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PEGylated polyaniline nanofibers: antifouling and conducting biomaterial for electrochemical DNA sensing

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ABSTRACT: Biofouling arising from nonspecific adsorption is a substantial outstanding challenge in diagnostics and disease monitoring, and antifouling sensing interfaces capable of reducing the nonspecific adsorption of proteins from biological complex samples are highly desirable. We present herein, the preparation of novel composite nanofibers through the grafting of polyethylene glycol (PEG) polymer onto polyaniline (PANI) nanofibers, and their application in the development of antifouling electrochemical biosensors. The PEGylated PANI (PANI/PEG) nanofibers possessed large surface area and remained conductive, and at the same time demonstrated excellent antifouling performances in single protein solutions as well as complex human serum samples. Sensitive and low fouling electrochemical biosensors for breast cancer susceptibility gene (BRCA1) can be easily fabricated through the attachment of DNA probes to the PANI/PEG nanofibers. The biosensor showed a very high sensitivity to target BRCA1 with a linear range from 0.01 pM to 1 nM, and it was also efficient enough to detect DNA mismatches with satisfactory selectivity. Moreover, the DNA biosensor based on the PEGylated PANI nanofibers supported the quantification of BRCA1 in complex human serum, indicating great potential of this novel biomaterial for application in biosensors and bioelectronics.

KEYWORDS: *polyaniline, polyethylene glycol, antifouling, nonspecific adsorption, biosensor*

1. INTRODUCTION

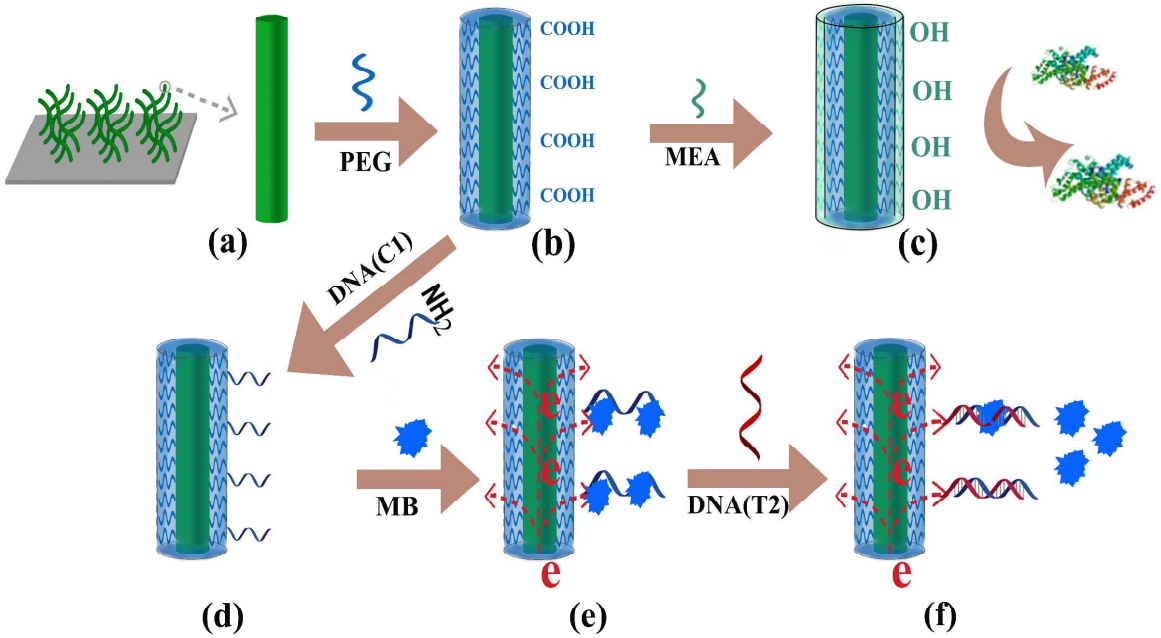
Biofouling from nonspecific protein adsorption is a persistent challenge for any interfaces that exposed to complex biological media. Such nonspecific protein adsorption can lead to decreased target diffusion to the sensing interface, which eventually leads to decreased sensor performances. Therefore, it is crucial to develop antifouling surfaces for sensors in many bioanalytical application fields, such as human disease biomarkers detection,[1-3] hormone determination,[4] and monitoring of drug serum levels[5-6].

Certain polymers such as polyurethane, polyvinyl chloride, poly(methacrylate) and poly(dimethylsiloxane), have been utilized to achieve selective transportation of targets to the sensing interface while they prevent biofouling materials from reaching the surfaces.[7-9] These reported polymers are suitable for clinical monitoring, but their stability and functions are greatly impaired when they are in contact with biological samples.[10-11] Polyethylene glycol (PEG), which is a nontoxic and highly hydrophilic biocompatible polymer, has been widely used to reduce nonspecific protein adsorption,[12] and it has been considered as "gold standard" of anti-biofouling polymers.[13-14] The protein resistance of long-chain PEG modified interfaces is ascribed to the "steric repulsion", and it is an entropic effect associated with the unfavorable change of free energy that associated with confinement and the dehydration of soft polymer chains.[15-17] Additionally, the antifouling effectiveness of PEG generally depends on the grafting technique as well as the polymer structure.[18-19] For instance, "grafting from" method requires relatively complicated chemical synthesis and tends to produce brushes without sufficiently high density.[20-22] However, another alternative, "grafting to", has proved to be an extraordinarily

effective method to create inert surface toward bimolecular adsorption.[23] PEG can be simply attached to surfaces through the formation of covalent bonds using "grafting to" methods to form well ordered polymer brushes. For example, PEG chains have been bound to a polylysine backbone with a high density, which was assembled on various oxide surfaces through electrostatic interactions, and the PEG branches were forced to be extended into the solution.[24-27]

Grafting of antifouling polymers with the long chains directly onto electrode surfaces, such as PEG that is not conductive, however, can lead to the formation of layers with high-impedance and cause decreases in electrode sensitivities. It is expected that the incorporation of PEG with certain conductive soft materials, such as conducting polymers, may be helpful to address this problem. Conducting polymers have promising applications in various fields including biosensors, electronic devices, integrated circuits, batteries etc., due to their excellent physical and chemical properties.[28-29] In particular, one-dimensional nanostructured conducting polymers, promising candidates as structural elements of nanoscale devices, have attracted enormous interest because of their large surface area, low power consumption, and great potential for the development of miniaturized devices.[30-34] For instance, polyaniline (PANI) nanofibers have been fabricated and utilized for the construction of a sensitive and selective electrochemical biosensor for hepatitis B virus gene.[35] Biosensors based on single PANI nanowire have been constructed to detect proteins such as myoglobin and immunoglobulin G.[36] However, these biosensors, though highly sensitive, are also highly susceptible to biofouling in complex biological media. If antifouling polymers like PEG were introduced into these systems, low fouling but highly sensitive assays may be developed. Unfortunately, the grafting of PEG derivatives onto conducting polymers has rarely been explored and ultrasensitive and antifouling sensing in natural biological samples is still challenging.

In this work, we aim to fabricate novel PANI/PEG nanofibers using "grafting to" approach to form a thin layer of PEG onto PANI nanofiber backbones. The resulting PEGylated PANI nanofibers, combining strong antifouling ability of PEG with the excellent electrochemical activity of PANI, provide an ideal substrate for the construction of low fouling electrochemical biosensors (Scheme 1). Herein, breast cancer susceptibility gene (BRCA1), a biomarker for human breast cancer,[37] has been detected as a model target. The marriage of conducting PANI/PEG nanofibers and sequence-specific DNA probes was envisaged to present both anti-biofouling ability and unique selectivity for the constructed electrochemical BRCA1 sensor.



Scheme 1. Illustration of the construction of PANI/PEG nanofibers antifouling interface and its application in ultrasensitive and low fouling DNA sensors. (a) PANI nanofibers deposited on the electrode, (b) PEG modification onto PANI nanofibers, (c) monoethanolamine (MEA) modification, (d) immobilization of capture DNA (C_1) onto GCE/PANI/PEG, (e) methylene blue (MB) interaction with capture DNA (C_1), and (f) hybridization with target DNA (T_2).

2. EXPERIMENTAL

2.1 Reagents

4-armed PEG terminated with carboxylic acid groups (2000 Da) was purchased from Suzhou Nord Derivatives Pharm-Tech Co., Ltd. Bovine serum albumin (BSA), human serum albumin (HSA), immunoglobulin G (IgG) and hemoglobin (Hb), were obtained from Beijing Xinkezhongjing Biological Technology Co. Ltd (Beijing, China). Aniline was bought from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). N-hydroxysuccinimde (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), methylene blue (MB) and monoethanolamine (MEA) were obtained from Aladdin Reagents (Shanghai, China). Human serum samples from healthy woman and woman with breast cancer were provided by the Eighth People's Hospital of Qingdao (Shangdong, China). 19-mer synthetic oligonucleotides, which were purified by liquid chromatography, were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The related sequences of DNA were as follows:

Capture DNA probe (C_1): 5' NH_2-GAT TTT CTT CCT TTT GTT C 3'; Target DNA (Complementary) (T_2): 5' GAA CAA AAG GAA GAA AAT C 3'; One-base mismatched DNA (M_1): 5' GAA CAA AAC GAA GAA AAT C 3'; Three-base mismatched DNA (M_2): 5' CAA CAA AAG CAA CAA AAT C 3'; Non-complementary DNA (M_3): 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).

