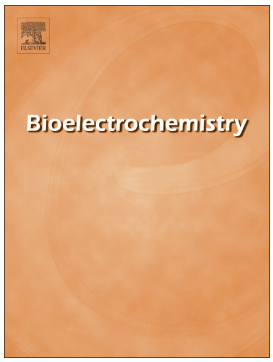

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Jiasheng Wang, Ni Hui

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Zwitterionic poly(carboxybetaine) functionalized conducting polymer polyaniline nanowires for the electrochemical detection of carcinoembryonic antigen in undiluted blood serum

Jiasheng Wang Ni Hui*

Qingdao Agricultural University, Qingdao266109, China

*Corresponding author. Tel: + 86 532 86080374; Fax: + 86 532 86080452; E-mail: huini1029@163.com

Abstract: biocompatible materials, ~~like~~ such as zwitterionic poly(carboxybetaine methacrylate) (polyCBMA), are of extraordinary importance in growth of bioelectronics and biosensors, because they not only greatly suppress nonspecific protein adsorption, but also have rich functional groups to facilitate the fixation of biological molecules. A novel nanocomposite was synthesized herein through modification polyCBMA onto conducting polymer polyaniline (PANI) nanowire surface. The prepared polyCBMA/PANI composite, integrating the good conductivity of PANI nanowires with the excellent antifouling capability of polyCBMA, provided a wonderful matrix for the growth of ultrasensitive and low fouling biosensor. Carcinoembryonic antigen (CEA), an important biomarker for a variety of cancers, was utilized as a model test. Furthermore its antibody was ~~antibodies were~~ fixed onto polyCBMA/PANI for the preparation of CEA biosensor. DPV was applied as a sensing principle with information at which peak potential and current the signals were recorded. ~~The fabricated immunosensor demonstrated ultrasensitive, favorable selectivity and ultralow detection limit. Assay linear range was from 1.0×10^{-14} g mL^{-1} to 1.0×10^{-10} g mL^{-1}~~ The peak currents were in inverse proportion to the logarithm of CEA concentration in the range from 1.0×10^{-14} g mL^{-1} to 1.0×10^{-10} g mL^{-1}, with a detect limit of 3.05 fg mL^{-1}. Furthermore, this low fouling, label-free biosensor has ~~biosensors have~~ been utilized for assaying in undiluted human serum samples with resisting serious nonspecific protein adsorption, demonstrating its feasible potential application in clinical analysis of CEA.

Keywords: poly(carboxybetaine methacrylate); polyaniline; antifouling;

carcinoembryonic antigen; immunosensor

1. Introduction:

Non-specific protein absorption, known as biofouling, is very important in numerous fields, including drug delivery systems, marine coatings, artificial organ implants, biomaterials and biosensors [1-4]. At present, only a few candidates available have been reported as "superlow fouling materials" and "nonfouling materials", such as poly(ethylene glycol) (PEG), and zwitterionic polymers [5-7]. It should be noted that PEG, a biocompatible and highly hydrophilic polymer, has widely been regarded as ~~a~~ gold standard for antifouling ~~unfouling~~ polymers [8]. However, PEG and its derivatives are susceptible to oxidative destruction in the presence of transition metal ions or oxygen existing in complex, real-world media [8, 9].

Zwitterionic materials, such as carboxybetaine (CB) and phosphorylcholine (PC), represent potentially excellent alternatives to common PEG because of their biocompatibility, antifouling performance and long-term stabilization [10-12]. It has been reported that zwitterionic surfaces form a hydration layer to resist nonspecific protein adsorption through electrostatic interactions and hydrogen bonding [12, 13]. Among them, poly(carboxybetaine methacrylate) ~~poly (carboxybetaine methacrylate)~~ (polyCBMA) has caught more and more interests as it can act as a biomimetic material with two roles of suppressing nonspecific protein adsorption effectively while supplying sufficient functional groups for immobilization of biological ligands ~~biorecognition units~~ [12]. CBMA has the similar structure to glycine betaine, which

exhibits an acid-base equilibrium. The abundant carboxylic groups of polyCBMA make it possible to covalently bind amino groups of proteins ~~PolyCBMA has been shown to be able to respond with protein functionalization through amino coupling chemistry reaction~~ in mild protein structure-protective environment [11, 12, 14]. Latest research results have demonstrated that polyCBMA can highly reduce platelet sticking and resist protein adsorption when used in undiluted blood serum and plasma [15, 16]. The experiment reports showed that polyCBMA offered ~~offer~~ great promise for utilization in complex blood media because of its effective antifouling ability and efficient functional performance.

In order to introduce an immobilization matrix for the antifouling material conjugation, polyaniline (PANI) nanowires were polymerized onto the surface and then antifouling materials containing carboxyl groups can be anchored to the PANI nanowires surface via covalent coupling chemistry. PANI, as an attractive conducting polymer, has shown important properties that can facilitate the development of high sensitive biosensors, because of its high electronic conductivity, high electrical appeal and low ionization potential [17-19]. It should be mentioned that PANI exhibits two redox couples in the convenient potential range which have been utilized as the sensing signal for the detection of DNA, carcinoembryonic antigen and alpha-fetoprotein [6, 20-22]. However, it has not been investigated that PANI nanowires ~~nanowire~~ not only provide ~~provides~~ the sensing signal using their ~~its~~ redox current, but also have ~~has~~ been utilized as a promising substrate for the fixation of antifouling materials.

