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Electrochemical determination of BCR/ABL fusion gene based on in-situ synthesized gold nanoparticles and cerium dioxide nanoparticles

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ABSTRACT

An efficient DNA electrochemical biosensor, based on the gold nanoparticles (GNPs) in-situ synthesized at the surface of multi-walled carbon nanotubes (MWCNTs), cerium dioxide (CeO_2) and chitosan (Chits) composite membrane, was developed for the detection of BCR/ABL fusion gene in chronic myelogenous leukemia (CML). The capture probe was attached onto the nanocomposite membrane modified glassy carbon electrode (GCE) through the conjugated structure. Owning to the synergistic effects of CeO_2 nanoparticles with a strong adsorption ability and MWCNTs with a large surface area and excellent electron transfer ability, the prepared composite membrane was demonstrated an efficient electron transfer ability. The biosensor was electrochemically characterized by cyclic voltammogram (CV) and differential pulse voltammetry (DPV), and the decrease of the peak currents upon hybridization was observed using methylene blue (MB) as the electroactive indicator. Under the optimized conditions, peak currents were linear over the range from 1×10^{-9} M to 1×10^{-12} M, with a detection limit of 5×10^{-13} M (based on the 3σ). And the proposed method was successfully applied for the detection of PCR real samples with satisfactory results. Furthermore, the developed DNA biosensor was demonstrated a good selectivity, a reasonable stability and a favorable reproducibility, which could be regenerated easily.

Keywords: Electrochemical DNA biosensor; BCR/ABL fusion gene; Cerium dioxide; In-situ synthesis; Methylene blue

1. Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder, resulting from the neoplastic transformation of the primitive hemopoietic stem cell [1-3]. CML is characterized by a reciprocal translocation between chromosome 9 and 22 with the formation of the Philadelphia (Ph') chromosome [4, 5]. Molecular analysis of this translocation has demonstrated that the breakpoints on chromosome 22 occur within 5.8 kilobases (kb) of the breakpoint cluster region (BCR) gene [6]. The abnormality usually occurs in more than 95% of CML patients [7]. Thus, the detection of BCR/ABL gene is of great importance for an early diagnosis, a better prognosis of the disease, and an improvement for detecting minimal residual leukemia cells in the CML patients, especially after the bone marrow transplantation [8]. Many methods have been used for the BCR/ABL fusion gene detection, for instance, chromosome analysis [9], flourescence in situ hybrization (FISH) [10], flow cytometry (FCM) [11], real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) [12] and so on. But drawbacks like the time-consuming, poor precision, and expensiveness still exist. Hence, a newly effective method to detect BCR/ABL fusion gene is much demanded.

In the last few years, electrochemical DNA biosensor, based on nucleic acid hybridization, has attracted considerable attentions, which provides a sensitive, accurate, rapid and cost-effective platform for a variety of practical applications in various fields including medical diagnostics, clinical genetic analysis, forensic identification and environmental monitoring [13]. Recently, nano-structured materials with unique physical and chemical properties have been explored intensively for electrocatalytic sensing applications [14, 15]. These kinds of materials like metal-oxides offer attractive features such as good electronic

conductivity, high specific surface area and large pore volume and size[16-18]. Among them, cerium dioxide (CeO_2) has been exploited as a promising material for the immobilization of enzyme [19], antibody [20], DNA [21] and other bio-materials owing to its unusual properties including large surface area, excellent biocompatibility, nontoxicity, high chemical stability and strong adsorption ability [22]. Besides, due to its uniquely high isoelectric point (IEP ≈ 9.2), CeO_2 would be helpful for the immobilization of negative charged biomolecules with low IEP via electrostatic interactions. For instance, CeO_2 nanoparticles are utilized to be the immobilizing carriers of DNA probe, which are becoming the focus of research due to unique properties derived from their low dimensionality and possible quantum-confinement effects. Lu et al have constructed an amperometric immunosensor for myeloperoxidase in human serum based on a multi-walled carbon nanotubes-ionic liquid-cerium dioxide film-modified electrode [23]. Anees A et al have employed the sol-gel derived nano-structured cerium oxide composite membrane for the immobilization of cholesterol oxidase [24]. Feng et al have prepared a nanoporous CeO_2/chitosan composite matrix for the immobilization of single-strand DNA probes for the detection of cancer gene [21]. And Zhang et al have fabricated an ionic liquid supported CeO_2 nanoshuttles-carbon nanotubes composite as a platform for the detection of phosphoenolpyruvate carboxylase gene [25].

The immobilization of modified materials onto the electrode surface is a crucial step for the construction of an electrochemical biosensor. Thus, searching for an effective and simple immobilizing method is of considerable importance. GNPs have received much attentions because of its large specific surface area, good biocompatibility and high surface free energy [26, 27]. Most of the GNPs solutions, reported in articles, were synthesized by the reduction

of $HAuCl_4$ solution with sodium citrate [28]. Chitosan (Chits), possessing characteristics of excellent biodegradability and outstanding biocompatibility, is a naturally occurred polysaccharide [29], which is extremely suitable for the reduction of $HAuCl_4$ solution and also very befitting for the stabilization of the formed GNPs. No environmental toxicant or biological hazardous materials would be introduced by this method [30]. Both Haizhen Huang et al [30] and Bo Wang et al [31] have successfully demonstrated a facile method for synthesizing GNPs by incubation of Chits films in $HAuCl_4$ solution. GNPs prepared in this way are more stable and showed no obvious aggregation within two months[30]. As far as we know, the in-situ synthesized technology of GNPs at the other nano-materials using the chitosan as the reduction reagent and applied for DNA biosensors has not been reported before. Therefore, in our experiment, we combined the in-situ synthesized technology of GNPs at the surfaces of other materials, together with the excellent nanoparticles, to improve the performance of the DNA biosensor.

Mehthylene blue (MB) is a well-known hybridization indicator of DNA, which can be largely adsorbed onto the oligonucleotides owing to its strong affinity with the electronegative phosphate skeletons and free guanine bases. However, in the double-stranded DNA with a double helix structure, bases of capture DNA probe and target DNA were covalently conjugated, resulting in a remarkable reducing of free exposed guanine bases, consequently leading to the reducing of MB binding amount in the dsDNA [32]. Herein, we reported a simple electrochemical DNA biosensor for the determination of DNA hybridization related to BCR/ABL fusion gene by employing MB as an external redox indicator. In this paper, the in-situ synthesized technology of GNPs in the presence of chitosan was introduced into a

novel membrane comprising of CeO_2 nanoparticles and MWCNTs. The prepared GNPs/MWCNTs/CeO_2-Chits nanocomposite membrane integrated the strong adsorption ability of CeO_2 to the DNA probes with the high conductivity of GNPs, and the large surface area and excellent electron-transfer ability of MWCNTs. Besides, the thiol group of the probe was simultaneously introduced which could be used for the assembly of GNPs onto the surface of the modified electrode surface. The remarkable synergistic effects of this nanocomposite could greatly enhance the loading amount of DNA probes and hence markedly improve the detection sensitivity.