2.2 Methods

Scanning electron microscope (SEM) with the model of JEOL JSM-7500F (Hitachi High-Technology Co., Ltd., Japan) was used to characterize nanostructures and morphologies of prepared PANI and PEGylated PANI nanofibers. XPS analysis was performed using a Thermo-VG Scientific ESCALAB 250 spectrometer with a Al monochromatic source operated at a voltage of 15 kV. Static water contact angle measurements (CA) were carried out using a JC2000 goniometer (Shanghai Zhongchen Instrument Co., China). Fourier transform infrared spectroscopy (FTIR) was carried out using a BRUKER TENSOR 70 spectrometer made by Bruker Optics (Germany). Electrochemical experiments were carried out using a CHI660E Electrochemical Workstation (Shanghai CH Instrument Co., China). The modified glassy carbon electrode (GCE, diameter 3.0 mm) was used as the working electrode, and the used reference and auxiliary electrodes were Ag/AgCl electrode and platinum wire, respectively. EIS and CVs of the bare electrode and the modified electrodes were recorded in phosphate buffered saline (PBS, 0.2 M, pH 7.4 if not otherwise stated) containing 5.0 mM redox probe $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. EIS conditions: frequency range from 1 to 100,000 Hz; the amplitude of the applied sine was set at 5 mV; and the current potential was set at 0.20 V; CV conditions: scanning range from - 1.2 to 1.2 V with scan rate of 0.10 V/s.

2.3 Synthesis of PANI/PEG nanofibers

4-armed PEG was immobilized covalently onto the PANI nanofibers using EDC and NHS (Scheme S1). GCE was firstly polished and washed, and then eletrochemically pretreated in PBS (0.2 M, pH 7.4).[38] For the synthesis of PANI/PEG nanofibers, firstly, PANI nanofibers were

electrodeposited onto the pretreated electrode through the galvanostatic technique in 1.0 M $HClO_4$ solution containing 0.5 M aniline, and the current density was controlled at 0.6, 0.3 and 0.15 mA cm^{-2} for the period of 0.5 h, 3 h and 3 h, respectively. The prepared PANI nanofibers were washed with water. Subsequently, in order to graft 4-armed PEG onto the PANI nanofiber surfaces, PANI nanofibers modified electrode was immersed into 2.0 mL PBS containing 0.4 M EDC, 0.1 M NHS, and 2.0 mg mL^{-1} 4-armed PEG (terminated with carboxyl groups) for 3 h. The electrode was then washed with PBS, and stored in PBS when not in use. The incubation of 4-armed PEG solution on PANI nanofibers modified electrode resulted in the formation of covalent bonds between amino groups of PANI and carboxyl groups of PEG. As a result, PANI/PEG nanofibers on the GCE surface were obtained.

2.4 Characterization of antifouling ability

As some of the carboxyl groups activated by EDC/NHS were not reacted with amino groups, the PEG/PANI modified GCEs were immersed into 1.0 M MEA for 10 minutes, and allowed the activated carboxyl groups to react with excess MEA, leaving a largely neutral charged, hydroxyl group terminated PEGylated interface.[39-41] To assess the antifouling performance of PEGylated PANI nanofibers, different protein samples including four single protein solutions (1.0 mg mL^{-1}) and complex media (human serum) were tested. Differential pulse voltammetry (DPV) technique was employed to measure or monitor the nonspecific adsorption on PANI nanofibers, and the DPV performing parameters were as follows: potential increment of 4.0 mV; amplitude of 50 mV; and pulse period of 0.5 s. The DPV responses of modified electrodes before and after incubation (incubated with single protein solution or

complex media for 30 min, and followed by washing with PBS and water) were recorded in PBS (0.2 M, with the pH 5.7), and the changes of the peak current and potential were compared.

2.5 Attachment of capture DNA

As shown in Scheme S1, electrodes modified with PEGylated PANI nanofibers (GCE/PANI/PEG) were incubated in 1.0 μM amino-functionalized oligonucleotides (C_1) solution (prepared with 0.2 M PBS, pH 7.4) containing 0.4 M EDC, 0.1 M NHS for 1 h. During this process the covalent attachment of oligonucleotides (C_1) was allowed to the carboxyl-PEG by the amide formation. The obtained DNA sensor was denoted as GCE/PANI/PEG/C_1. The obtained sensors were washed with PBS, and stored in PBS when not in use.

2.6 Sensing with the DNA sensor

For the hybridization and electrochemical detection, the DNA sensor was dipped into 1 mL of MB (20 μM) for 10 min to incorporate MB into the DNA sequence, followed by rinse with PBS and water. The hybridization reaction was completed by dipping the MB incorporated DNA sensor into PBS containing different concentration of target analyte (T_2) for 30 minutes at ambient room temperature. The MB reduction signals of the DNA sensor before and after hybridization were measured by DPV in blank PBS (0.2 M, pH 7.4) in the range of -0.6 to 0.1 V. To test the specificity of the assay, one-base mismatched DNA (M_1), three-base mismatched DNA (M_2) and totally non-complementary DNA (M_3) sequences were used as controls.

a

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of PANI/PEG nanofibers

The synthetic process of PANI nanofibers was carried out in 1.0 M $HClO_4$ solution containing 0.5 M aniline using galvanostatic technique. These PANI nanofibers were surface modified with carboxyl group terminated 4-armed PEG (see Scheme S1 for details), and the covalent attachment through the formation of amide was verified using FTIR (Figure S1). The FTIR spectrum of PANI (Figure S1A) shows clear benzenoid and quinoid ring stretching bands (C=C) at 1493 and 1560 cm^{-1}, respectively. Those bands at 1305 and 1143 cm^{-1} are ascribed to the C-N stretching of the secondary aromatic amine, and bands at 822 and 743 cm^{-1} are ascribed to the out-of-plane bending of C-H. These characteristic bands are in good agreement with those previously reported for PANI, indicating that PANI nanofibers were successfully formed.[42-44] For the FTIR spectrum of PEG modified PANI (Figure S1B), new bands appear at 1644 and 1247 cm^{-1}, which are ascribed to amide bands.[45-46] These results clearly revealed the covalent attachment of PEG onto PANI through the formation of amide bonds.

Typical SEM images of the PANI and PANI/PEG nanofibers are shown in Figure 1. Clearly, the prepared PANI nanofibers, with diameters of about 150-200 nm, formed a porous network on the electrode surface, and they presented a very rough microstructure with many particles protruding from the surface (Figure 1A and B). Interestingly, after the modification with PEG, it was found that the PANI nanofibers were all uniformly covered by PEG, and their network-like structure almost remained unchanged (Figure 1C and D). Compared with PANI nanofibers, the obtained PANI/PEG nanofibers exhibited much smoother surfaces and wider diameters (increased to about 300 nm), indicating successful covering of PANI nanofibers with PEG and the formation of PEGylated PANI nanofibers.

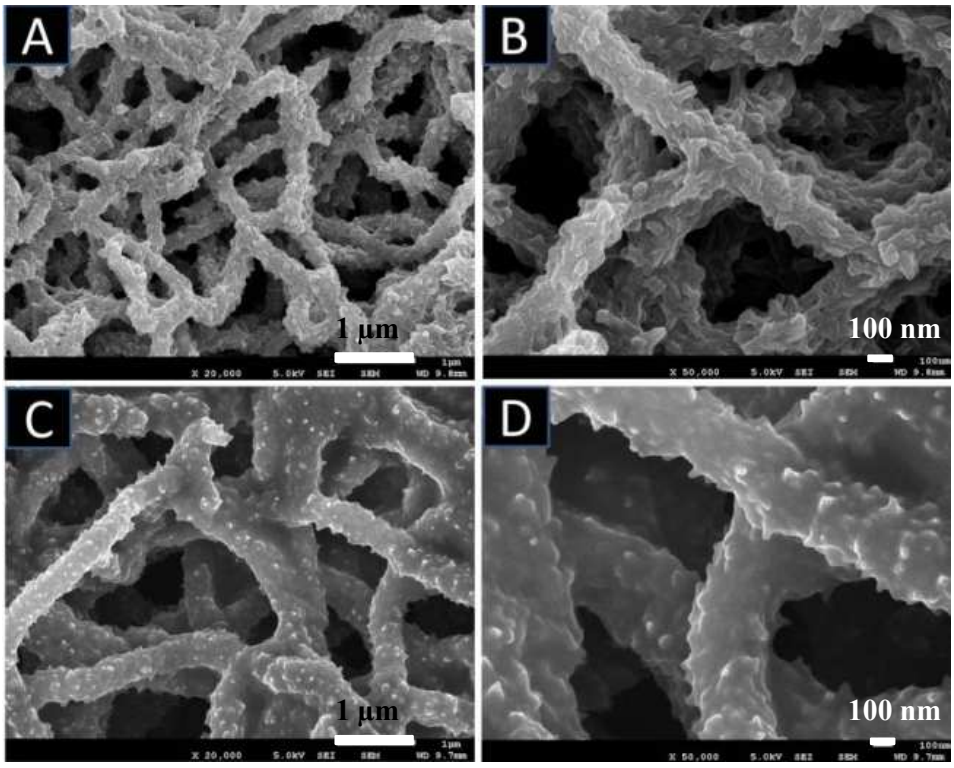


Figure 1. SEM images of the PANI nanofibers (A, B) and PEGylated PANI nanofibers (C, D). Figures A and C are in lower magnification, and figures B and D are in higher magnification.