In our work, we aim to prepare polyCBMA/PANI nanocomposites through photopolymerization way to wrap thin polyCBMA layer onto electrochemically polymerized PANI nanowires surface. The prepared polyCBMA/PANI nanowires, integrating stable antifouling performance of polyCBMA with the remarkable reactivity of PANI, supply a perfect matrix for the development of an ultrasensitive and nonfouling biosensor, as shown in ~~Scheme 1~~ Schematic 1. Here, Carcinoembryonic antigen (CEA), an important biomarker for a variety of cancers [23, 24], has been detected as a typical analyte. PolyCBMA/PANI and CEA antibody were married together, which supply biosensors with obvious merits such as high sensitivity and selectivity. CEA biosensors present ultralow ~~ultra-small~~ fouling interference to sustain a highly steady response even in human serum samples.

Schematic 1.

2. EXPERIMENTAL SECTION

2.1 Regents

Human serum albumin (HSA), lysozyme (LZ), human hemoglobin (Hb), Bovine serum albumin (BSA), CEA antibody and antigen, trypsinogen (Try) and Alpha fetoprotein (AFP) were bought from Xinkezhongjing Company ~~company~~ (Beijing, China). DNA sequence (5' GAA CAA AAG GAA GAA AAT C 3') was purchase by Sangon ~~biotechnology company~~ Biotechnology Company~~,~~ (Shanghai, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), aniline and N-hydroxysuccinimde (NHS) were obtained from Sigma Aldrich. β-propiolactone and

2-(dimethylamino) ethyl methacrylate were bought from WuXi AppTec ~~company~~ Company (Shanghai, China). Blood serum was supplied by the Qingdao People's Hospital (Qingdao, China). All reagents were of analytical grade ~~analytical reagents~~. Water used in the experiment was obtained from a Milli-Q water purifying system.

CBMA ~~Carboxybetaine methacrylate (CBMA)~~ monomer was prepared in accordance with the literature [25]. The mixed anhydrous acetone containing 2 mM β-propiolactone and 10 mM 2-(dimethylamino) ethyl methacrylate was stirred continuously at 15°C for 5 h under nitrogen surroundings. Then the white precipitate was washed using anhydrous acetone and ether respectively before being dried and stored up for use.

2.2 Apparatus

Electrochemical experiments were carried out on a CHI660E Electrochemical Workstation ~~workstation~~ (Shanghai CH Instrument Company, China). A traditional three-electrode system was utilized ~~utlized~~ with the modified glassy carbon electrode (GCE, diameter 3.0 mm) as the working electrode, an ~~a~~ Ag/AgCl electrode (filled with 1.0 M KCl) as the reference electrode and a platinum wire as the counter electrode. ~~Wetting conducts of the modified surfaces were estimated by contact angle detector~~ Static water contact angle measurements were carried out using a JC2000D1 goniometer (Shanghai Zhongchen Instrument Company ~~instrument company~~, China). Electron microscope (SEM) with the model of JEOL JSM-7500F (Hitachi High-Technology Co., Ltd., Japan) was utilized to characterize morphologies of prepared PANI nanowires and polyCBMA/PANI nanowires.

2.3 Surface modification

GCEs were polished with alumina slurries (1.0, 0.3 and 0.05 mm) in turn. They were cleaned in water, ethanol, and water for 3 min each ultrasonically, respectively. Then GCEs were electrochemically oxidized in phosphate buffered saline (PBS) according to literature [26]. The pretreated GCEs were dried in the protection of nitrogen gas.

As shown in ~~Scheme~~ Schematic 1, the CEA immunosensor was constructed step-wise. Firstly, PANI nanowires were electrodeposited onto the oxidized GCE by constant current method in 1.0 M $HClO_4$ and 0.5 M aniline mixed solution (The current density of 0.06, 0.03, 0.015 $mA \cdot cm^{-2}$ for 0.5 h, 3h, 3h was regulated, respectively). Secondly, the PANI nanowires ~~nanowire~~ modified electrodes incubated in a mixed solution containing 0.5 mM CBMA, 0.4 M EDC and 0.1 M NHS for 3 hours, in order to immobilize CBMA monomers to the PANI nanowire surface. Thirdly, a photopolymerization solution was prepared including CBMA (17.2 mg mL^{-1}), ethylene glycol dimethacrylate (0.2 mg mL^{-1}), and 2-hydroxy-2-methylpropiophenone (1.17 mg mL^{-1}) [27]. 5.0 μL of the photopolymerization solution was dropped onto the surface of the modified electrode. To start polymerization through the modified surface, the modified electrode was exposed under 254 nm light for half an hour. Fourthly, the polyCBMA/PANI/GCE was then hatched in 1.0 μg mL^{-1} CEA antibody, 0.4 M EDC and 0.1 M NHS for 3h, after immersing in PBS (10 mM, pH 7.4, 24 h) to balance the zwitterionic polymer. Lastly, prior to analysis the modified electrodes were immersed in BSA for 60 min

(100 μM), washed vastly with buffer, so as to deactivate the residual activated carboxyl units on the modified surface. The fabricated CEA immunosensors (Ab/polyCBMA/PANI/GCEs) were sufficiently rinsed and stored in PBS at room temperature.

2.4 Electrochemical tests.

Cyclic voltammetry (CV) measurements were carried out in 0.2 M PBS (pH 5.7) in the potential from -0.4 V to 0.8 V at scan rate of 0.1 V s^{-1}. In order to perform ~~performance~~ the immunoreaction and electrochemical measurement, the modified biosensor (Ab/polyCBMA/PANI/GCE) was first hatched in different concentration of CEA standard solutions or human serum for 1 h at room temperature, then followed by rinsing with water and PBS repeatedly, and differential pulse voltammetry (DPV) was accomplished in PBS (0.2 M, pH 5.7) to register typical peaks of PANI nanowires electrodeposited onto the modified electrode. In order to calculate the recovery rate, the CEA standard solutions were added into serum samples; subsequently the same electrochemical tests were recorded. The DPV parameter was set in a potential range of -0.4 to 0.6 V, along with the pulse width of 0.05 s and the amplitude of 0.05 V.