2. Experimental

2.1. Reagents and materials

Methylene blue (MB) and $HAuCl_4 \cdot 4H_2O$ were purchased from Sigma (USA). Cerium dioxide (CeO_2, spherical, particle diameter: 20 nm) was obtained from Beijing Nachen S&T Co. (Beijing, China). Multi-walled carbon nanotubes (MWCNTs) were purchased from Shenzhen Nanoport Port. Co. Ltd. (Shenzhen, China). Tris-Base and Chitosan (Chits) were obtained from the Shanghai Sangon Biotechnology Co. (Shanghai, China). The 18-mer synthetic oligonucleotides designed according to the BCR/ABL fusion gene were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China), and their base sequences were as follows:

Immobilized probe HS-ssDNA (S_1): 5'-HS-AGA GTT CAA AAG CCC TTC-3'

Target ssDNA (S_2, complementary to S_1): 5'-GAA GGG CTT TTG AAC TCT-3'

One-base mismatched ssDNA (S_3): 5'-GAA GGG CAT TTG AAC TCT-3'

Non-complementary to S_1 (S_4): 5'-ACG TGG TCC CCA GCT CTC-3'

All oligonucleotides stock solutions (100 μM) were prepared with TE buffer solution (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0) and kept frozen. The 20 μM MB buffer solutions were prepared in a 20 mM Tris-HCl, 50 mM NaCl buffer solution (pH 7.2). All other chemicals were of analytical reagent grade.

2.2. Apparatus and instrumentations

A CHI 660D electrochemical analyzer (Shanghai Chenhua Instrument Company, Shanghai, China), which was in connection with a glassy carbon modified working electrode (diameter = 3mm), an Ag/AgCl reference electrode and a platinum wire counter electrode, was used for electrochemical measurements. All potentials herein were referred to this reference electrode. Differential pulse voltammetry (DPV) was carried out from -0.60 V to 0.10 V with an amplitude of 50 mV. The UV-vis spectroscopy was measured by a ND-1000 spectrophotometer (NanoDrop Technologies, USA). The pH values of all solutions were measured by a model pHS-25 digital acidimeter (Shanghai Leici Factory, Shanghai, China). A scanning electron microscopy (SEM) system (FEI Nova-400, USA) and a transmission electron microscopy (TEM) system (Hitachi-7500, Japan) were used to characterize the morphology of the synthetic nanocomposite. PCR was carried out using a My Cycler thermal cycler (Bio-Rad Laboratories, USA). Gel images were recorded on an imaging system (Bio-Rad Laboratories, USA).

2.3. Preparation of GNPs/MWCNTs/CeO_2-Chits solution

Chits solution of 5 mL (1.0 %, w/w) was firstly prepared by dissolving Chits powder into 1.0 % (v/v) acetic acid solution. Then 5 mg of CeO_2 were dispersed into the above Chits solution with the aid of ultrasonication for 1.5 h to obtain 1.0 mg/mL homogeneous ivory suspension. Then 5 mg of MWCNTs were added to the CeO_2 suspension and the resulted suspension was ultrasonicated until the uniform suspension was obtained. After that, 65 μL $HAuCl_4$ aqueous solution (1.0 %, w/w) was added to the resultant MWCNTs/CeO_2-Chits solution. The homogeneous mixture was then incubated at 80 ℃ for 1.5 h until a purple solution was obtained [30-33], which indicated that GNPs was successfully in-situ synthesized at the surface of MWCNTs/CeO_2-Chits homogeneous composite.

2.4. Fabrication of the modified electrode

Prior to each modification, the glassy carbon electrode (GCE, diameter 3.0 mm) was freshly polished with 0.05 μm alumina powder and sonicated in double distilled water (DDW), dehydrated ethanol and DDW respectively. After dried at room temperature, 10 μL of the above resulted GNPs/MWCNTs/CeO_2-Chits solution was pipetted onto the surface of the clean GCE and dried at room temperature, thus a uniform membrane coated electrode (GNPs/MWCNTs/CeO_2-Chits/GCE) was obtained. The other modified electrodes were fabricated with the similar procedure. The fabrication procedures were illustrated in Scheme.1.

2.5. The immobilization of the ssDNA onto the modified electrode

After the electrode modified and dried, 10 μL of 10 μM HS-ssDNA (S_1) probe was

pipetted onto the surface of the GNPs/MWCNTs/CeO_2-Chits/GCE to achieve the immobilization of S_1. Then the electrode was dried at room temperature and followed by washed with 20 mM Tris-HCl buffer solutions (pH 7.2) to remove the unspecific adsorbed oligonucleotides.

2.6. DNA hybridization on probe S_1-immobilized electrode

The S_1-modified electrode was immersed in 20 mM Tris-HCl buffer containing complementary S_2, single-base mismatch S_3 or non-complementary S_4 at 40 ℃ water bath for 45 minutes. After hybridization, the electrode surface was washed with 20 mM Tris-HCl buffer solutions and DDW to remove unbounded oligonucleotides.

2.7. MB accumulation onto the hybrid-modified GCE

MB was accumulated onto the surface of hybrid-modified GCE by immersing the electrode into a stirring 20 mM Tris-HCl buffer solution (pH 7.2) containing 20 μM MB and 50 mM NaCl for 10 min without applying any potential. After that, the electrode was rinsed with 20 mM Tris-HCl buffer (pH 7.2) for 10 s to remove the non-specifically bounded MB and transferred into the blank buffer solution (20 mM Tris-HCl containing 50 mM NaCl, pH 7.2).

2.8. Electrochemical measurements

Cyclic voltammogram (CV) was adopted to electrochemically characterize the modified electrodes obtained during the stepwise modification . The measurements were performed in

the 10 mM $K_3[Fe(CN)_6]$ solutions containing 0.1 M KCl as the supporting electrolyte. Signals were collected between -0.20 V and 0.80 V, with a scan rate of 50 mV s^{-1}. The DNA hybridization process was assessed by the differential pulse voltammetry (DPV). The DPVs were collected from -0.60 V to 0.10 V, with an amplitude of 5 mV.

3. Results and discussion

3.1. The morphology of GNPs/MWCNTs/CeO_2-Chits composite

The morphology of GNPs/MWCNTs/CeO_2-Chits composite was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) respectively. MWCNT was observed as a tubular shape with a length of about 600 nm according to the solid arrow (Fig. 1A). MWCNTs/CeO_2-Chits composite (Fig. 1B) was successfully synthesized which displayed a relatively homogeneous dendritic morphology. After the reaction of $HAuCl_4$, GNPs with a uniform diameter of about 20 nm was successfully in-situ synthesized at the surface of MWCNTs/CeO_2-Chits composite (Fig. 1C). TEM was also used to confirm the microstructures of MWCNTs/CeO_2-Chits composite (Fig. 1D). It could be seen from Fig. 1E, with the successful synthesis of GNPs, plenty of small dark nanoparticles with about 20 nm were well distributed on the surface of the MWCNTs/CeO_2-Chits composite. Fig. 1F was the magnifying view of GNPs at 200 nm. The formed GNPs/MWCNTs/CeO_2-Chits composite provided numerous binding sites for the efficient immobilization of DNA probes. A SEM image of CeO_2 was provided in the supplementary material. As can be seen from the Fig. S1, CeO_2 nanoparticle exhibited a rough and wrinkled shape with a finely nanostructured surface, which could greatly increase the

loading amount of probe-ssDNA [34]. This result was also confirmed by the TEM photo of CeO_2 (Fig. S2).

