



Short communication

An enhanced sensing platform for ultrasensitive impedimetric detection of target genes based on ordered FePt nanoparticles decorated carbon nanotubes



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ARTICLE INFO

Article history:

Received 26 July 2012

Received in revised form

7 October 2012

Accepted 15 October 2012

Available online 26 October 2012

Keywords:

Carbon nanotubes

FePt nanoparticles

DNA biosensor

Cyclic voltammetry

Electrochemical impedance spectroscopy

PML/RARA fusion gene

ABSTRACT

We demonstrate a novel high-performance DNA hybridization biosensor with a carbon nanotubes (CNTs)-based nanocomposite membrane as the enhanced sensing platform. The platform was constructed by homogeneously distributing ordered FePt nanoparticles (NPs) onto the CNTs matrix. The surface structure and electrochemical performance of the FePt/CNTs nanocomposite membrane were systematically investigated. Such a nanostructured composite membrane platform could combine with the advantages of FePt NPs and CNTs, greatly facilitate the electron-transfer process and the sensing behavior for DNA detection, leading to excellent sensitivity and selectivity. The complementary target genes from acute promyelocytic leukemia could be quantified in a wide range of 1.0×10^{-12} mol/L to 1.0×10^{-6} mol/L using electrochemical impedance spectroscopy, and the detection limit was 2.1×10^{-13} mol/L under the optimal conditions. In addition, the DNA electrochemical biosensor was highly selective to discriminate single-base or double-base mismatched sequences.

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1. Introduction

Rapid, simple and sensitive determination of specific DNA sequences is extremely important in clinical diagnostics, mutation detection, gene therapy, food safety monitoring, and a variety of other biomedical researches (Sassolas et al., 2008; Teles and Fonseca, 2008). Therefore, it is highly desirable to develop DNA analysis techniques with high sensitivity and accuracy. In the past few years, different techniques have been developed for DNA detection such as acoustic, gravimetric, fluorescence, surface plasmon resonance, and quartz crystal microbalance (Gabl et al., 2004; Prusty and Herrmann, 2010; Altintas et al., 2012). Nevertheless, these techniques suffer from some disadvantages that include relatively complex operation processes, time-consuming, and high cost. DNA biosensors based on electrochemical transduction have attracted broad attention and been widely developed due to their advantages such as simple and inexpensive instrumentation, fast response, precise and sensitive measurements, and low production cost as well as compatibility with microfabrication technology (Dong et al., 2010; Jayakumar et al., 2012). Currently,

much effort has been devoted to promote the detection sensitivity and selectivity of electrochemical DNA biosensors (Li et al., 2012).

Due to its unique and desirable physicochemical properties, such as large surface area, excellent conductivity and electrocatalytic activity, fine biocompatibility and robust mechanical strength, carbon nanotubes (CNTs) and its based composite materials had been widely used in the electrochemical biosensor field (Jacobs et al., 2010; Nie et al., 2011; Yu et al., 2012; Mokhtari et al., 2012). For instance, Yu et al. used a versatile potentiostatic double-pulse technique to electrodeposit Ag nanoparticles (NPs) on CNTs multilayer films which are preassembled on indium tin oxide (ITO) electrode via layer-by-layer self-assembly technique, and the prepared Ag/CNTs composite films exhibited excellent electrocatalytic activity to the reduction of hydrogen peroxide (Yu et al., 2012). Mokhtari et al. demonstrated the construction of a CNTs paste electrode chemically modified by incorporation of vinylferrocene for simultaneous voltammetric determination of morphine and diclofenac in biological and pharmaceutical samples (Mokhtari et al., 2012).

Our group is extremely interested in the electrochemical DNA biosensor field (Zhang et al., 2012a, 2012b). It is known that the sensitivity of DNA detection can be improved using nanomaterials. We were inspired from previously published work to take advantage of the benefits of CNTs based nanocomposite materials to improve the sensitivity and selectivity for the sequence-specific detection of DNA. Acute promyelocytic leukemia (APL)

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is a kind of acute leukemia, which often goes with bleeding severely. The bleeding mechanism of the APL patients is very complex. Almost all of the APL patients are characterized by chromosome reciprocal translocation, resulting in the generation of fusion gene between promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA), which forms PML/RARA fusion gene (Wei et al., 2009). Here, we report, for the first time, an innovative design of an ultrahigh performance label-free impedimetric biosensor for the detection of PML/RARA fusion gene based on ordered FePt NPs decorated CNTs. The FePt/CNTs nanocomposite membrane combined the very large surface area of two kinds of nanomaterials and their excellent conductivity with the attractive biocompatibility of FePt NPs to DNA molecules. The synergistic effect of the sensing composite membrane could enhance the DNA attachment quantity and the detection sensitivity of DNA sequence.