For regeneration of the immunosensor, the biosensor toward 1.0×10^{-10} g mL^{-1} CEA was exposed to a solution of 7 M urea for 5 min, and washed with water adequately.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the modified electrodes.

Fig. 1.

The surface images of PANI nanowires and polyCBMA/PANI nanowires were characterized by SEM. The obtained PANI nanowires presented a very neat form on the electrode surface (Fig. 1A and B). The diameter of aligned PANI nanowires was about 50 nm, and ~~it was observed that~~ all of them were oriented perpendicular to the electrode. It was observed that the PANI nanowires were uniformly wrapped by polyCBMA, and the structure of uniform nanowire array almost kept unchanged (Fig. 1C and D). Compared with PANI nanowires, the diameter of the polyCBMA/PANI nanowires expanded to 150 nm, suggesting successful wrapping of PANI nanowires with polyCBMA.

In order to describe the hydrophilicity of the different surface, contact angles of PANI nanowires and polyCBMA/PANI nanowires modified surfaces were estimated. With the modification of PANI nanowires and polyCBMA, the contact angle greatly reduced (Fig. 2 and Table S1,). The PANI nanowires modified surface demonstrated moderate hydrophilicity with the contact angle of 56.3°. Predictably, after further wrapping of PANI nanowires with polyCBMA, the contact angle of polyCBMA/PANI decreased ~~greatly changed from 56.3°~~ to 12.5°, indicating good hydrophilicity of the polyCBMA/PANI nanowires. The contact angle of polyCBMA/PANI nanowires is smaller than that of previous antifouling materials, such as PEG/PANI (22.4°) and the peptide SAM (19.6°) [28, 29]. The nonfouling mechanism of zwitterionic materials have indicated that a nonfouling surface around the zwitterionic carboxybetaine groups can be obtained when the surface possesses a nanometer-scale consubstantial

hybrid of balanced charge groups from zwitterionic substance since they are strongly hydrated via ionic solvation, which is the key to their antifouling ability [30, 31]. Thus polyCBMA/PANI nanowires with super-hydrophilicity can effectively suppress protein nonspecific adsorption in the biological medium and support an ideal matrix for the development of antifouling biosensor.

Fig. 2.

3.2 Electrochemical characterization of the modified electrodes

For the construction and measurement ~~measurment~~ of the CEA immunosensor, the bare GCEs were modified with PANI, CBMA, polyCBMA and CEA antibody, and hatched with CEA subsequently, and the whole process was investigated by CV and DPV. As shown in Fig. 3A, a pair of redox peaks of PANI were observed in the CV curves, which are ascribed to the redox transition of PANI between the leucoemeraldine and emeraldine [32, 33]. After the immobilization of CBMA onto PANI nanowires and the formation of polyCBMA, the oxidation peak current signal decreased because of the fact that the polyCBMA retarded the electron transport to some degree. Following the immobilization of CEA antibodies onto the polyCBMA/PANI/GCE ~~PANI/polyCBMA/GCE~~ surface, the peak current of oxidation slightly reduced (curve d), proving that CEA antibodies were attached onto polyCBMA/PANI. What's more interesting is that the specific binding of target CEA antibody to the immunosensor has caused an even greater decrease in the peak current when it's attached to the electrodes (curve e), as the non-conductive protein thin layers

on the biosensor surface can hinder the electrochemical redox reaction of the PANI.

DPV is used as the detection technology because its sensitivity is superior to that of CV. As shown in Fig. 3B, after polyCBMA is attached to PANI nanowires, the value of DPV signals decreased; but the DPV peaks of polyCBMA/PANI were still seen (curve b and c). These results proved that polyCBMA coated PANI nanowires remained conductive. Similar to the CV results described above, the peak current signals ~~signal~~ decreased after the modification of CEA antibody and CEA (curve d and e). Obviously, based on the regular variation of DPV signal with the specific combination of CEA target to its antibody specifically, a biosensor for the quantitative CEA testing can be triumphantly fabricated.

Fig. 3.

3.3 Optimization of experimental conditions

To improve the sensitivity and rapidity of the immunoreaction, the conditions of pH and incubation time were investigated. Fig. 4A showed that as pH increased to 5.7, the current responses ~~reach~~ reached the maximization. Thus in this experiment pH 5.7 PBS was selected as the testing medium. When antigens arrived at the antibodies immobilized on the immunosensors, it need take some time to form ~~from~~ the specific antibody-antigen immunocomplexes. Clearly it can be seen that after 45 min the current response reached a plateau, exhibiting a saturated binding between the CEA antigen and the antibody on electrode (Fig. 4B). Therefore, 45 min was utilized as the proper incubation step time of the measurements.

Fig. 4.

3.4 Sensing performance of the immunosensor

The electrochemical response of Ab/polyCBMA/PANI/GCE has been investigated as a function of CEA concentration. DPV is employed as a sensitive analytical technique to study electrochemical changes induced by antibody-antigen interaction on the biosensor surface in optimal conditions. Clearly, DPV signal of the immunosensor fell with the increase of CEA concentration (Fig. 5A). There is a linear relationship between the peak current of the immunosensor and the logarithm of the target CEA concentration ranging from 1.0×10^{-14} g mL^{-1} to 1.0×10^{-10} g mL^{-1} (Fig. 5B). The linear regression equation can be described as I_P (μA) = -3.973-2.601 Log C (R^2=0.9940), and the detection limit was figured out to be as low as 3.05 fg mL^{-1} (S/N=3), which is much lower than those of other CEA modified biosensor [22, 34-36]. This good performance of the biosensor may be ascribed to the excellent biocompatibility of the obtained hydrophilic polyCBMA/PANI nanocomposite, which can supply an appropriate micro-environment for the fixed CEA antibody to show strong specific binding affinity toward CEA.