3.2. UV-vis spectroscopy

The UV-vis absorption spectra of the in-situ synthesized GNPs on different systems were shown in Fig. 2. Chits and MWCNTs-Chits solution showed no absorption peaks (Curve (a) and Curve (e)), while GNPs-Chits solution and CeO_2-Chits solution exhibited absorption peaks at 525 nm (Curve (b)) and 328 nm (Curve (d)), respectively. Those peaks were the characteristic absorption peaks of GNPs and CeO_2. After GNPs successfully in-situ synthesized on the surface of MWCNTs/CeO_2-Chits solution (Curve (c)), both 525 nm and 328 nm showed the absorption peaks, which indicated that the composite had been successfully prepared.

3.3. Electrochemical characteristics of GNPs/MWCNTs/CeO_2-Chits/GCE

Cyclic voltammogram (CV) is an effective and convenient technique to investigate electrochemical behaviors, which can be used to monitor the electrochemical characteristics of different components modified electrodes in this paper. The CVs of different modified electrodes, recorded from -0.2 V to 0.80 V in 10 mM $K_3[Fe(CN)_6]$ solution containing 0.1 M KCl at a scan rate of 50 mV s^{-1}, were presented in Fig. 3. A pair of well-defined peaks were shown in Curve (a) which reflected the reversible redox reaction of ferricyanide ions on the bare glassy carbon electrode. After modified with CeO_2-Chits composite membrane (Curve (c)), peak current increased greatly, which indicated that CeO_2-Chits film enhanced the

electrochemical response of the $[Fe(CN)_6]^{4-/3-}$. This was mainly attributed to the excellent electronic conductivity of cerium oxide. Apparently, after MWCNTs added into CeO_2-Chits suspension (Curve (d)), the response became greater, with a small peak-to-peak separation (ΔEp). This increase in current response was the distinct evidence of the fact that MWCNTs could greatly promote electron-transfer reactions. After GNPs in-situ synthesized on the surface of MWCNTs/CeO_2-Chits membrane (Curve (e)), peak currents kept on increasing with a slightly increased peak-to-peak separation (ΔEp). This phenomenon reflected that GNPs had been successfully synthesized at the surface of MWCNTs/CeO_2-Chits membrane. Compared to the former results, peak current of DNA probe immobilized electrode significantly decreased, only higher than that of bare GCE (Curve (b)). This was due to the obvious obstruction of DNA probe with negative charged skeletons, which could hinder the $[Fe(CN)_6]^{4-/3-}$ participating electron transfer reaction [35]. Those results suggested that DNA probes had been successfully immobilized onto the surface of GNPs/MWCNTs/CeO_2-Chits/GCE.

3.4. Electrochemical behaviors of MB on the modified electrodes.

Fig. 4 showed the Cyclic voltammograms of the electrodes modified with different components incubating in 20 mM Tris-HCl buffer solution containing 50 mM NaCl (pH 7.2), with a scan rate of 50 mV s^{-1}. It could be clearly seen that no peaks of MB were observed both on the bare GCE (Curve (a)) and GNPs/MWCNTs/CeO_2-Chits/GCE (Curve (b)). After ssDNA immobilized on the modified electrode (S_1/GNPs/MWCNTs/CeO_2-Chits/GCE), oxidoreduction peak of MB (Curve (c)) was found largely increased. This oxidoreduction

peak was attributed to the adsorption of MB which owned a strong affinity to electronegative phosphate skeletons and free guanine bases exposed out from the capture probes . However, the hybridized DNA chain possesses a double helix structure, resulting in a remarkable reducing of free exposed guanine base, which consequently leading to the less binging of MB on the dsDNA, so the greatest amounts of MB were accumulated on the ssDNA modified electrode. Therefore, after hybridized with target DNA, the voltammetric reduction signal of MB greatly decreased owing to the inaccessibility of the guanine bases in dsDNA (Curve (d)).

3.5. Electrochemical influence of CeO_2 to MB reduction signals

Electrochemical responses of hybridization with the complementary target DNA in the absence and presence of CeO_2 were studied. As shown in Fig. 5, Curve (b) was the MB reduction signal of the ssDNA modified electrode in the absence of CeO_2, after hybridized with the complementary target DNA (Curve (a)), DPV signal of MB was reduced to about 0.5 μA. Curve (d) was responding to the MB signal of ssDNA modified electrode in the presence of CeO_2, similarly, after hybridized with the target DNA (Curve (c)), reduction signal decreased as well, while the difference of these two peak currents was 5.8 μA. With the presence of CeO_2, peak current was maximal 12 times higher than that of the absence, which was contributed to the unusual properties of CeO_2 of large surface area, excellent biocompatibility, high chemical stability and strong adsorption capability.

3.6. Electrochemical comparison of different modified GCEs

The surface coverage amount of probe-ssDNA, hybridization efficiency and sensitivity of

GNPs/CeO_2-Chits, GNPs/MWCNTs-Chits, MWCNTs/CeO_2-Chits and GNPs/MWCNTs/CeO_2-Chits modified GCE were tested under the same conditions respectively, and the results were shown in Fig. S3. Gray columns represented the DPV responses of MB before the hybridization, while the black ones represented the results after hybridization with the target DNA of same concentration. Three different conclusions could be drawn from the results: 1. Before the hybridization, signals of MB were closely related to the amounts of ssDNA, which were mainly attributed to the surface condition of nanomaterial modified GCE. Clearly, the GNPs/MWCNTs/CeO_2-Chits modified GCE showed the highest response than others, which demonstrated the maximum coverage amount of probe-ssDNA. 2. When hybridized to the target DNA with the same concentration, due to the formation of dsDNA, accumulated MBs onto the nucleic acids were decreased, leading to the signal decreased in different levels, which also reflected the hybridization efficiency. 3. With the maximum immobilizing amounts of ssDNA, the GNPs/MWCNTs/CeO_2-Chits modified GCE adsorbed the maximum amounts of MB, thus obtained the highest DPV signal before the hybridization. Moreover, the value after the hybridization decreased significantly, which is also the maximum decrease among the four modified GCEs. This phenomenon clearly demonstrated that the proposed method in this paper owned the best sensitivity than DNA biosensors based on the individual nano-material or other combination of nano-materials.

3.7. The selectivity and sensitivity of electrochemical DNA biosensor

3.7.1. The selectivity of electrochemical DNA biosensor

The selectivity of the developed DNA electrochemical biosensor was investigated by

adopting the S_1-GNPs/MWCNTs/CeO_2-Chits/GCE as the capture probe to hybridize various DNA sequences related to BCR/ABL fusion gene and using the MB as an electrochemical hybridization indicator (Fig. 6). DPV peak current presented the highest signal response about 26.3 μA without hybridization (Curve a), which was due to the greatest amounts of accumulated MB. After hybridized with the non-complementary sequence (S4), signal slightly decreased to about 25.5μA (Curve b), indicating that no obvious changes occurred during the hybridization. After hybridized with one-base-mismatched oligonucleotide (S3), significantly decreased voltammertric signal of 18.2 μA was obtained (Curve c), implying that the hybridization occurred incompletely due to the existence of the one-base mismatch. After hybridized with the complementary oligonucleotide (S2), an remarkable decrease in peak current at about 10.9 μA was observed (Curve d), suggesting that hybrids (dsDNA) were formed at the electrode surface, and the MB interaction with guanine residues of the probe was prevented by the formation of duplex. The RSD was 3.3%. These results showed that the proposed electrochemical DNA biosensor owned a good selectivity for hybridization detection with a satisfactory sensitivity.