2. Materials and methods

2.1. Apparatus and materials

All the electrochemical measurements were carried out using an EC500 electrochemical workstation (Wuhan GaossUnion Company, China). A conventional three-electrode system was used with glassy carbon electrode (GCE) or the modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt wire as the counter electrode. Scanning electron microscopy (SEM) was carried out using a JSM-6700 F machine (JEOL, Japan). Transmission electron microscopy (TEM) was conducted on a JEM-2000EX machine (JEOL, Japan).

The synthesis of FePt/CNTs nanocomposite and DNA sequences used in experiments can be seen in [supplementary materials](#).

2.2. Preparation of FePt/CNTs/GCE

Before modification, the GCE was freshly polished prior to each experiment with 0.3 μm and 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ paste, respectively, and rinsed with ultrapure water after each polishing step, finally cleaned ultrasonically in ethanol and water, respectively, for 3 min. 10 μL of 2.0 mg/mL FePt/CNTs suspension was dropped on the GCE and allowed to dry at room temperature, thus a uniform membrane modified electrode (FePt/CNTs/GCE) was obtained.

2.3. Immobilization and hybridization of DNA

Immobilization of ssDNA probes was performed by immersing the FePt/CNTs/GCE into 2.0 mL PBS buffer solution (pH 7.0) containing 1.0×10^{-6} mol/L ssDNA probes for 2 h at room temperature, followed by washing the electrode with 0.5% sodium dodecylsulfate (SDS) solution and then rinsing it with ultrapure water to remove the unimmobilized ssDNA, and this probe-captured electrode was denoted as ssDNA/FePt/CNTs/GCE.

Further, the hybridization reaction was carried out by transferring 10 μL of hybridization solution (PBS buffer, pH 7.0) containing cDNA onto the probe-modified electrode, followed by thoroughly washing the electrode with 0.5% SDS solution to remove the unhybridized DNA. The hybridizations of probe ssDNA with single-base mismatched DNA, double-base mismatched DNA and ncDNA were carried out through similar procedure.

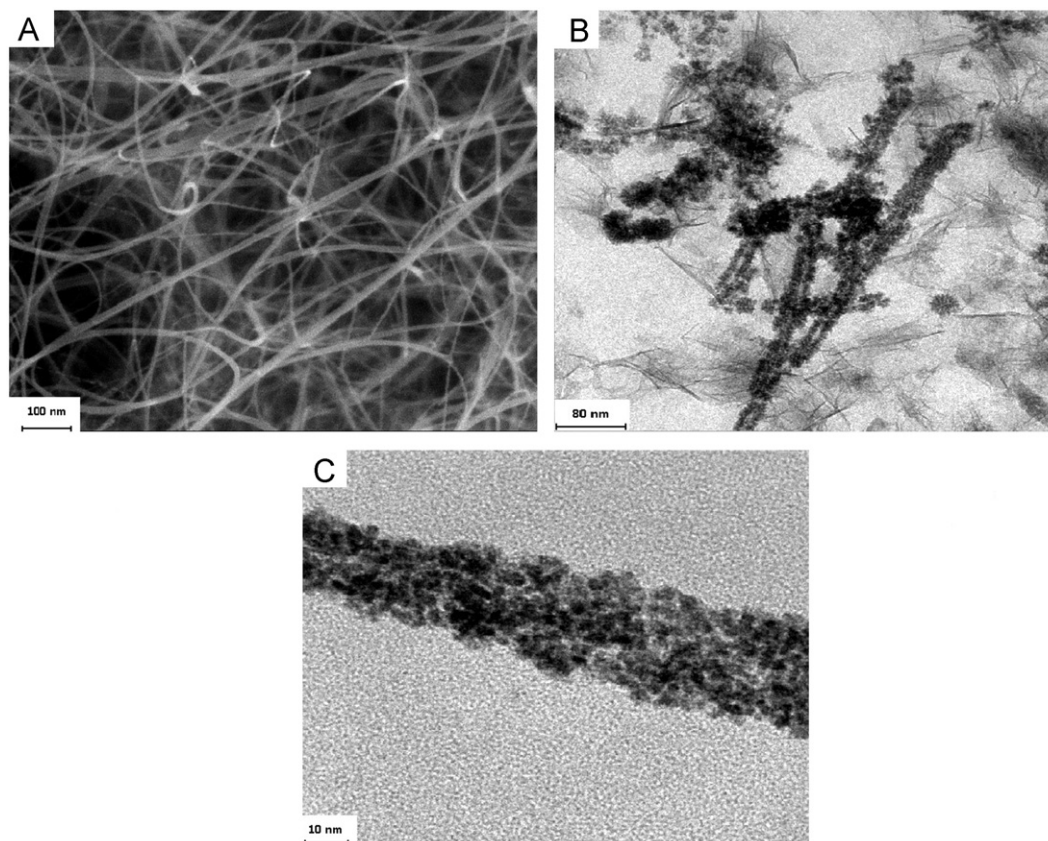


Fig. 1. (A) SEM image of pristine CNTs, (B) low-magnification and (C) high-magnification TEM images of the synthesized FePt/CNTs.