Fig. 5.

3.5 Antifouling, Specificity and reproducibility property of the biosensor

To assess the antifouling ability of CEA biosensors, their DPV signal changes after immersing in singe protein solution (BSA, HSA, Hb, LZ) and complex biological media (cow's milk, saliva, bovine fetal serum and human serum) were

investigated, respectively (Fig. 6, Fig. S1). Only before soaking in serum samples CEA biosensors were firstly saturated in standard CEA solution to excluded possible response change related to target binding. ~~The biosensors were soaked in serum samples in order to estimate their antifouling property.~~ Clearly, compared with only PANI nanowires modified electrode, the biosensor demonstrated significantly smaller DPV signal change ~~variety~~, whether after incubating in singe protein solution or complex biological media, suggesting good antifouling capacity of the sensing interface in both single protein solution and complex human serum samples.

The selectivity and reproducibility are key criteria ~~criterions~~ to evaluate a successful immunosensor for practical use. So as to investigate the reproducibility of the CEA immunosensor, six biosensors constructed independently were utilized to detect target CEA (10 pg mL^{-1}), and the relative standard deviation (RSD) was 6.6%. The CEA immunosensors were utilized to assay the same target CEA repetitively for six times, and the RSD was 2.85%, indicating good reproducibility.

To investigate the selectivity of the CEA immunosensor towards target CEA, the DPV responses (change in current) of the CEA biosensor were estimated in different solution such as BSA, PSA, AFP, HSA, ssDNA sequence and target antigen (CEA). As demonstrated in Fig. 7, the biosensors demonstrated negligible changes ~~change~~ in current to BSA, HSA, PSA, AFP, ssDNA even at very high artificially concentrations, proving outstanding selectivity of the biosensors ~~biosensor~~ to target CEA. These results may be due to two facts: (i) the strong specific bioaffinity between CEA antibody and CEA antigen, and (ii) the antifouling ability of the polyCBMA/PANI

nanowires, which can efficiently restrain the nonspecific protein attachment.

The possibility of reusing the immunosensor for five times through a regeneration step was estimated. The immunosensor (toward 1.0×10^{-10} g mL^{-1} CEA) was immersion in 7.0 M urea for 5 min (to disassociate the antigen-antibody immunocomplex~~-immune-complex~~) [37] and followed by thorough washing with PBS (pH=7.4). As shown in Fig. S2, the current response of the biosensor is stable after five repetitions.

An analysis of sensor stability was investigated by continuous CV measurement in PBS (0.2 M, pH 5.7), with the potential range from -0.4 to 0.8 V. As shown in Fig. S3, the transducing signal is largely unchanged after 50 cycles. A longer term analysis of peak current across 7 days shows a retention of >90% signal (Fig. S4)

Fig. 6.

Fig. 7.

3.6 The analysis of real samples

To assess the practical application of the prepared biosensor, the CEA concentration in different serum was perceived (The serum samples were diluted 10 times by 0.01 M pH 7.4 buffers before detection). As summarized in Table 1, the recovery rates were between from 94.3% to 104.2%, and the RSD was between 4.5%-5.9%. Furthermore, the application of the proposed immunosensor was also compared with a clinically approved electrochemiluminescence immunoassay. The proposed method is in good agreement with those by the electrochemiluminescence

immunoassay (ECI), demonstrating that the developed immunosensor can be utilized in the determination of CEA in serum samples.

Table 1.

4. Conclusion

In summary, an electrochemical CEA biosensor based on the novel biomaterial of polyCBMA/PANI nanowires was constructed ~~fortunately~~. The prepared polyCBMA/PANI nanowires demonstrated good conductivity and antifouling property from single protein solutions (BSA, HSA, Hb, LZ) and~~,~~ complex media (cow's milk, human serum, bovine fetal serum, saliva), because of their excellent hydrophilicity. And polyCBMA/PANI nanowires contained numerous carboxylic groups on their surface that can be employed for the attachment of biomolecules. Utilizing the special performance of polyCBMA/PANI nanowires, such as inherent electroactivity, good conductivity, nanowire microstructure (large specific surface area), excellent biocompatibility and fouling-resistant property, an ultrasensitive, label-free, and antifouling electrochemical biosensor for a model tumor marker CEA was developed, and it exhibited promising clinical applications in complex serum samples. It is expected that polyCBMA/PANI nanowires can be utilized for the development of other nonfouling electrochemical biosensors and bioelectronics.

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Captions

Schematic 1. Schematic illustration of the construction process of the CEA biosensor. (a) PANI nanowires electrodeposited onto the GCE, (b) covalently bound CBMA on PANI nanowires, (c) a photopolymerized polyCBMA layer on PANI nanowire, (d) the immobilization of CEA antibody on the polyCBMA/PANI composite, (e) specific CEA capturing and the antifouling towards nonspecific protein (f) DPV current signal recording.

Fig. 1. SEM morphologies of electrodes modified with PANI nanowire arrays (A, B) and polyCBMA/PANI nanocomposites (C, D).

Fig. 2. Contact angle images of (A) ITO, (B) PANI/ITO and (C) polyCBMA/PANI/ITO surfaces.