We also tested our electrochemical DNA biosensor with other oligonucleotides and biomolecules including DNA fragments of MRSA and Escherichia coli O111, BSA and HCV. (Fig. S5). DPV responses after treated with the same concentration of those interferents exhibited no obvious differences compared with the probe modified electrode, which demonstrated the good specificity of the DNA biosensor.

3.7.2. The sensitivity of the electrochemical DNA biosensor

The sensitivity of this DNA biosensor was explored by hybridizing with different concentrations of complementary oligonucleotides. As shown in Fig.7, signals of MB peak current gradually increased with the increasing concentration levels of target DNA, and the current responses at about -0.26 V increased in proportion to the amount of target sequence. The values of MB cathodic peak current were linear with the concentrations of target DNA in the range from 1×10^{-12} M to 1×10^{-9} M. The linear regression equation was calculated as I_p (μA) = 1.66 (log (C/pM)) + 2.17 (r = 0.9981). A detection limit of 5×10^{-13} M could be estimated using 3σ (where σ was the standard deviation of the blank solution, n = 10).

The analytical performance of our DNA biosensor has been compared to those reported previously, as shown in Table S1. It can be seen that the proposed method displayed a better performance than the other methods in analytical range or detection limit.

3.8. Detection of PCR real samples

This electrochemical DNA biosensor has been used for assay of PCR products with satisfactory result. Fig. S4A represents the DPV signal responses to MB obtained when the probe for CML immobilized and hybridized with real PCR samples in the hybridization step. DPV signals obtained from the probe modified GCE gave a mean average signal of 18.8 μA (Curve a). DPV signals obtained from the hybridization with the negative and positive real sample gave the mean average signals of 16.6 μA and 11.4 μA, respectively (Curve b and c). DPV peak current of the un-hybridization condition was higher than that of probe modified GCE after hybridized with positive real sample, and an obvious decrease in the DPV signal was observed with the positive real sample. This decrease indicated that the hybridization on

the GCE surface occurred and the accumulated MB reduced owing to the formation of DNA hybrids. When hybridized with negative real sample, the peak current was higher than that of positive sample because of the absence of complementary target sequence.

A gel electrophoresis method was performed, and the result was in good agreement with the DPV results obtained from the electrochemical DNA biosensor (Fig. S4B).

3.9. Reproducibility, stability and regenerability of the developed DNA biosensor

The reproducibility of the proposed DNA biosensor was investigated by the multiple and repetitive measurements of the probe modified electrode in 1×10^{-11} M target DNA solution under the same conditions. The results showed a relative standard deviation (R.S.D.) about 6.58% (n = 6), which indicated that this system owned a satisfying reproducibility.

The stability of the modified electrode was also tested. No obvious significant changes in cathodic peak currents were observed for more than 15 complete CV cycles. The long-term test lasted for 1 month, and the biosensor retained more than 96% of its initial current response.

The regeneration of the DNA biosensor was evaluated by rinsing the hybridization surfaces with hot DDW for 5 min, following by repetitive rapid cooling in icy bath. After 10 regeneration and hybridization, the electrode reserved 95.2% of its origin current response.

4. Conclusion

In summary, we developed an electrochemical DNA biosensor for the detection of BCR/ABL fusion gene of CML based on GNPs/MWCNTs/CeO_2-Chits composite membrane

modified electrode. The GNPs were in-situ synthesized at the surface of MWCNTs and CeO_2 nanoparticles composite membrane, which could greatly enhance the DNA immobilization amount and the hybridization efficiency. The DNA biosensor showed a good selectivity and sensitivity, which was successfully used to detect the PCR real samples with satisfactory results. Importantly, since this approach didn't require complicated fabrication procedure, the strategy proposed here likely to be versatile for simplifying the fabrication of other electrochemical DNA biosensors.

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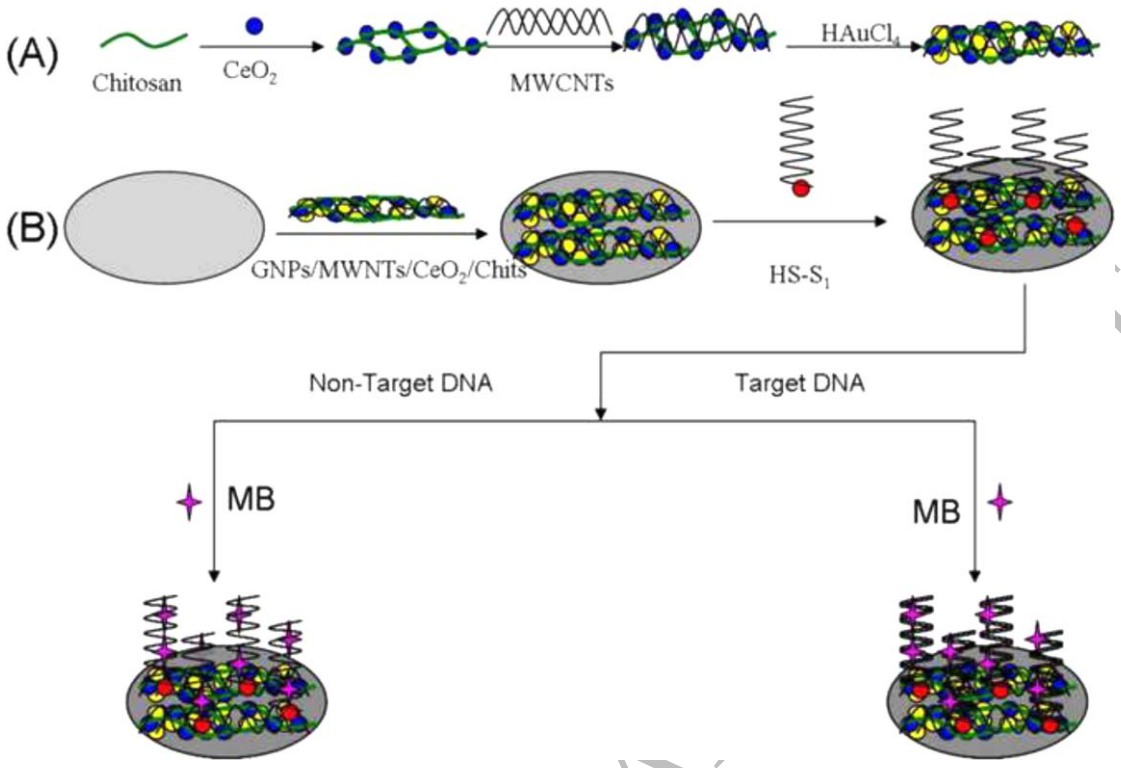

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3 Scheme 1. Schematic illustration of the preparation procedure of