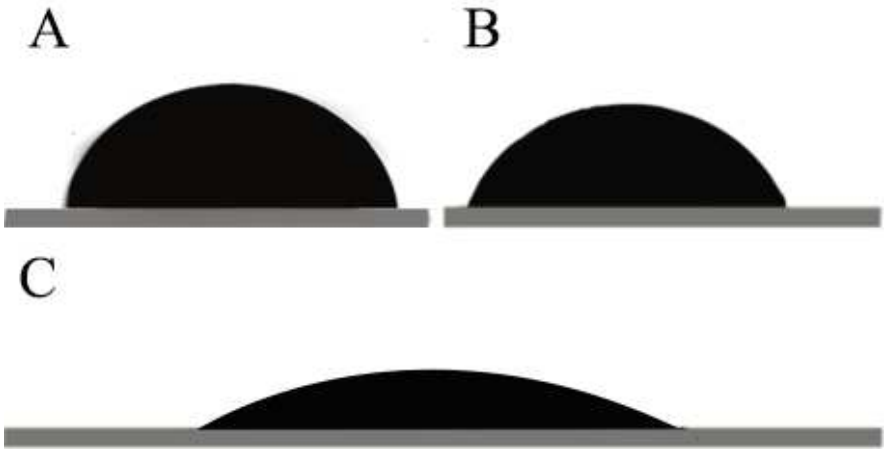


Figure 2. Contact angle images of (A) ITO, (B) PANI and (C) PANI/PEG coated ITO surfaces.

The water contact angle is commonly used to characterize the surface relative wetting property and hydrophilicity. As shown clearly in Figure 2 and Table S1 (see Supporting Information), after coating with PANI nanofibers, the contact angle of the surface decreased from 76.6° to 55°, indicating moderate hydrophilicity of the PANI nanofibers modified surface. As expected, after further modification of PANI nanofibers with PEG, the contact angle significantly decreased to 22.4°, suggesting excellent hydrophilicity of the PEGylated PANI nanofibers. The contact angle of PEGylated PANI nanofibers is close to that of other materials with antifouling ability reported in previous literature, such as the zwitterionic peptide SAM (e.g., CRERERE, 19.6°),[47] and poly(β-peptoid) (17°).[48] Previous reports have proved that, surface modification or functionalization with hydrophilic materials was an effective strategy to improve the antifouling ability of synthesized polymer materialss.[49] Therefore, the hydrophilicity of PEGylated PANI nanofibers may be beneficial for the formation of a hydration layer to prevent nonspecific protein adsorption.

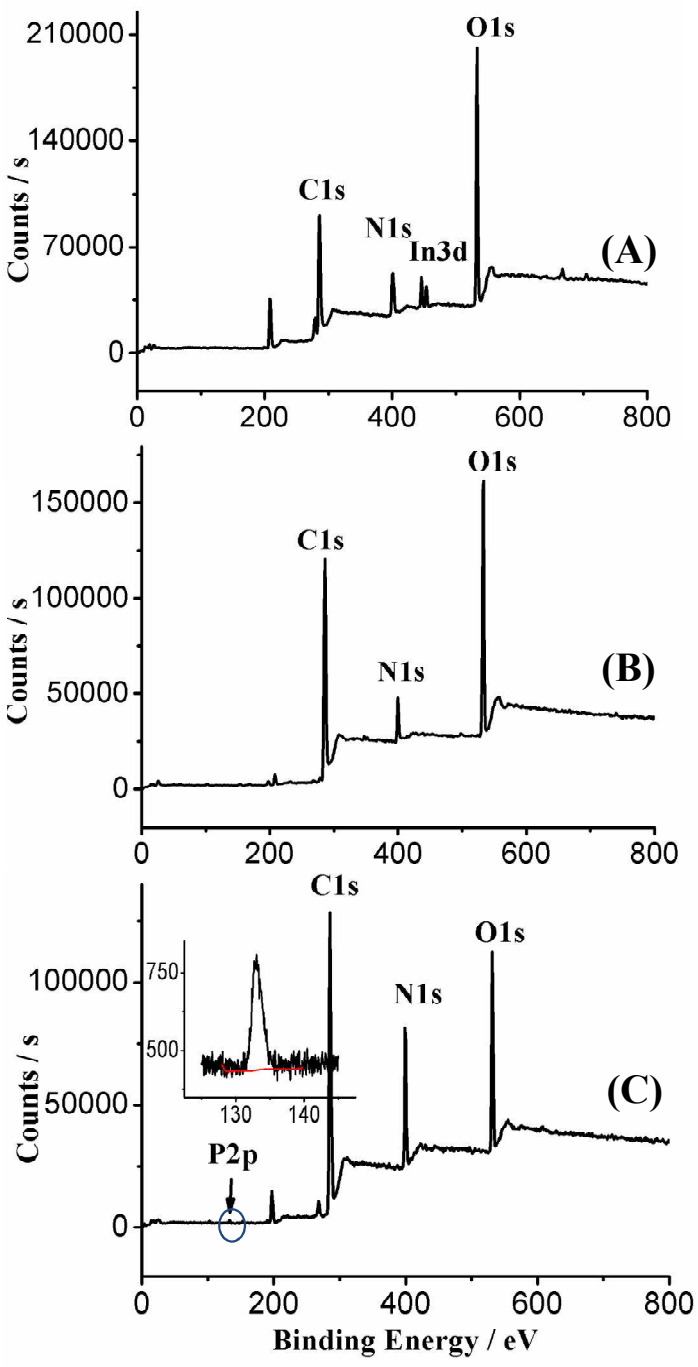


Figure 3. X-ray photoelectron spectra of (A) PANI, (B) PANI/PEG, and (C) PANI/PEG/C_1 Coated Indium tin oxide (ITO) surface.

To further verify the successful modification of PANI nanofibers with PEG and the immobilization of DNA capture probe, X-Ray photoelectron spectroscopy (XPS) analysis was performed after each modification steps. As shown in Figure 3, for the PANI nanofibers modified surface, C 1s and N 1s peaks corresponding to PANI were observed. After the covalent binding of PEG onto PANI nanofibers, C 1s peak increased apparently corresponding to PEG (Figure 3B). Subsequently, after DNA capture probes (C_1) were attached to the PEGylated PANI nanofibers, the N 1s peak significantly increased, together with the appearance of a new P 2p peak, indicating the successful immobilization of DNA capture probes. The quantitative analysis of the surface chemical composition was shown in Table S2. The sequential decrease of the O 1s and In 3d peaks, and increase of the C 1s and P 2p peaks indicated the successful decoration of PEG and capture probe (C_1) onto the PANI surface.

3.2. Nonspecific protein adsorption on PANI and PANI/PEG surfaces

As a typical conducting polymer, PANI can undergo electrochemical redox reaction easily and show a sharp cathodic peak at about 0.2 V corresponding to the transition of leucoemeraldine/emeraldine.[50] To assess the antifouling ability of electrode surfaces modified with PANI and PANI/PEG nanofibers, DPV responses of PANI and PANI/PEG modified electrodes were compared before and after soaking in various protein solutions, such as HSA, BSA, Hb, and IgG. In a typical experiment, the modified electrodes were immersed in PBS (0.2 M, pH 5.7) to establish stable DPV base responses, and then their DPV responses were recorded in the same PBS again after incubation in aqueous solutions with 1.0 mg mL^{-1} of different proteins for 30 min, respectively. As shown in Figure 4A, the DPV signals (peak current and potential) of the PANI nanofibers modified electrodes significantly changed, with the peak

currents decreased and peak potentials shifted greatly after the incubation in protein solutions. As shown in Figure S2, the current response of the PANI modified electrodes retained 30.18% of the original value after incubation in IgG; and the peak potential changed into 228.93% due to adsorption of HSA. This is ascribed to the fact that after incubation in protein solutions, proteins will adsorb onto the surface of electrode and block the electrochemical reaction, leading to changes in the DPV responses. These results indicated that a large amount of single proteins (BSA, HSA, Hb, IgG) were adsorbed onto the PANI nanofibers without protein-resistence coating. In contrast, Figure 4B showed that electrodes modified with PEGylated PANI nanofibers can significantly reduce the protein adsorption, and very tiny changes in both peak currents and potentials were observed after incubation in protein solutions. As can be seen in Figure S2, the largest current response change to IgG was 92.56% and the largest potential change to HSA was 103.71%. Therefore, PANI/PEG nanofibers were proved to be able to resist various protein adsorptions, indicating excellent antifouling ability of these PEGylated nanofibers.