Fig. 3. CV (A) and DPV (B) recorded at (a) PANI/GCE, (b) CBMA/PANI/GCE, (c) polyCBMA/PANI/GCE, (d) Ab/polyCBMA/PANI/GCE and (e) CEA/Ab/polyCBMA/PANI/GCE in 0.2 M PBS (pH 5.7). The concentration of CEA is 1.0×10^{-12} g mL^{-1}.

Fig. 4. Effects of the (A) pH value and (B) CEA incubation time on the DPV responses of the immunosensor towards 10^{-14} g mL^{-1} CEA. Error bars represent the standard deviations of the change in peak current acquired from three repeated

measurements.

Fig. 5. (A) DPV responses of the biosensor towards different concentrations of target CEA and (B) the dynamic response range of the biosensor. Fig. A, curve a represents the blank solution and curves b–j refers to CEA at the concentration from 10^{-16} to 10^{-8} g mL^{-1}. Fig. B, from low to high, each point represents a concentration of 10^{-4} to 10^{5} fg mL^{-1}. The inset in Fig. B is the calibration curve of the biosensor. Error bars represent the standard deviations of the change in peak current acquired from three repeated measurements.

Fig. 6. The comparison of the antifouling property between PANI/GCE (black column) and Ab/polyCBMA/ PANI/GCE (red column) towards single protein solution (1: BSA; 2: HSA; 3: Hb; 4: LZ; $1.0x10^{-4}$ mg mL^{-1} each) (A), huaman serum (B), bovine fetal serum (C) and cow's milk (D). Note: only before soaking in serum samples, CEA biosensors were firstly saturated in standard CEA solution.

Fig. 7. The selectivity of the CEA biosensor towards BSA, PSA, AFP, HSA, DNA and target antigen CEA.

Schematic 1.

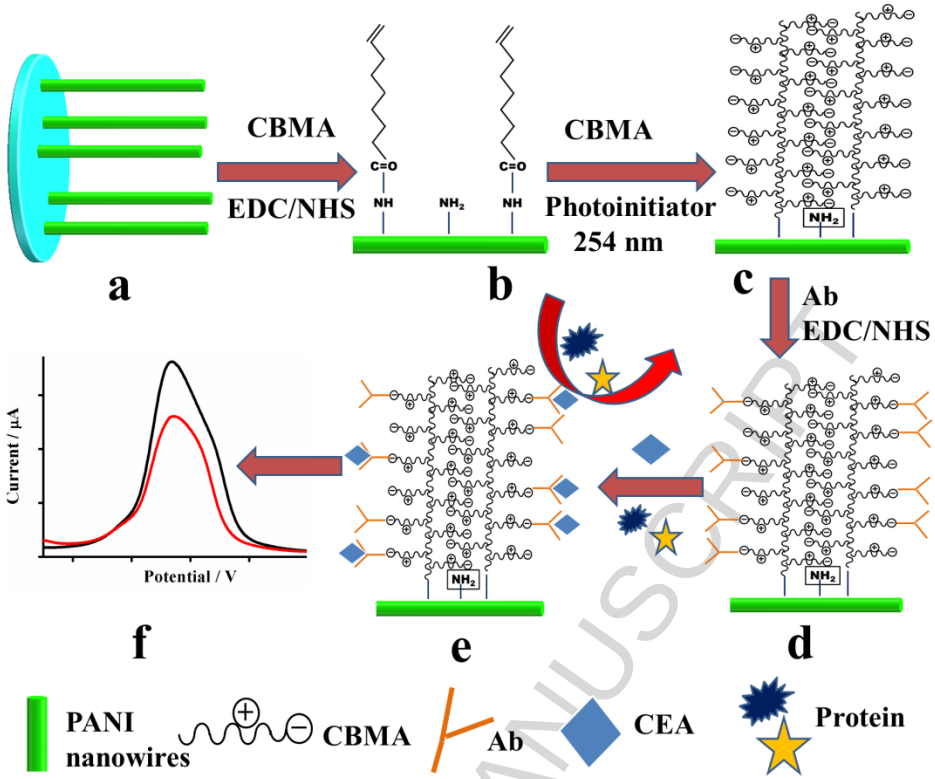


Fig. 1.