4 GNPs/MWCNTs/CeO_2-Chits composite (A), and the fabrication process of electrochemical

5 DNA biosensor (B).

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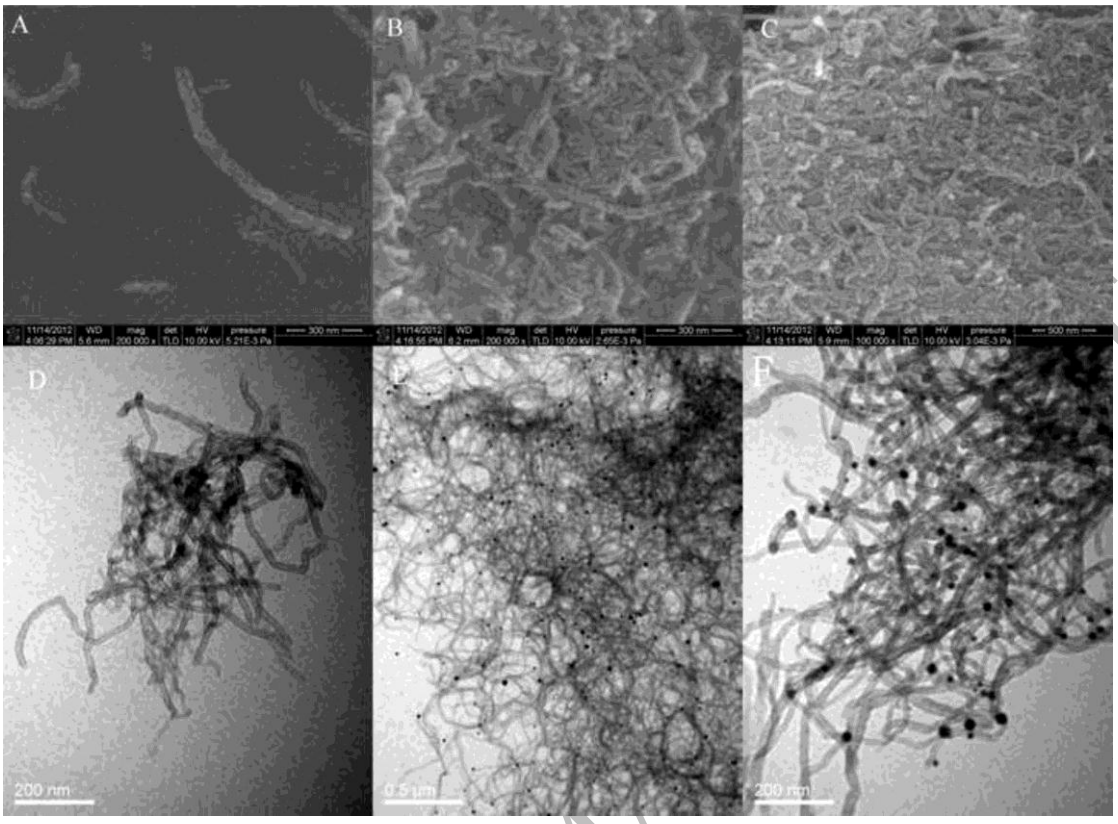


Fig. 1. SEM images of MWCNTs (A), MWCNTs/CeO_2-Chits composite (B), GNPs/MWCNTs/CeO_2-Chits composite (C) and TEM images of MWCNTs/CeO_2-Chits composite (D), GNPs/MWCNTs/CeO_2-Chits composite (E), (F) is the enlarged view of (E).

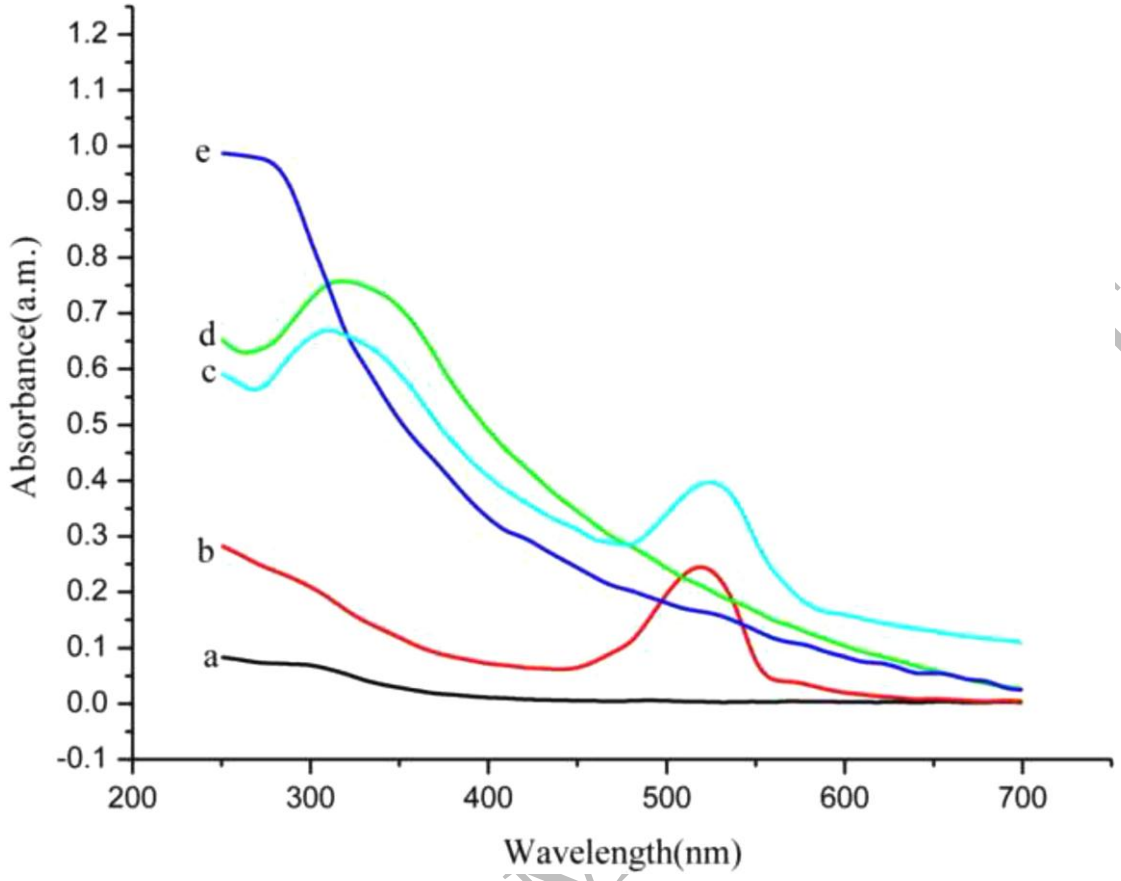


Fig. 2. UV-vis absorption spectra of the Chits (a), GNPs-Chits (b), GNPs/MWCNTs/CeO_2-Chits (c), CeO_2-Chits (d), MWCNTs-Chits (e).