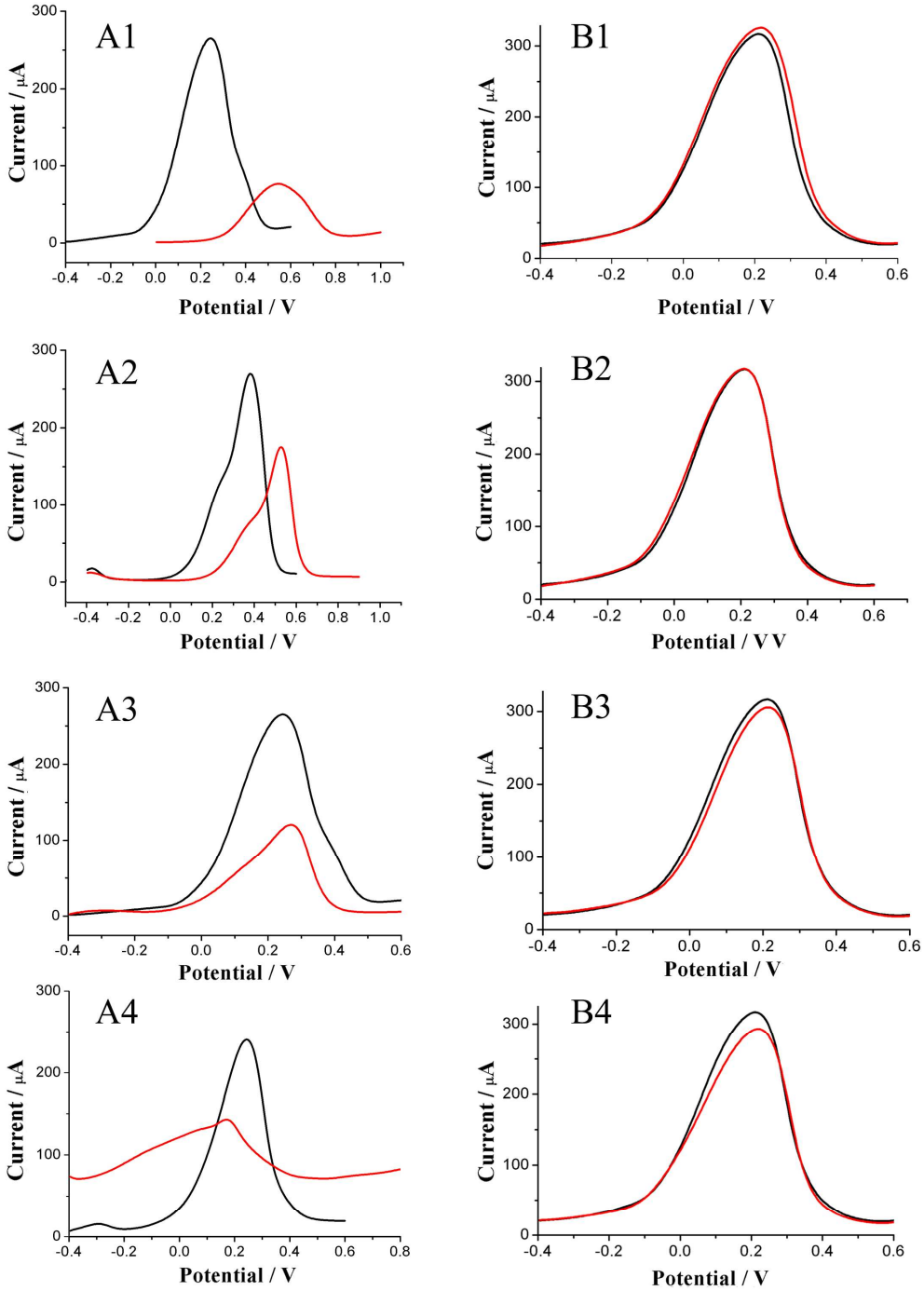
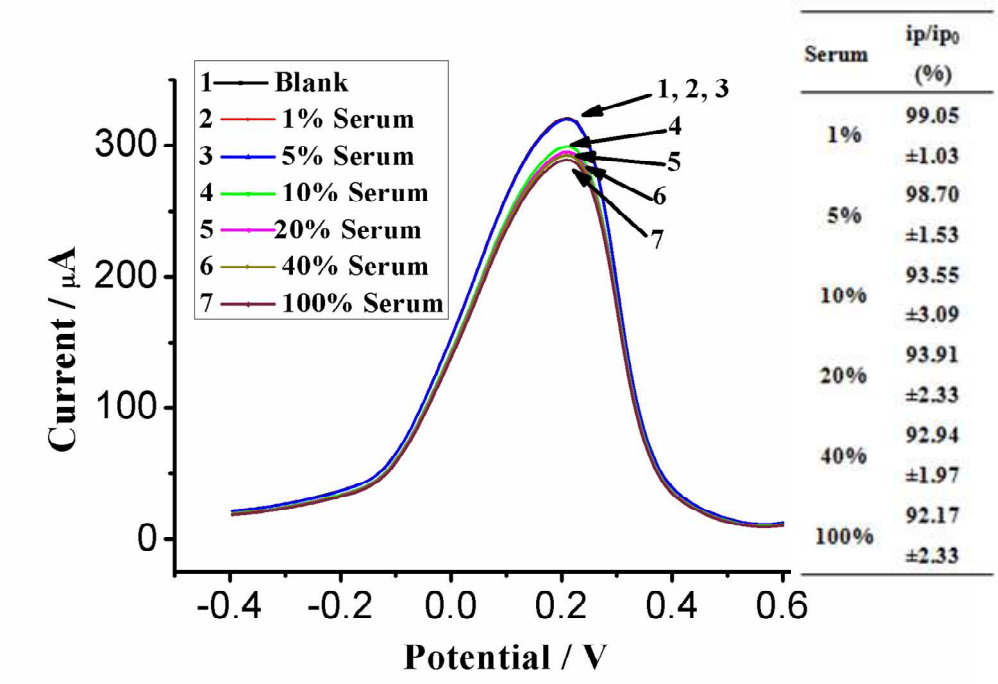


Figure 4. DPV responses of PANI (A) and PANI/PEG (B) before (black line) and after (red line) incubation in different protein solutions (1: HSA; 2: BSA; 3: Hb; 4: IgG; 1.0 mg mL^{-1} each).



Serum	ip/ip$_0$ (%)
1%	99.05 ±1.03
5%	98.70 ±1.53
10%	93.55 ±3.09
20%	93.91 ±2.33
40%	92.94 ±1.97
100%	92.17 ±2.33

Figure 5. DPV responses of PANI/PEG before and after incubation in different concentration human serum solutions (The curves for blank, 1% serum and 5% serum are overlapped. The inset table shows the value of i_p/i_{p0}).