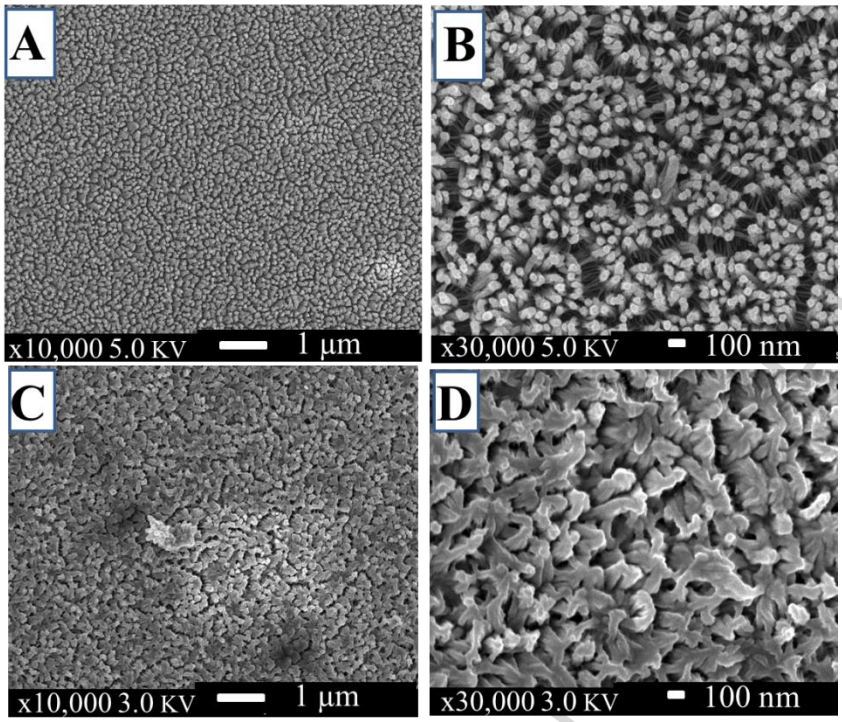
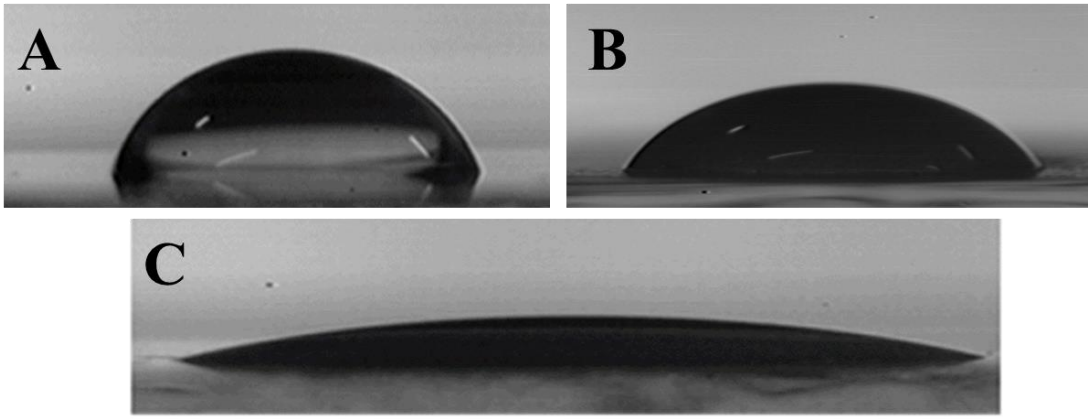


Fig. 2.



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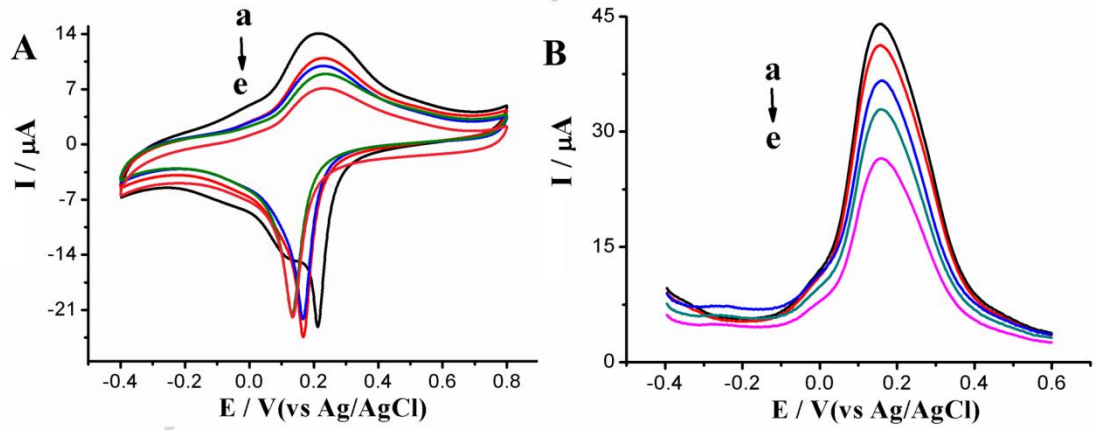


Fig. 4.

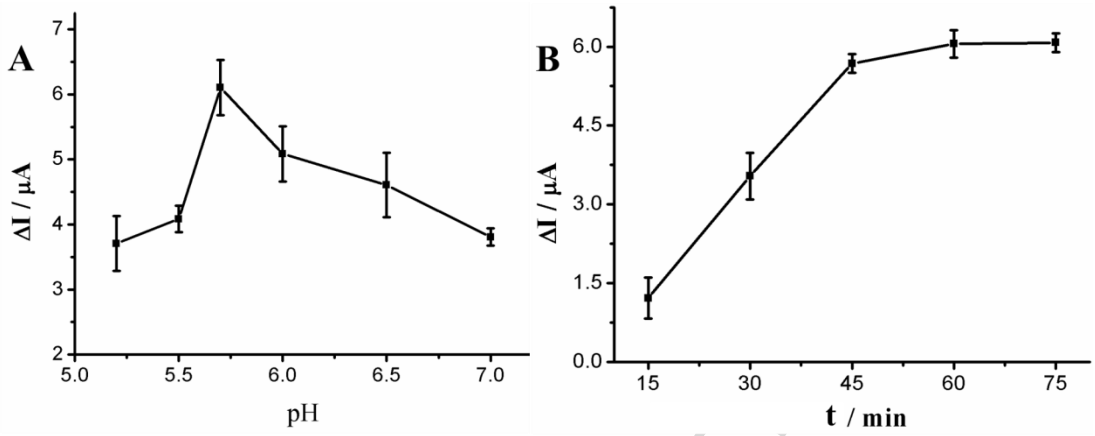


Fig. 5.

