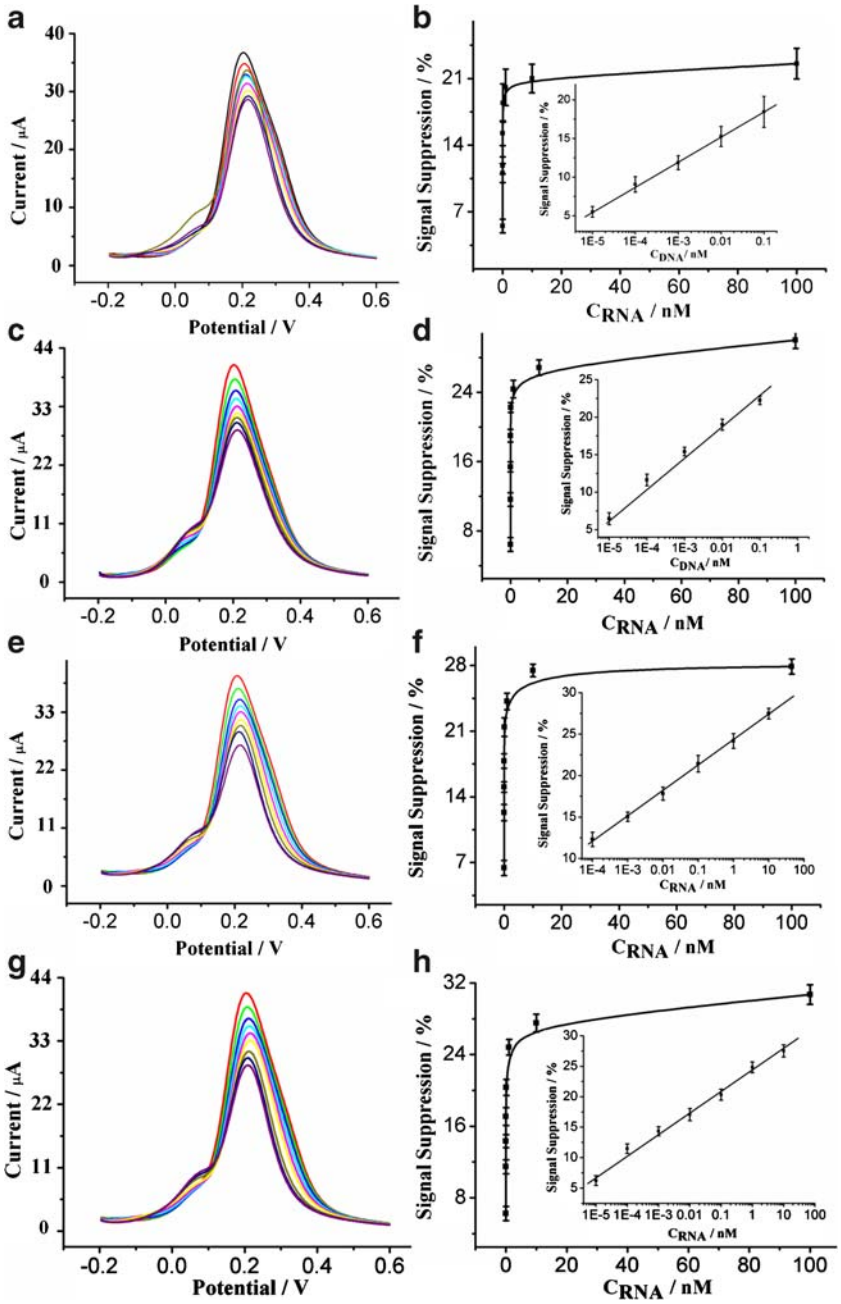


Fig. 6 DPV curves of DNA/PANI/GCE (**a**), DNA/MCH/PANI/GCE (**c**), DNA/PEG/PANI/GCE (**e**), and DNA/Pep/PANI/GCE (**g**) toward different concentrations of target miRNA24 (T2) at the optimized conditions. Curves correspond to miRNA24 at the concentrations from 0, 10^{-14} to 10^{-7} mol L^{-1}. Signal suppression of DNA/PANI/GCE (**b**), DNA/MCH/PANI/GCE (**d**), DNA/PEG/PANI/GCE (**f**), and DNA/Pep/PANI/GCE (**h**) after immersion with different concentration of miRNA24 in PBS (0.2 M, pH 5.7). Inset showed the corresponding calibration curve

results proved that the DNA/Pep/PANI/GCE has the strongest ability to distinguish target miRNA, and this is mainly due to the combination of the unique DNA/miRNA recognition and the excellent antifouling ability of the Pep/PANI. These antifouling molecules can greatly suppress the nonspecific adsorption of the biomolecules onto the surface of the biosensors. Overall, the incorporation of these antifouling molecules has demonstrated to be advantageous; all of them are effective in preventing nonspecific binding and interactions of T2 to the sensor surface.