Besides single protein solutions, the antifouling property of PEGylated PANI nanofibers was further investigated in complex biological media, human serum. As shown in Figure 5, after incubation in 1% human serum, the current responses of the PANI/PEG modified electrodes only changed slightly by 0.95%. The curves for blank, 1% serum and 5% serum are overlapped. After incubation in 20% human serum, the current responses retained 93.91% of their initial values, and more interestingly, even after incubation in the whole serum, the current responses retained 92.17% of their initial currents. These results demonstrated that PANI nanofibers coated with PEG can form an excellent antifouling layer to resist protein adsorption in these natural complex media, because PEG with strong hydrophilicity can effectively reduce protein adsorption onto surfaces through the hydrophobic interaction.[22]

3.3. Performance of the DNA biosensor based on PEGylated PANI nanofibers

3.3.1. Electrochemical characterization of the DNA biosensor

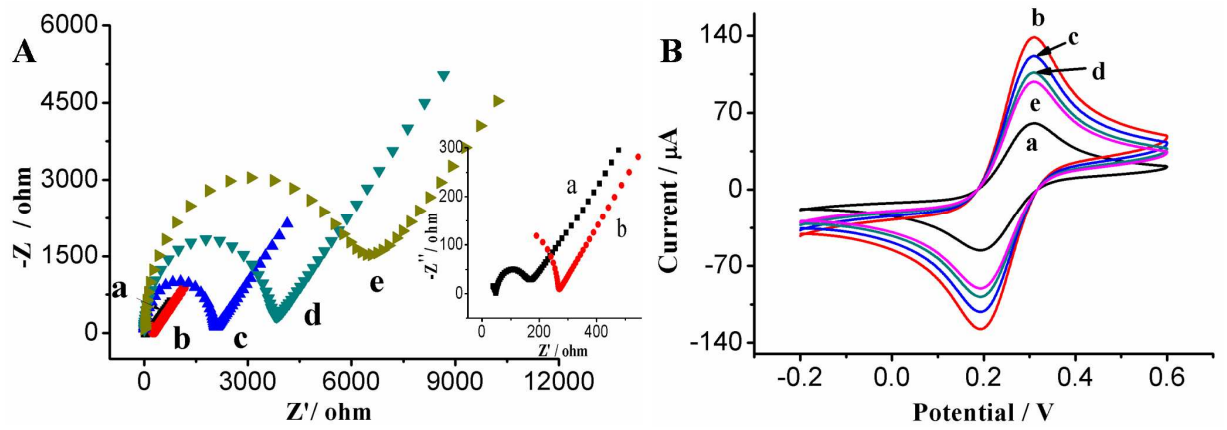


Figure 6. (A) EIS and (B) CVs recorded in PBS containing 5.0 mM redox probe $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. EIS conditions: frequency range from 1 to 100,000 Hz; the amplitude of the applied sine was set at 5 mV; and the current potential was set at 0.20 V; CV conditions: scanning range from - 1.2 to 1.2 V with scan rate of 0.10 V/s. (a: the bare electrode; b: GCE/PANI; c: GCE/PANI/PEG; d: GCE/PANI/PEG/C_1; e : GCE/PANI/PEG/C_1/T_2).

Electrochemical impedance spectroscopy (EIS) is a valuable and facile technique for characterizing the stepwise fabrication of a modified electrode.[51] In the Nyquist impedance plot, the semicircle portion (at higher frequency range) reflects the resistance of electron transfer (R_{et}), which controls the kinetics of redox probes at the modified electrode interface. Characteristic EIS plots of various electrodes were illustrated in Figure 6A. It was observed that PANI nanofibers modified GCE (curve b) showed an almost straight line, while the R_{et} of bare GCE was 180 Ω (curve a), which distinctly demonstrated the good electron transfer property of PANI nanofibers modified electrode. The R_{et} increased after the grafting of PEG onto PANI nanofibers (~2.0 kΩ, curves c), as non-conductive PEG will block the electron transfer to some degree.

However, this value is much smaller than that of pure PEG coated electrode (~120 kΩ),[52] indicating that PEGylated PANI nanofibers are still conductive. Predictably, there was a sharp increase in R_{et} as DNA capture probes were further immobilized onto the electrode surface, because the negatively charged phosphoric acid backbones of DNA probes repelled the negative $[Fe(CN)_6]^{3-/4-}$ probe and inhibited the electron transfer (curve d).[53] Interestingly, a further significant increase in R_{et} (~6.5 kΩ, curve e) could be observed when probe hybridized with its complementary target DNA, due to the formation of double-strand DNA. The result demonstrated that selective DNA hybridization was favorably occurred on the capture probe modified electrode.

Cyclic voltammetry (CV) technique was further used to characterize the modification process of the modified electrode. An obvious increase in peak current was observed after PANI nanofibers were modified on the electrode (Figure 6B curve b). As expected, the peak currents decreased slightly after modification with PEG, C_1 and T_2. These results were in accordance with the EIS characterization. To further verify that PEGylated PANI nanofibers remained electroactive, CV measurements in PBS were also used to study the electrochemical performances of GCEs modified with different PANI nanofibers, as shown in Figure S3. Clearly, a sharp oxidation peak at about 0.2 V and a reduction peak at about - 0.4 V were observed for the PANI nanofibers modified GCE due to the oxidation and reduction of PANI.[54] After the covalent bonding of PEG onto PANI nanofibers, although the magnitude of the oxidation peak current decreased, the oxidation peak of PEGylated PANI was still observed (curve b). This result indicated that PEG coated PANI nanofibers remained conductive.

3.3.2. DNA sensing with an indicator

The detection of BRCA1 was carried out by DPV transduction of the hybridization reaction between the immobilized capture DNA probe and the target BRCA1 (T_2), using MB as an electrochemical indicator to enhance the sensing signal. Figure 7 shows typical DPV curves of the GCE/PANI/PEG/C_1 before (curve a) and after (curve b) hybridization with BRCA1. Well-defined DPV peaks at about - 0.31 V, related to the electrochemical reduction of the indicator MB, were clearly observed,[55] and the reduction current i_p of MB significantly decreased after BRCA1 hybridization. Two possible mechanism of MB interaction with DNA sequence have been reported: through electrostatic interaction with the negatively charged DNA backbone (with many phosphate groups), or by interaction with DNA guanine bases. It has also been reported that MB showed a much higher affinity to single-strand DNA rather than double-strand DNA.[56-57] For instance, Yang and coworkers have reported the evidence of the direct interaction of MB with DNA guanine bases.[58] Erdem *et al* have reported that MB specifically interacted with guanine bases, and the DNA hybridization reaction limited the interaction between MB and guanine, because guanines were wrapped in duplex structure.[59] Therefore, along with the specific binding of target DNA to the immobilized DNA capture probe, a decrease in MB reduction current was expected, as shown in Figure 7, and this current change, in turn, can be used for the assay of target DNA.

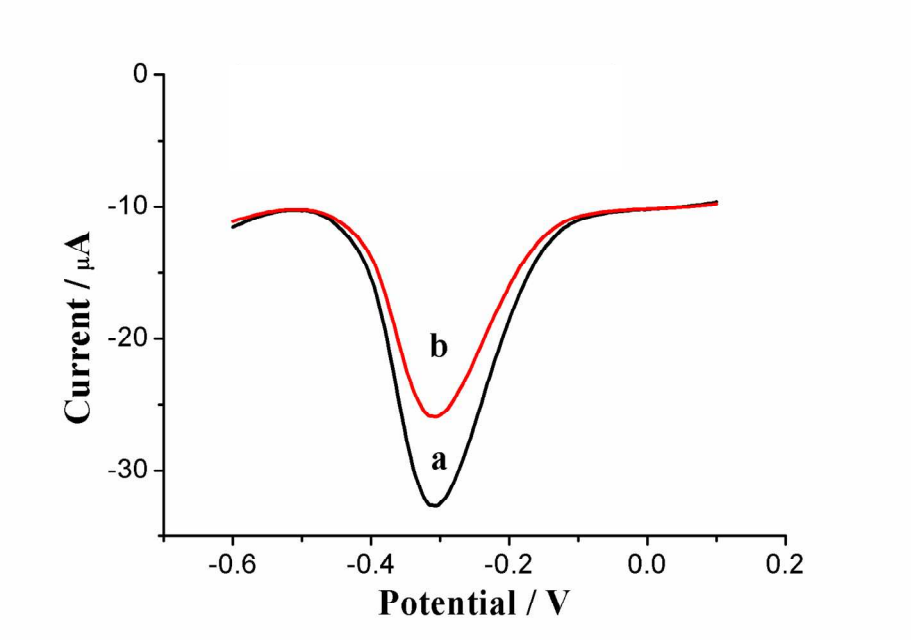


Figure 7. DPV curves of the DNA sensor before (curve a, black line) and after (curve b, red line) target BRCA1 (T_2) hybridization. The MB concentration was 20 μM, and the adsorption time for MB was 10 min; the added concentration of T_2 was 1 nM and the hybridization time was 30 min.

3.3.3. Optimization of conditions for the DNA detection

In order to select optimal conditions for BRCA1 detection with the prepared DNA sensor, the effects of MB concentration and MB interaction time with DNA, reaction time for DNA probe immobilization, and DNA hybridization time on the sensor response were selectively investigated in detail. The concentration of MB showed significant effect on the current change Δi_p before and after BRCA1 hybridization, and the Δi_p reached maximum value at 20 μM MB (Figure S4A). At a fixed interaction time, the increase in concentration of MB can accelerate the dynamic interaction between MB and the DNA sequence, resulting in an enhancement of the electrochemical response. When the concentration of MB was higher than 20 μM, the adsorption

of MB reached saturation, and therefore 20 μM MB was selected as the optimum concentration for MB. At a fixed concentration of MB (20 μM), the interaction of MB and DNA reached saturation at the time period of 10 min (Figure S4B). The immobilization time for capture probe immobilization and the target DNA hybridization time were also optimized, and the selected immobilization time for capture DNA probe was 1 h (Figure S4C), while the optimum DNA hybridization time was 30 min (Figure S4D).