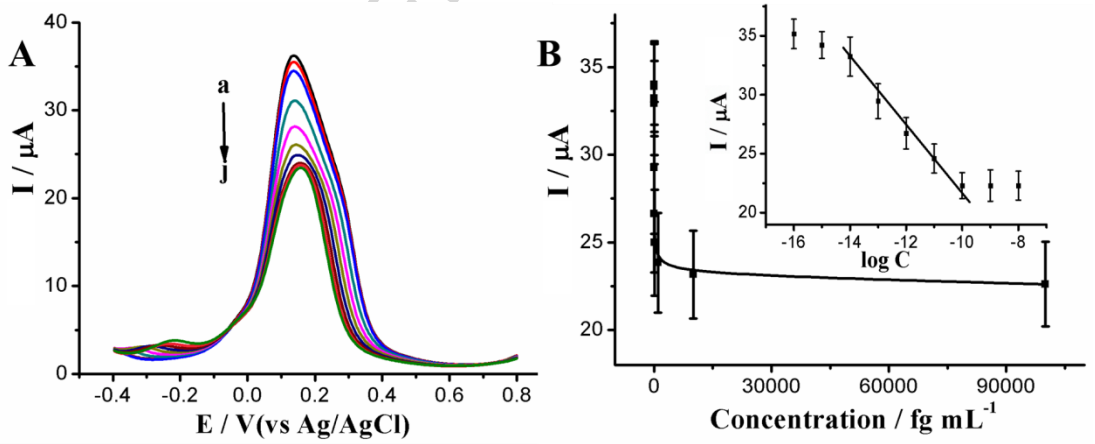


Fig. 6.

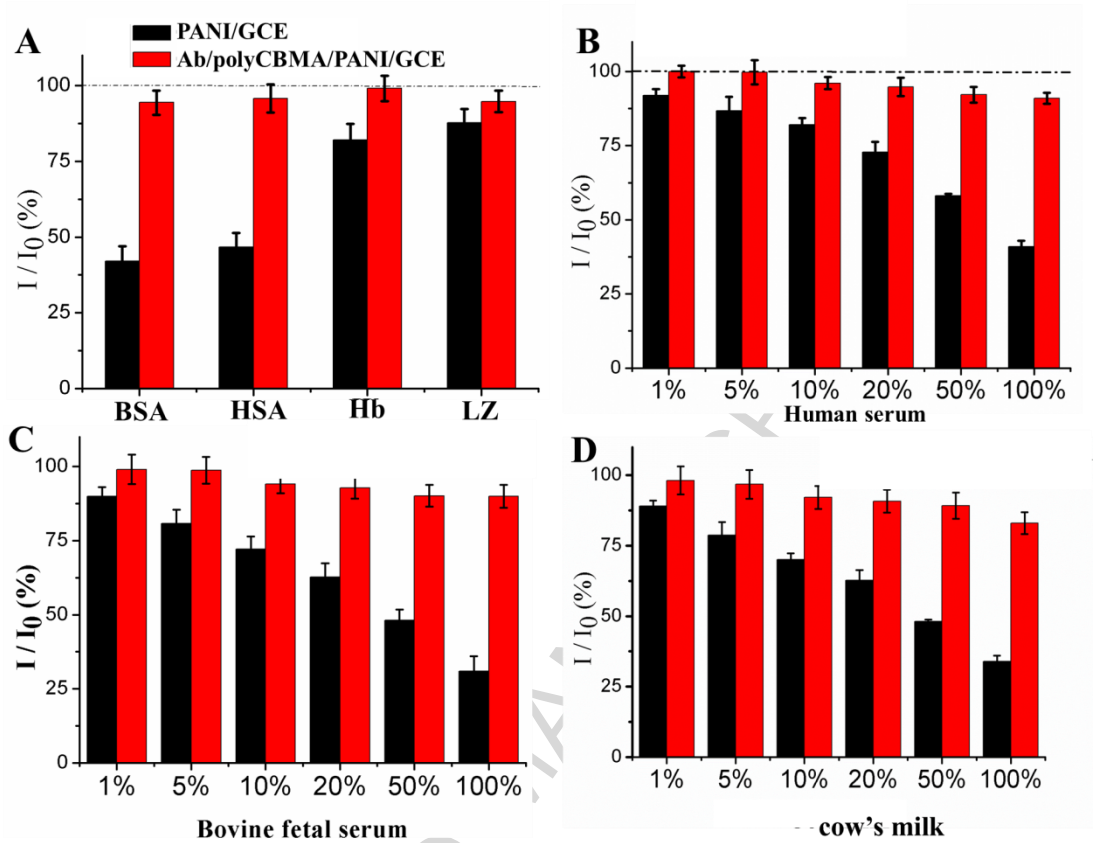


Fig. 7.