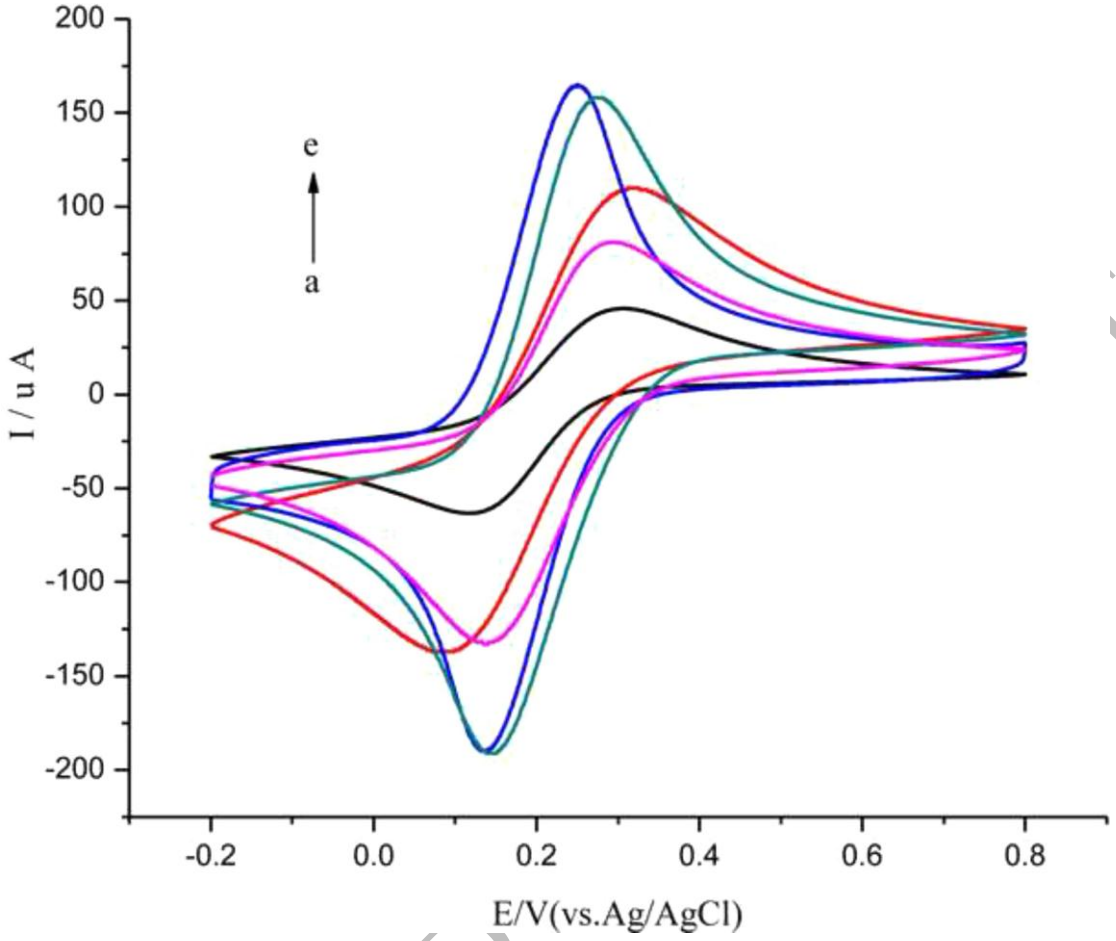


Fig. 3. Cyclic voltammograms of the Bare GCE (a), ssDNA immobilization (b), CeO_2-Chits/GCE (c), MWCNTs/CeO_2-Chits/GCE (d), GNPs/ MWCNTs/CeO_2-Chits/GCE (e) in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution (pH 7.0) containing 0.1 M KCl; scan rate: 50 mV s^{-1}.

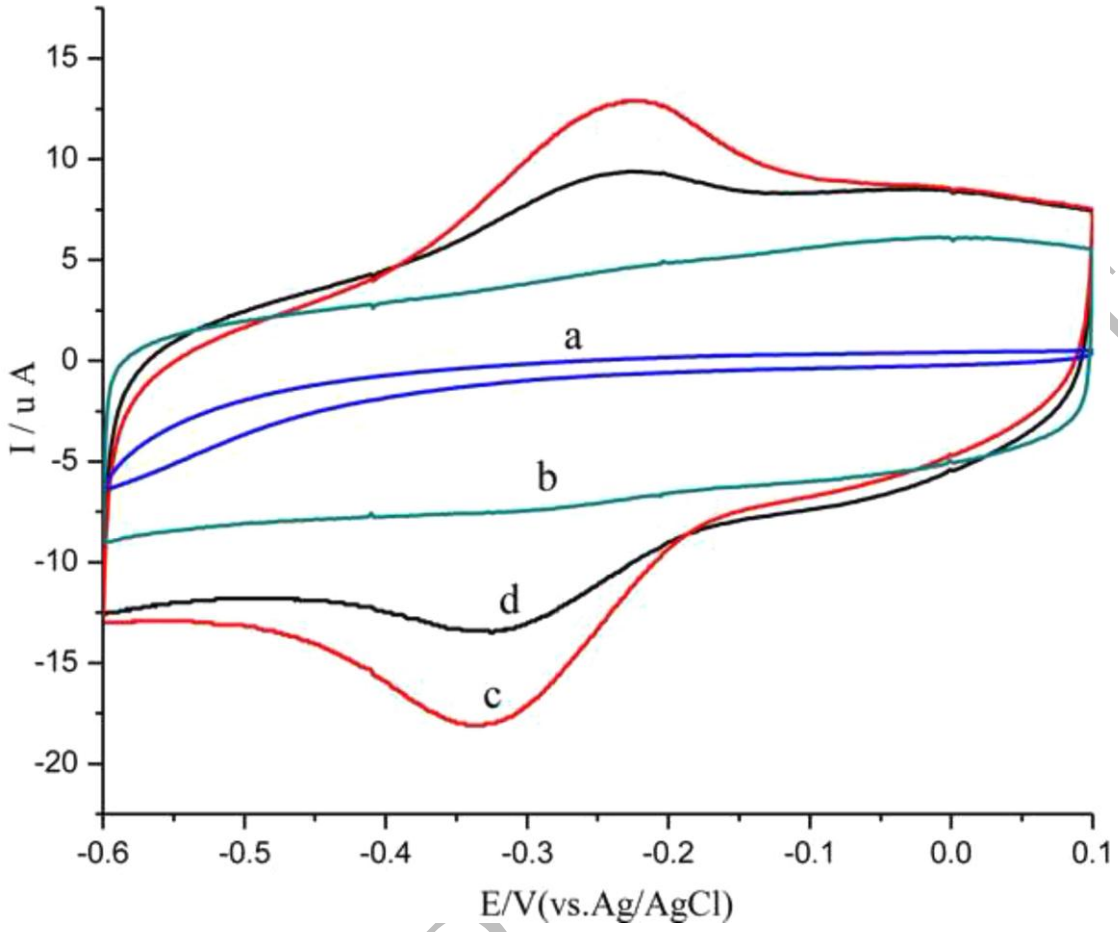


Fig. 4. Cyclic voltammograms of the Bare GCE (a), GNPs/MWCNTs/CeO_2-Chits/GCE (b), S_1/GNPs/MWCNTs/CeO_2-Chits/GCE (c), S_1-S_2/GNPs/MWCNTs/CeO_2-Chits/GCE (d) in 20 μM MB of 20 mM Tris-HCl solution containing 50 mM NaCl (pH 7.2); scan rate: 50 mV s^{-1}.