3.3.4. Analytical performances of the DNA sensor

The performance of the developed biosensor for the sensing of target BRCA1 was initially studied in PBS using DPV. The current response of the biosensor decreased along with the increase of BRCA1 concentration (Figure S5), owing to the selective hybridization of target DNA and the formation of dsDNA. As shown in Figure 8A, the calibration curve exhibited a linear relationship between the measured DPV peak currents and logarithm values of the target concentrations within the range from 0.01 pM to 1 nM, and the corresponding regression equation was $\Delta i_p = 14.81 + 0.6583 \log C$, with the correlation coefficient (R^2) of 0.993. The limit of detection (LOD) was calculated to be 0.0038 pM (S/N = 3). According to the summarized performances of various biosensors (Table S3), the LOD of this developed biosensor is significantly lower than that of many previously reported assays.[60-65] It was clear that the target binding efficacy and the interfacial sensitivity resolved in this work was sufficient for the PEGylated PANI nanofibers based sensing interface to be detectably responsive to its target BRCA1 at very low levels.

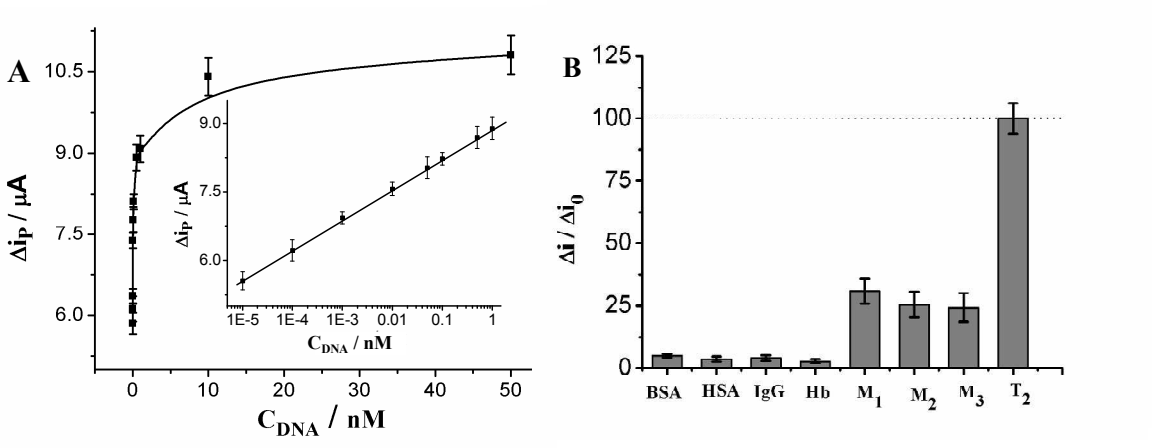


Figure 8. (A) Peak current change of the DNA sensor after incubation with controlled concentrations of BRCA1 in PBS (0.2 M, pH 7.4). Inset showed the corresponding calibration curve (R = 0.993). (B) Responses of the DNA sensor to 0.1 μM BSA, 0.1 μM HSA, 0.1 μM IgG, 0.1 μM Hb, 0.1 μM M_1, 0.1 μM M_2, 0.1 μM M_3, and 10.0 pM T_2. Error bars here represented the standard deviations across three repeated measurements.

To evaluate the specificity of the developed biosensor toward its target DNA, the response $\Delta i/\Delta i_0$ (%) of the DNA sensor was tested in different solutions containing BSA, HSA, IgG, Hb, M_1, M_2 or M_3 separately. As shown in Figure 8B, the biosensor exhibited good selectivity toward the target probe T_2, with the BSA, HSA, IgG and Hb showed almost no response, and the M_1, M_2 and M_3 showed relatively large responses. Since the tested concentration of BSA (and HSA, IgG, Hb, M_1, M_2 and M_3) is ten thousand times of the target probe (T_2), the selectivity of this sensor is acceptable (in a previous work, the Yao group has reported that single base-mismatched target RNA generated 60% signal as the target).[55] The specificity may be ascribed to the strong bioaffinity of the capture DNA probe to its target, and the good antifouling ability of PEGylated PANI nanofibers that effectively reduces the nonspecific adsorption. Five DNA sensors prepared independently were used to test 100 pM target DNA, and the measured result revealed a small relative standard deviation (RSD) of about 5.8%, indicating excellent reproducibility.

In this work, the stability of GCE/PANI/PEG and GCE/PANI/PEG/C_1 electrodes has been investigated by performing 50 cycles CV measurements in PBS (0.2 M, pH 5.7) with the potential range from -0.2 V to 0.8 V. As shown in Figure S6, the anodic and cathodic peaks of PANI are almost unchanged, indicating that both GCE/PANI/PEG and GCE/PANI/PEG/C_1 have a sufficient stability. The effect of scan rate on the electrochemical response of GCE/PANI/PEG and GCE/PANI/PEG/C_1 electrodes has also been investigated, as shown in Figure S7 and Figure S8. The cathodic peak currents of the GCE/PANI/PEG and GCE/PANI/PEG/C_1 electrodes showed linear relationship with the scan rate, demonstrating that the redox processes of the GCE/PANI/PEG and GCE/PANI/PEG/C_1 electrodes were surface confined processes with quick electron transfer kinetics. Additionally, the long-term stability of the GCE/PANI/PEG/C_1 biosensor was also evaluated by measuring its peak current of MB within ten days (Figure S9), and it retained approximately 92.14% of its original signal after ten days, showing very good stability.

The potential practical application of the developed DNA biosensor was estimated by detecting BRCA1 in human serum samples from healthy people and people with breast cancer, which were carefully prepared by adding accurate concentrations of BRCA1 into these samples. The analytical results were summarized in Table S4, with recoveries distribution between 95.4% to 105.6% and the RSD ranging from 3.58% to 8.14%. The assaying performance of the DNA sensor in human serum is generally acceptable, demonstrating that the PEGylated PANI nanofiber-based biosensor has great potential for DNA detection in complex real samples.

4. CONCLUSION

In summary, novel PEGylated PANI nanofibers were prepared by covalent grafting of PEG onto PANI nanofibers. The PEGylated PANI nanofibers remained conductive, and at the same time showed excellent antifouling ability to prevent the nonspecific adsorption from simple protein solutions and complex biological media. These nanofibers also possessed high immobilization capacity for capture probes, ensuring them to be used as promising matrices for the fabrication of antifouling biosensors. A novel ultrasensitive and low fouling electrochemical DNA hybridization biosensor has been developed to detect a breast cancer marker, BRCA1 related DNA sequence, based on the PEGylated PANI nanofibers. The DNA sensor was capable of assaying targets in human serum without suffering from nonspecific adsorption in complex biological media, owing to the excellent antifouling property of PEGylated PANI nanofibers. It is expected that highly sensitive and low fouling biosensors made on this basis will potentially find broad applications in the practical assay of human disease markers.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: Materials including scheme for covalent immobilization of 4-armed PEG and capture DNA onto PANI nanofibers, FTIR of PANI and PANI/PEG, optimization conditions of DNA sensor fabrication and sensing, and analytical results for BRCA1 in real samples.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wang, W.; Fan, X.; Xu, S.; Davis, J. J.; Luo, X. Low Fouling Label-free DNA Sensor Based on Polyethylene Glycols Decorated with Gold Nanoparticles for the Detection of Breast Cancer Biomarkers. *Biosens. Bioelectron.* **2015**, *71*, 51-56.

(2) Masson, J. F.; Battaglia, T. M.; Khairallah, P.; Beaudoin, S.; Booksh, K. S. Quantitative Measurement of Cardiac Markers in Undiluted Serum. *Anal. Chem.* **2007**, *79*, 612-619.

(3) Luo, X.; Xu, Q.; James, T.; Davis, J. J. Redox and Label-Free Array Detection of Protein Markers in Human Serum. *Anal. Chem.* **2014**, *86*, 5553-5558.

(4) Chen, L.; Ye, R.; Zhang, W.; Hu, C.; Zhou, B.; Peterson, D. R.; Au D. W. T.; Lam P. K. S.; Qian, P. Y. Endocrine Disruption throughout the Hypothalamus-Pituitary-Gonadal-Liver (HPGL) Axis in Marine Medaka (Oryzias Melastigma) Chronically Exposed to the Antifouling and Chemopreventive Agent, 3, 3′-Diindolylmethane (DIM). *Chem. Res. Toxicol.* **2016**, *29*, 1020-1028.

(5) Danelian, E.; Karlen, A.; Karlsson, R.; Winiwarter, S.; Hansson, A.; Lofas, S.; Lennernas, H.; Hamalainen, M. D. SPR Biosensor Studies of the Direct Interaction between 27 Drugs and a Liposome Surface: Correlation with Fraction absorbed in Humans. *J. Med. Chem.* **2000**, *43*, 2083-2086.