Sensing performance of miRNA biosensors

The electrochemical responses of all four kinds of biosensors have been investigated as a function of miRNA24 concentration. Clearly, the DPV peak currents of all sensors decreased along with the increase of the target miRNA24 concentration, because of the selective hybridization reaction of target miRNA24 and the formation of DNA/miRNA on electrode-sensing interfaces (Fig. 6). The good linear relationships between the peak currents and the logarithm of the target miRNA24 concentrations were obtained. The method of

Table 1 Comparison of two methods for miRNA24 detection in human serum (5%, V/V)

Samples	miRNA measured with fluorescence quantitative PCR instrument (Roche 48,011) (nM)	RSD (%)	miRNA measured with this biosensor (nM)	RSD (%)
1	58.4	2.52	55.9	3.21
2	58.8	1.70	57.2	4.12
3	56.4	2.03	55.7	2.98
4	57.0	5.71	56.8	4.22

LOD calculation can be seen in the ESM. As shown in Table S3 (see ESM), the analytical performance of the four sensors is compared. These results demonstrated that the introduction of the antifouling materials will not hinder the target binding and does not significantly change the sensitivity of biosensors, and the biosensor based on the DNA/Pep/PANI has the widest linear range amongst them. This excellent sensing performance of the DNA/Pep/PANI-based biosensor may be ascribed to the good biocompatibility of the hydrophilic Pep/PANI, which can supply a suitable micro-environment for the fixed DNA probes to show strong specific binding affinity toward miRNA.

The selectivity of the biosensor

To assess the selectivity of the constructed sensors toward their target miRNA24, the responses (SS%) of all four kinds of biosensors were evaluated in different solutions containing BSA, HSA, IgG, Hb, DNA, or miRNA24, respectively. As shown in Fig. S6 (see ESM), the DNA/PANI/GCE exhibited the worst selectivity, while the DNA/MCH/PANI/GCE, DNA/PEG/PANI/GCE, and DNA/Pep/PANI/GCE biosensors all showed excellent selectivity, and they showed negligible signal suppression toward DNA or proteins even at high concentrations. The good selectivity may be due to the specific binding between the capture DNA probes and the target miRNAs, and the excellent antifouling capabilities of the antifouling molecules (Pep, PEG, and MCH) modified PANI interfaces that can efficiently suppress the nonspecific protein adsorption.

The real sample detection

To investigate the DNA/Pep/PANI/GCE for possible practical detection, four human serum samples (5%, V/V) have been tested in parallel using both PCR and the new proposed biosensor. Four samples were analyzed with fluorescence quantitative PCR instrument (Roche 48011) and then detected with the prepared biosensor. As shown in Table 1, the measured results by the biosensor are very close to those of the PCR measurements.

Conclusion

In conclusion, we have successfully developed ultralow fouling miRNA biosensors that are specific and sensitive by introducing antifouling materials into the conducting polymer PANI surface. Compared with MCH and PEG utilized in this work, the designed zwitterionic peptide is the most efficient antifouling materials in restraining nonspecific protein adsorption, and the combination of the peptide with conducting polymer and DNA probe ensures the development of electrochemical biosensors with enhanced specificity and selectivity. The DNA/Pep/PANI-based biosensor is capable of assaying miRNA targets in 5% serum samples, and the introduction of antifouling materials into the sensing interfaces does not significantly affect the biosensor sensing performances. The designed zwitterionic peptides, with antifouling capability superior to that of commonly used materials like PEG and MCH, have a promising potential to be used in the construction of other biosensor and bioelectronics capable of performing in complex biological systems.

Authors' contributions Dongwei Wang: Conceptualization, methodology, software, investigation, and writing original draft. Jiasheng Wang: Validation, formal analysis, visualization, and software. Zhiling Song: Validation, formal analysis, visualization, and software. Ni Hui: Resources; writing—review and editing; supervision; and data curation.

Funding This research was financially supported by the National Natural Science Foundation of China (21705088), 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).

Compliance with ethical standards

This study involved five human serum samples. All these samples come from healthy people. All human serum experiments were conducted according to the ethical guidelines of Qingdao Agricultural University and the "3R" principle. All experiments and the research project hereby are approved by the Ethics Committee of Qingdao Agricultural University.

Conflict of interest The authors declare that they have no conflict of interests.

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