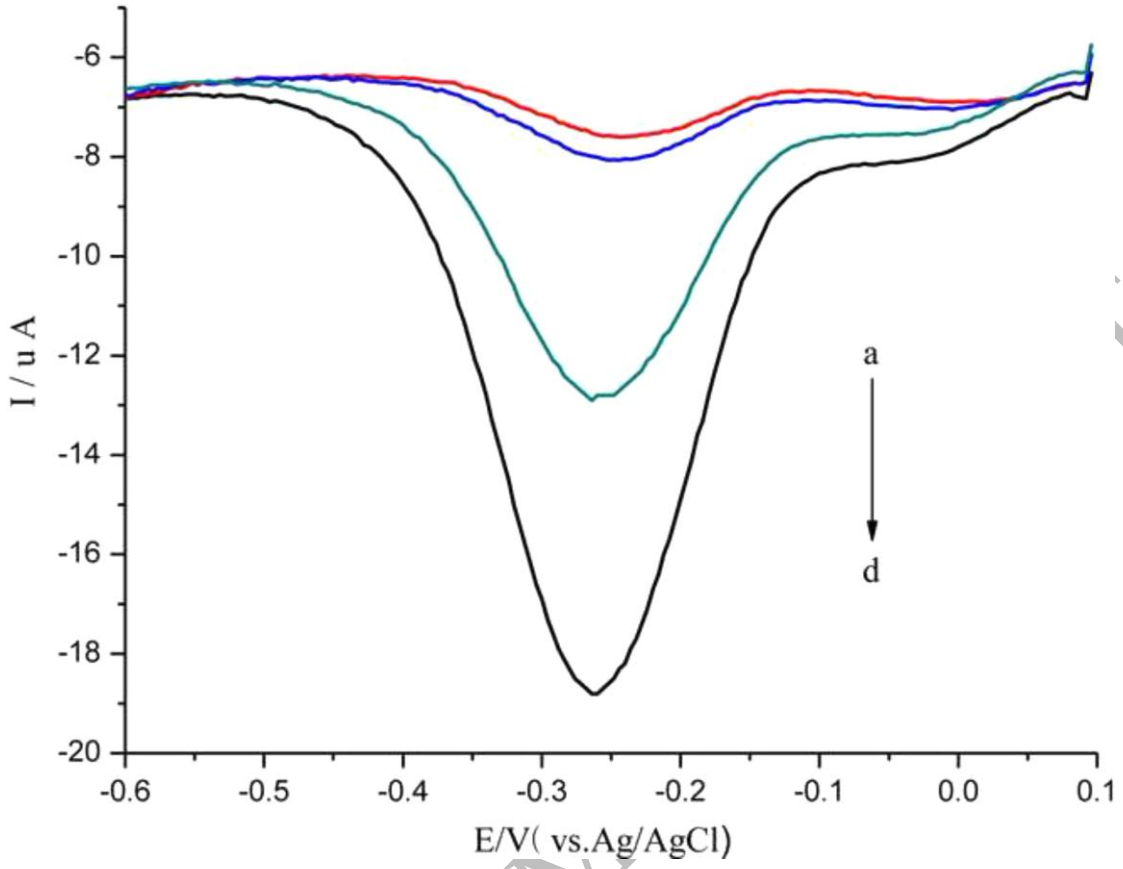


Fig. 5. DPVs for 20 μM MB as redox indicator at a probe modified electrode without CeO_2 (b) and with CeO_2 (d); after hybridization with the 1×10^{-11} M complementary target DNA (a) and (c), respectively.

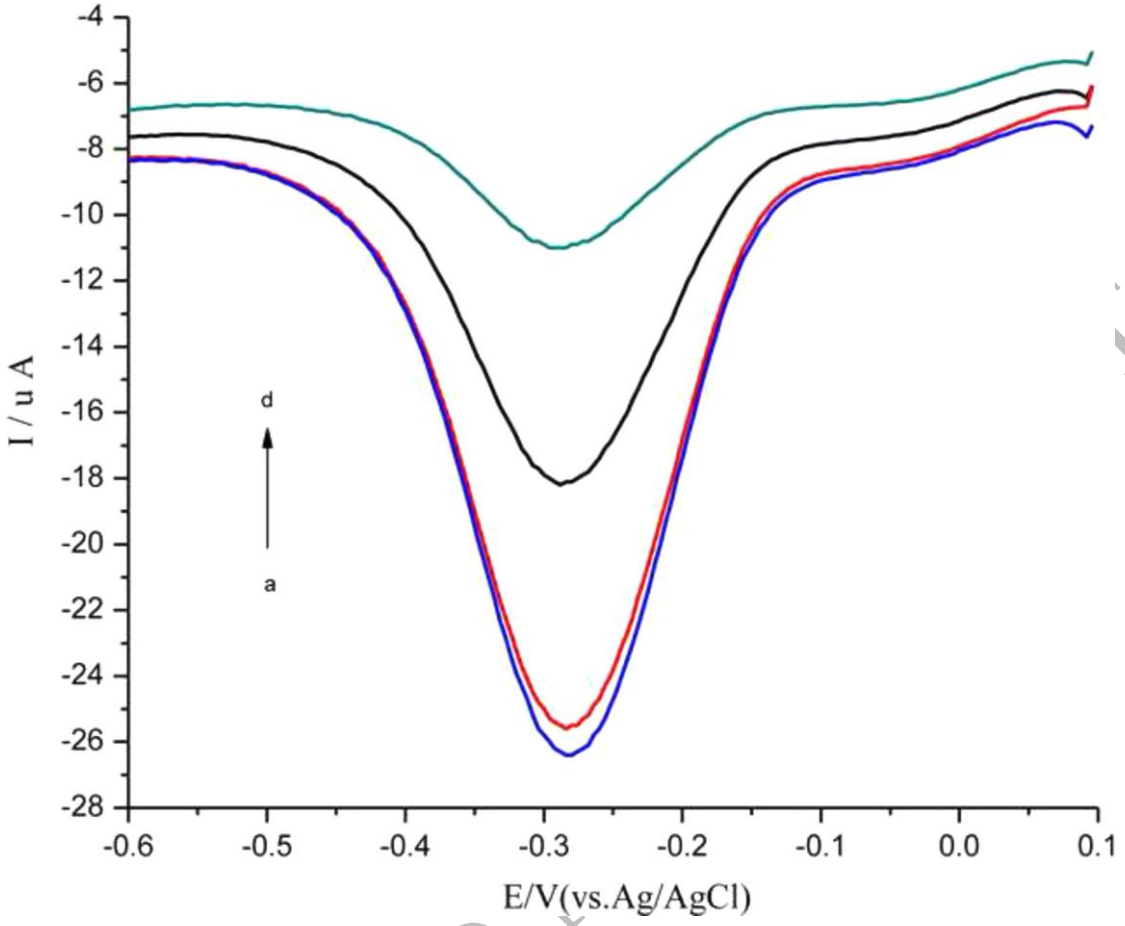


Fig. 6. DPVs of ssDNA modified electrode (a) and after hybridization with the non-complementary sequence (b), one-base-mismatch sequence (c), and complementary target sequence (d) using 20 μM MB as redox indicator in 20 mM Tris-HCl buffer solution (pH 7.2) containing 50 mM NaCl.