(6) Sun, Q.; Yan, F.; Yao, L.; Su, B. Anti-Biofouling Isoporous Silica-Micelle Membrane Enabling Drug Detection in Human Whole Blood. *Anal. Chem.* **2016**, *88*, 8364-8368.

(7) Kang, S.; Lee, M.; Kang, M.; Noh, M.; Jeon, J.; Lee, Y.; Seo, J. H. Development of Anti-biofouling Interface on Hydroxyapatite Surface by Coating Zwitterionic MPC Polymer Containing Calcium-binding Moieties to Prevent Oral Bacterial Adhesion. *Acta Biomater.* **2016**, *40*, 70-77.

(8) Salomon, S.; Leichle, T.; Dezest, D.; Seichepine, F.; Guillon, S.; Thibault, C.; Vieu, C.; Nicu, L. Arrays of Nanoelectromechanical Biosensors Functionalized by Microcontact Printing. *Nanotechnology* **2012**, *23*, 495-501.

(9) Wang, N.; Burugapalli, K.; Song, W.; Halls, J.; Moussy, F.; Ray, A.; Zheng, Y. Electrospun Fibro-porous Polyurethane Coatings for Implantable Glucose Biosensors. *Biomaterials* **2013**, *34*, 888-901.

(10) Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Bio-smart Hydrogels: Co-joined Molecular Recognition and Signal Transduction in Biosensor Fabrication and Drug Delivery. *Biosens. Bioelectron.* **2002**, *17*, 973-981.

(11) Pérez, J. H.; López-Ruiz, B.; López-Cabarcos, E. Synthesis and Characterization of Microparticles Based on Poly-methacrylic Acid with Glucose Oxidase for Biosensor Applications. *Talanta* **2016**, *149*, 310-318.

(12) Fan, X., Su, Y., Zhao, X., Li, Y., Zhang, R., Ma, T., Jiang, Z. Manipulating the Segregation Behavior of Polyethylene Glycol by Hydrogen Bonding Interaction to Endow Ultrafiltration Membranes with Enhanced Antifouling Performance. *J. Membr. Sci.* **2016**, *499*, 56-64.

(13) Pino, P.; Yang, F.; Pelaz, B.; Zhang, Q.; Kantner, K.; Hartmann, R.; Soenen, S. J. Basic Physicochemical Properties of Polyethylene Glycol Coated Gold Nanoparticles That Determine Their Interaction with Cells. *Angew. Chem., Int. Ed.* **2016**, *55*, 5483-5487.

(14) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angew. Chem., Int. Ed.* **2010**, *49*, 6288-6308.

(15) Hucknall, A.; Rangarajan, S.; Chilkoti, A. In Pursuit of Zero: Polymer Brushes That Resist the Adsorption of Proteins. *Adv. Mater.* **2009**, *21*, 2441-2446.

(16) Banerjee, I.; Pangule, R. C.; Kane, R. S. Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Adv. Mater.* **2011**, *23*, 690-718.

(17) Meyerbröker, N.; Kriesche, T.; Zharnikov, M. Novel Ultrathin Poly(ethylene glycol) Films as Flexible Platform for Biological Applications and Plasmonics. *ACS Appl. Mater. Interfaces* **2013**, *5*, 2641-2649.

(18) Lau, K. H. A.; Ren, C.; Park, S. H.; Szleifer, I.; Messersmith, P. B. An Experimental-Theoretical Analysis of Protein Adsorption on Peptidomimetic Polymer Brushes. *Langmuir* **2012**, *28*, 2288-2298.

(19) Satulovsky, J.; Carignano, M. A.; Szleifer, I. Kinetic and Thermodynamic Control of Protein Adsorption. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 9037-9041.

(20) Tugulu, S.; Klok, H. A. Stability and Nonfouling Properties of Poly (poly (ethylene glycol) methacrylate) Brushes under Cell Culture Conditions. *Biomacromolecules* **2008**, *9*, 906-912.

(21) Stadler, V.; Kirmse, R.; Beyer, M.; Breitling, F.; Ludwig, T.; Bischoff, F. R. PEGMA/MMA Copolymer Graftings: Generation, Protein Resistance, and a Hydrophobic Domain. *Langmuir* **2008**, *24*, 8151-8157.

(22) Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A. "Non-Fouling" Oligo (ethylene glycol)-Functionalized Polymer Brushes Synthesized by Surface-Initiated Atom Transfer Radical Polymerization. *Adv. Mater.* **2004**, *16*, 338-341.

(23) Emilsson, G.; Schoch, R. L.; Feuz, L.; Höök, F.; Lim, R. Y.; Dahlin, A. B. Strongly Stretched Protein Resistant Poly (ethylene glycol) Brushes Prepared by Grafting-to. *ACS Appl. Mater. Interfaces* **2015**, *7*, 7505-7515.

(24) Mazzotta, F.; Johnson, T. W.; Dahlin, A. B.; Shaver, J.; Oh, S.-H.; Höök, F. Influence of the Evanescent Field Decay Length on the Sensitivity of Plasmonic Nanodisks and Nanoholes. *ACS Photonics* **2015**, *2*, 256-262.

(25) Huang, N. P.; Michel, R.; Voros, J.; Textor, M.; Hofer, R.; Rossi, A.; Elbert, D. L. Poly (L-lysine)-g-Poly (ethylene glycol) Layers on Metal Oxide Surfaces: Surface-analytical Characterization and Resistance to Serum and Fibrinogen Adsorption. *Langmuir* **2001**, *17*, 489-498.

(26) Flavel, B. S.; Jasieniak, M.; Velleman, L.; Ciampi, S.; Luais, E.; Peterson, J. R.; Griesser, H. J.; Shapter, J. G.; Gooding, J. J. Grafting of Poly(ethylene glycol) on Click Chemistry Modified Si(100) Surfaces. *Langmuir* **2013**, *29*, 8355-8362

(27) Jo, S.; Park, K. Surface Modification Using Silanated Poly (ethylene glycol). *Biomaterials* **2000**, *21*, 605-616.

(28) Weathers, A.; Khan, Z. U.; Brooke, R.; Evans, D.; Pettes, M. T.; Andreasen, J. W.; Crispin, X.; Shi, L. Significant Electronic Thermal Transport in the Conducting Polymer Poly(3,4-ethylenedioxythiophene). *Adv. Mater.* **2015,** *27*, 2101-2106.

(29) Wu, Z. S.; Parvez, K.; Li, S.; Yang, S.; Liu, Z.; Liu, S.; Feng, X.; Müllen, K. Alternating Stacked Graphene-Conducting Polymer Compact Films with Ultrahigh Areal and Volumetric Capacitances for High-Energy Micro-Supercapacitors. *Adv. Mater.* **2015**, *27*, 4054-4061.

(30) Bai, S.; Sun, C.; Wan, P.; Wang, C.; Luo, R.; Li, Y.; Liu, J.; Sun, X. Transparent Conducting Films of Hierarchically Nanostructured Polyaniline Networks on Flexible Substrates for High-Performance Gas Sensors. *Small* **2015**, *11*, 306-310.

(31) Lu, X.; Zhang, W.; Wang, C.; Wen, T.; Wei, Y. One-dimensional Conducting Polymer Nanocomposites: Synthesis, Properties and Applications. *Prog. Polym. Sci.* **2011**, *36*, 671-712.

(32) Tran, H. D.; Li, D.; Kaner, R. B. One-Dimensional Conducting Polymer Nanostructures: Bulk Synthesis and Applications. *Adv. Mater.* **2009**, *21*, 1487-1499.

(33) Huang, J.; Virji, S.; Weiller, B. H.; Kaner, R. B. Polyaniline Nanofibers: Facile Synthesis and Chemical Sensors. *J. Am. Chem. Soc.* **2003**, *125*, 314-315.

(34) Ishikawa, F. N.; Chang, H. K.; Curreli, M.; Liao, H. I.; Olson, C. A.; Chen, P. C.; Zhang, R.; Roberts, R. W.; Sun, R.; Cote, R. J.; Thompson, M. E.; Zhou, C. Label-free, Electrical Detection of the SARS Virus N-protein with Nanowire Biosensors Utilizing Antibody Mimics as Capture Probes. *ACS Nano* **2009**, *3*, 1219-1224.

(35) Hao, Y.; Zhou, B.; Wang, F.; Li, J.; Deng, L.; Liu, Y. N. Construction of Highly Ordered Polyaniline Nanowires and their Applications in DNA Sensing. *Biosens. Bioelectron.* **2014**, *52*, 422-426.

(36) Lee, I.; Luo, X.; Cui, X. T.; Yun, M. Highly Sensitive Single Polyaniline Nanowire Biosensor for the Detection of Immunoglobulin G and Myoglobin. *Biosens. Bioelectron.* **2011**, *26*, 3297-3302.

(37) Siegel, R.; Naishadham, D.; Jemal, A. Cancer Statistics, 2013. *CA Cancer J. Clin.* **2013**, *63*, 11-30.