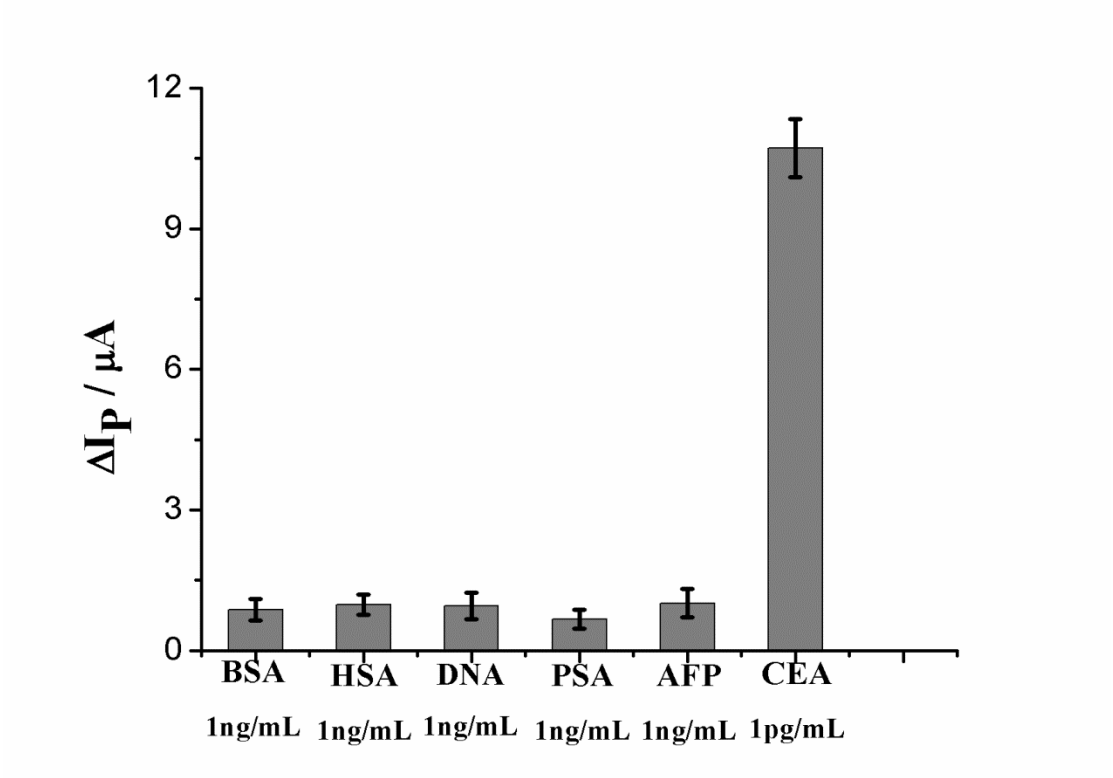
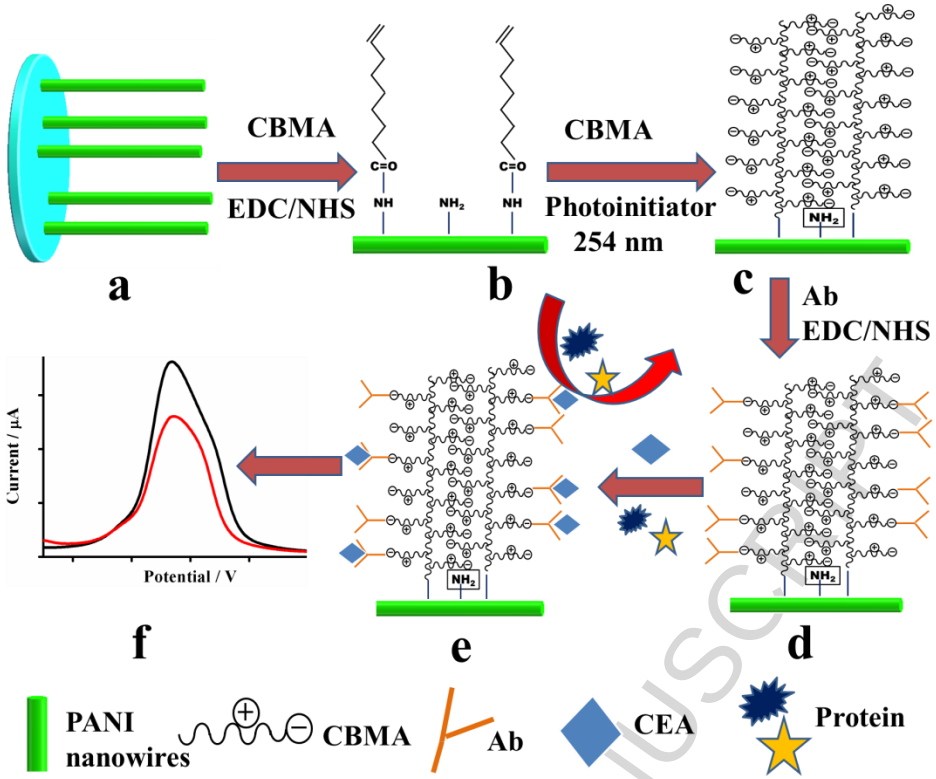


Table 1. Determination of CEA in human serum samples.

Samples	CEA value measured by ECI (ng mL^{-1})	The prepared immunosensor			
		Measured CEA value (ng mL^{-1})	RSD (%)	Added CEA (ng mL^{-1})	Recovery (%)
1	0.251	0.252	5.1	0.15	96.5
2	0.455	0.457	4.5	0.20	97.1
3	0.848	0.854	5.9	0.10	104.2
4	0.591	0.587	4.7	0.15	94.3

Note: Human serum samples were diluted 10 times by 0.01 M pH 7.4 PBS before detection.



A novel composite nanowires were synthesized using photopolymerization approach to form a layer of poly(carboxybetaine methacrylate) (polyCBMA) onto conducting polymer polyaniline (PANI) nanowire surface. The prepared polyCBMA/PANI composite, integrating the good conductivity of PANI nanowires with the excellent antifouling capability of polyCBMA, provided a wonderful matrix for the growth of ultrasensitive and low fouling biosensor. The CEA biosensor based on polyCBMA/PANI nanowires has been constructed for assaying in serum samples without suffering from serious nonspecific protein adsorption.

Highlights

- A highly sensitive electrochemical biosensor for carcinoembryonic antigen was developed.

- A novel nanocomposite polyCBMA/PANI was synthesized through modification polyCBMA onto conducting polymer PANI nanowires.

- The prepared polyCBMA/PANI composite was used as biosensor substrate.

- The biosensor based on polyCBMA/PANI exhibited promising clinical applications in complex serum samples.