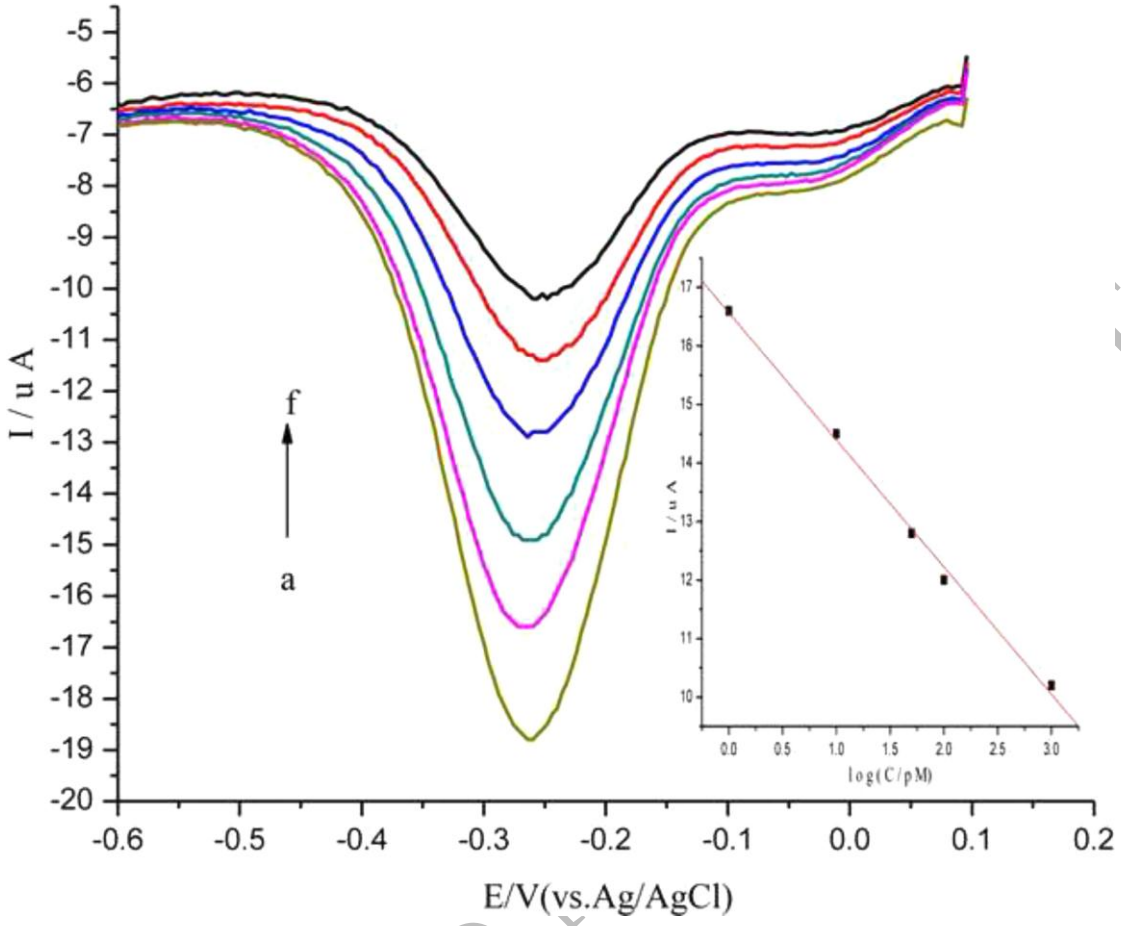
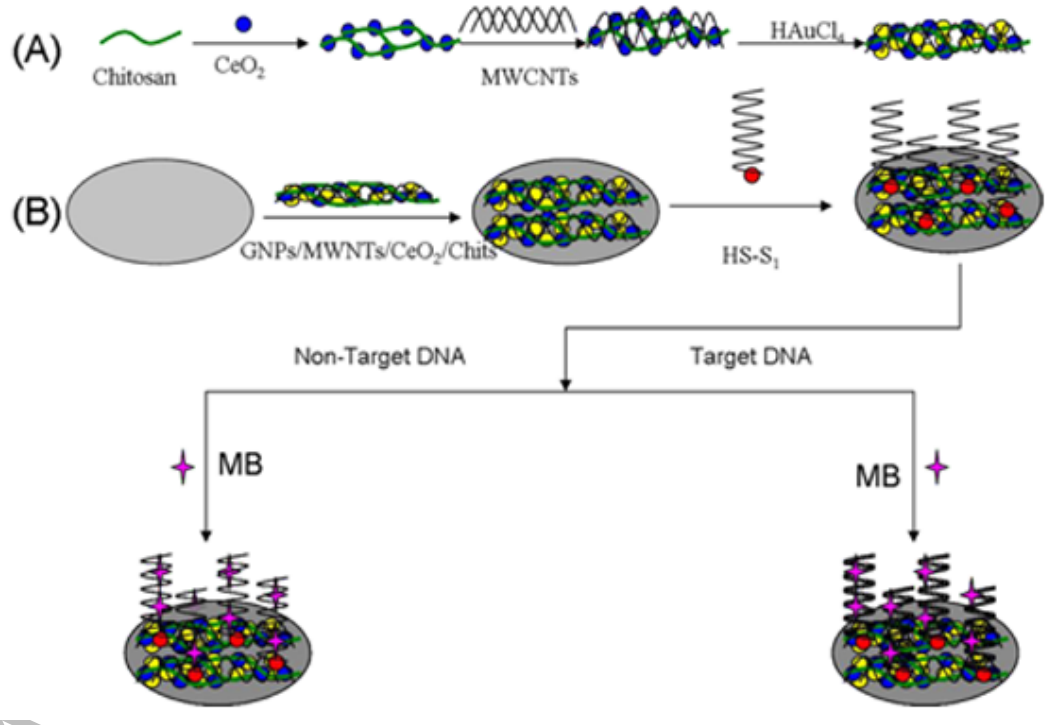


Fig. 7. DPVs of MB signals after hybridized with different concentrations of target sequence: probe (a), 1×10^{-12} M (b), 1×10^{-11} M (c), 5×10^{-11} M (d), 1×10^{-10} M (e), 1×10^{-9} M (f). The inset shows the calibration curve of DPV peak currents (μA) with different concentrations of target DNA (log(C/pM)).

Graphical abstract

GNPs/MWCNTs/CeO_2-Chits composite membrane modified glass electrode was constructed for detecting BCR/ABL fusion gene in CML. The methylene blue was used to as an electroactive indicator to provide decreased electrochemical signal upon hybridization of the probe with the target DNA. (A) Preparation procedure of the GNPs/MWCNTs/CeO_2-Chits composite. (B) Fabrication process of the electrochemical DNA biosensor.



Highlights

- A novel strategy for the detection of BCR/ABL fusion gene in CML has been constructed.
- The GNPs/MWCNTs/CeO2–Chits composite was prepared which could greatly improve the electron-transfer ability of electroactive species toward the electrode surface.
- The proposed method offers a good selectivity, stability, reproducibility and could be regenerated easily.