(38) Luo, X.; Killard, A. J.; Smyth, M. R. Nanocomposite and Nanoporous Polyaniline Conducting Polymers Exhibit Enhanced Catalysis of Nitrite Reduction. *Chem. Eur. J.* **2007**, *13*, 2138-2143.

(39) Briand, E.; Salmain, M.; Herry, J. M.; Perrot, H.; Compère, C.; Pradier, C. M. Building of an Immunosensor: How can the Composition and Structure of the Thiol Attachment Layer Affect the Immunosensor Efficiency. *Biosens. Bioelectron.* **2006**, *22*, 440-448.

(40) Zamfir, L. G.; Geana, I.; Bourigua, S.; Rotariu, L.; Bala, C.; Errachid, A.; Jaffrezic-Renault, N. Highly Sensitive Label-free Immunosensor for Ochratoxin A based on Functionalized Magnetic Nanoparticles and EIS/SPR Detection. *Sens. Actuators, B* **2011**, *159*, 178-184.

(41) Bryan, T.; Luo, X.; Forsgren, L.; Morozova-Roche, L. A.; Davis, J. J. The Robust Electrochemical Detection of a Parkinson's Disease Marker in Whole Blood Sera. *Chem. Sci.* **2012**, *3*, 3468-3473.

(42) Zhao M.; Wu X.; Cai C. Polyaniline Nanofibers: Synthesis, Characterization, and Application to Direct Electron transfer of Glucose Oxidase. *J. Phys. Chem. C* **2009**, *113*, 4987-4996.

(43) Reddy, K. R.; Sin, B. C.; Ryu, K. S.; Kim, J. C.; Chung, H.; Lee, Y. Conducting Polymer Functionalized Multi-walled Carbon Nanotubes with Noble Metal Nanoparticles: Synthesis, Morphological Characteristics and Electrical Properties. *Synth. Met.* **2009**, *159*, 595-603.

(44) Guo, Q.; Yi, C.; Zhu, L.; Yang, Q.; Xie, Y. Chemical Synthesis of Cross-linked Polyaniline by a Novel Solvothermal Metathesis Reaction of P-dichlorobenzene with Sodium Amide. *Polymer* **2005**, *46,* 3185-3189.

(45) Matharu, Z.; Sumana, G.; Arya, S. K.; Singh, S. P.; Gupta, V., Malhotra, B. D. Polyaniline Langmuir-Blodgett Film based Cholesterol Biosensor. *Langmuir* **2007**, *23*. 13188-13192.

(46) Devi, R.; Yadav, S.; Pundir, C. S. Electrochemical Detection of Xanthine in Fish Meat by Xanthine Oxidase Immobilized on Carboxylated Multiwalled Carbon Nanotubes/Polyaniline Composite Film. *Biochem. Eng. J.* **2011**, *58*, 148-153.

(47) Ye, H.; Wang, L.; Huang, R.; Su, R.; Liu, B.; Qi, W.; He, Z. Superior Antifouling Performance of a Zwitterionic Peptide Compared to an Amphiphilic, Non-Ionic Peptide. *ACS Appl. Mater. Interfaces* **2015**, *7*, 22448-22457.

(48) Lin, S.; Zhang, B.; Skoumal, M. J.; Ramunno, B.; Li, X.; Wesdemiotis, C.; Liu, L.; Jia, L. Antifouling Poly(β-peptoid)s. *Biomacromolecules* **2011**, *12*, 2573-2582.

(49) Chen, S.; Li, L.; Zhao, C.; Zheng, J. Surface Hydration: Principles and Applications toward Low-Fouling/Nonfouling Biomaterials. *Polymer* **2010**, *5*, 5283-5293.

(50) Lai, G.; Zhang, H.; Tamanna, T.; Yu, A. Ultrasensitive Immunoassay Based on Electrochemical Measurement of Enzymatically Produced Polyaniline. *Anal. Chem.* **2014**, *86*, 1789-1793.

(51) Elshafey, R.; Siaj, M.; Zourob, M. DNA Aptamers Selection and Characterization for Development of Label-free Impedimetric Aptasensor for Neurotoxin Anatoxin-a. *Biosens. Bioelectron.* **2015**, *68*, 295-302.

(52) Xu, M.; Luo, X.; Davis, J. J. The Label Free Picomolar Detection of Insulin in Blood Serum. *Biosens. Bioelectron.* **2013**, *39*, 21-25.

(53) Wang, L.; Hua, E.; Liang, M.; Ma, C.; Liu, Z.; Sheng, S.; Liu, M.; Xie, G.; Feng, W. Graphene Sheets, Polyaniline and AuNPs based DNA Sensor for Electrochemical Determination of BCR/ABL Fusion Gene with Functional Hairpin Probe. *Biosens. Bioelectron.* **2014**, *51*, 201-207.

(54) Dey, A.; Kaushik, A.; Arya, S. K.; Bhansali, S. Mediator Free Highly Sensitive Polyaniline-Gold Hybrid Nanocomposite based Immunosensor for Prostate-Specific Antigen (PSA) Detection. *J. Mater. Chem.* **2012**, *22*, 14763.

(55) Li, F.; Peng, J.; Zheng, Q.; Guo, X.; Tang, H.; Yao, S. Carbon Nanotube-Polyamidoamine Dendrimer Hybrid-Modified Electrodes for Highly Sensitive Electrochemical Detection of MicroRNA24. *Anal. Chem.* **2015**, *87*, 4806-4813.

(56) Gangopadhyay, R.; Chowdhury, A. D.; De, A. Functionalized Polyaniline Nanofibers for Biosensing. *Sens. Actuators, B* **2012**, *171*, 777-785.

(57) Pan, D.; Zuo, X.; Wan, Y.; Wang, L.; Zhang, J.; Song, S.; Fan, C. Electrochemical Interrogation of Interactions between Surface-confined DNA and Methylene Blue. *Sensors* **2007**, *7*, 2671-2680.

(58) Yang, W. R.; Ozsoz, M.; Hibbert, D. B.; Gooding, J. J. Evidence for the Direct Interaction between Methylene Blue and Guanine Bases Using DNA-modified Carbon Paste Electrodes. *Electroanalysis* **2002**, *14*, 1299-1302.

(59) Erdem, A.; Ozsoz, M. Interaction of the Anticancer Drug Epirubicin with DNA. *Anal. Chim. Acta* **2001**, *437*, 107-114.

(60) Loo, A. H.; Sofer, Z.; Bouša, D.; Ulbrich, P.; Bonanni, A.; Pumera, M. Carboxylic Carbon Quantum Dots as a Fluorescent Sensing Platform for DNA Detection. *ACS Appl. Mater Interfaces*, **2016**, *8*, 1951-1957.

(61) Yun, W.; Jiang, J.; Cai, D.; Zhao, P.; Liao, J.; Sang, G. Ultrasensitive Visual Detection of DNA with Tunable Dynamic Range by Using Unmodified Gold Nanoparticles and Target Catalyzed Hairpin Assembly Amplification. *Biosen*. *Bioelectron*. **2016**, *77*, 421-427.

(62) Tian, J.; Liu, Q.; Shi, J.; Hu, J.; Asiri, A. M.; Sun, X.; He, Y. Rapid, Sensitive, and Selective Fluorescent DNA Detection Using Iron-based Metal-Organic Framework Nanorods: Synergies of the Metal Center and Organic Linker. *Biosen*. *Bioelectron*. **2015**, *71*, 1-6.

(63) Ren, X.; Yan, T.; Zhang, S.; Zhang, X.; Gao, P.; Wu, D.; Du, B.; Wei, Q. Ultrasensitive Dual Amplification Sandwich Immunosensor for Breast Cancer Susceptibility Gene Based on Sheet Materials. *Analyst* **2014**, *139*, 3061-3068.

(64) Chen, M.; Hou, C.; Huo, D.; Bao, J.; Fa, H.; Shen, C. An Electrochemical DNA Biosensor Based on Nitrogen-doped Graphene/Au Nanoparticles for Human Multidrug Resistance Gene Detection. *Biosen*. *Bioelectron*. **2016**, 85, 684-691.

(65) Fan, H.; Zhang, Y.; Wu, D.; Ma, H.; Li, X.; Li, Y.; Wang, H.; Li, H.; Du, B.; Wei, Q. Construction of Label-free Electrochemical Immunosensor on Mesoporous Carbon Nanospheres for Breast Cancer Susceptibility Gene. *Anal*. *Chim*. *Acta* **2013**, *770*, 62-67.

Table of content

PEGylated PANI nanofibers were fabricated using "grafting to" approach to form a thin layer of PEG onto PANI nanofiber surfaces. The resulting PEGylated PANI nanofibers, combining strong antifouling ability of PEG with the excellent electrochemical activity of PANI, provide an ideal substrate for the construction of a low fouling electrochemical biosensor for breast cancer susceptibility gene (BRCA1).