3. Results and discussion

3.1. Morphology of the synthesized CNTs and FePt/CNTs nanocomposite

The typical SEM image of pristine CNTs was shown in Fig. 1A, which was synthesized according to the reported method (Flahaut et al., 2003). It is clear that extremely abundant of CNTs could be obtained by this method. TEM images of the as-prepared FePt/CNTs sample were shown in Fig. 1B and C, and it can be seen that a dense layer of FePt NPs have been formed along the skeleton of individual CNTs to form FePt/CNTs nanocomposite. More importantly, few aggregations of FePt NPs were found in solution, indicating the high selective nucleation of FePt NPs along the functional CNTs surface.

3.2. Electrochemical characterization of the modified electrodes

Cyclic voltammograms of different modified electrodes in 1.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution were shown in Fig. 2A. A couple of well-defined redox peaks could be observed at the bare GCE (curve a). On the FePt/GCE an increased electrochemical response of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was observed (curve b), which could be attributed to the presence of FePt NPs with fine electrical conductivity on the electrode surface. On the CNTs/GCE the electrochemical response was further increased (curve c), indicating the presence of CNTs greatly improved the electrode performance. CNTs had been elucidated to have the advantages such as large surface area, excellent electrical conductivity, wide electrochemical window, strong adsorption ability, and fine biocompatibility (Jacobs et al., 2010). So the presence of CNTs on the electrode surface can accelerate the electron transfer rate between the redox couple in bulk solution and the electrode. On the FePt/CNTs/GCE the redox peak currents were further obviously increased (curve d), indicating that the synergistic amplification effects of FePt NPs and CNTs on enhancing the electrochemical reaction rate of the redox probe was outstanding.

The modified electrodes were further characterized by EIS and the interfacial electron transfer resistance (R_{et}) can be calculated from the diameter of Nyquist diagram (Musameh et al., 2011; Patil et al., 2012), so the Nyquist diagrams of different modified electrodes were recorded. As illustrated in Fig. 2B, the R_{et} value of bare GCE

was got as 885 Ω (curve a). On the FePt/GCE and CNTs/GCE the R_{et} values decreased to 592 Ω (curve b) and 442 Ω (curve c), respectively, indicating the presence of FePt NPs and CNTs on the electrode surface could obviously increase the conductivity of the electrode surface and decrease the impedance. On the FePt/CNTs/GCE a further decrease of R_{et} (317 Ω , curve d) appeared, indicating the highest conductivity of the electrode interface. So the synergistic effects of FePt NPs and CNTs in the membrane greatly decreased the resistance for the electron transfer. The agreement of EIS with CV results indicated that the FePt/CNTs nanocomposite membrane was an excellent platform for the electrode modification.

3.3. EIS characterization of probe DNA immobilization and hybridization

Fig. S1 (see supplementary materials) shows the Nyquist diagrams of 1.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the FePt/CNTs/GCE (curve a), ssDNA/FePt/CNTs/GCE (curve b) and the hybridized dsDNA/FePt/CNTs/GCE (curve c). After the ssDNA probes were immobilized on the FePt/CNTs composite membrane, the negatively charged phosphate backbone of probe ssDNA prevented $[\text{Fe}(\text{CN})_6]^{3-/4-}$ from reaching the electrode surface during the redox process, and therefore led to a larger R_{et} value (curve b) than that at the FePt/CNTs/GCE (curve a). When the probe DNA was hybridized with its complementary target DNA (cDNA) in solution, the R_{et} value increased greatly to a much larger value (curve c). After hybridization, the negative charges on the electrode surface increased remarkably and the surface membranes became thicker, which might raise the R_{et} value. Therefore, from the changes of the R_{et} value, the immobilization and hybridization of DNA on this FePt/CNTs/GCE platform could be understood clearly.

3.4. Study on selectivity and sensitivity of this impedance-based DNA biosensor

The selectivity of this DNA biosensor was investigated by using the ssDNA probe to hybridize with different DNA sequences related to the PML/RARA fusion gene. After hybridization of the ssDNA probe, the changes of the Nyquist diagram of 1.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 mol/L KCl were shown in Fig. 3A. Curve a was the Nyquist diagram at the probe ssDNA/FePt/CNTs/GCE. After hybridization of the ssDNA probe with the cDNA, the change of the

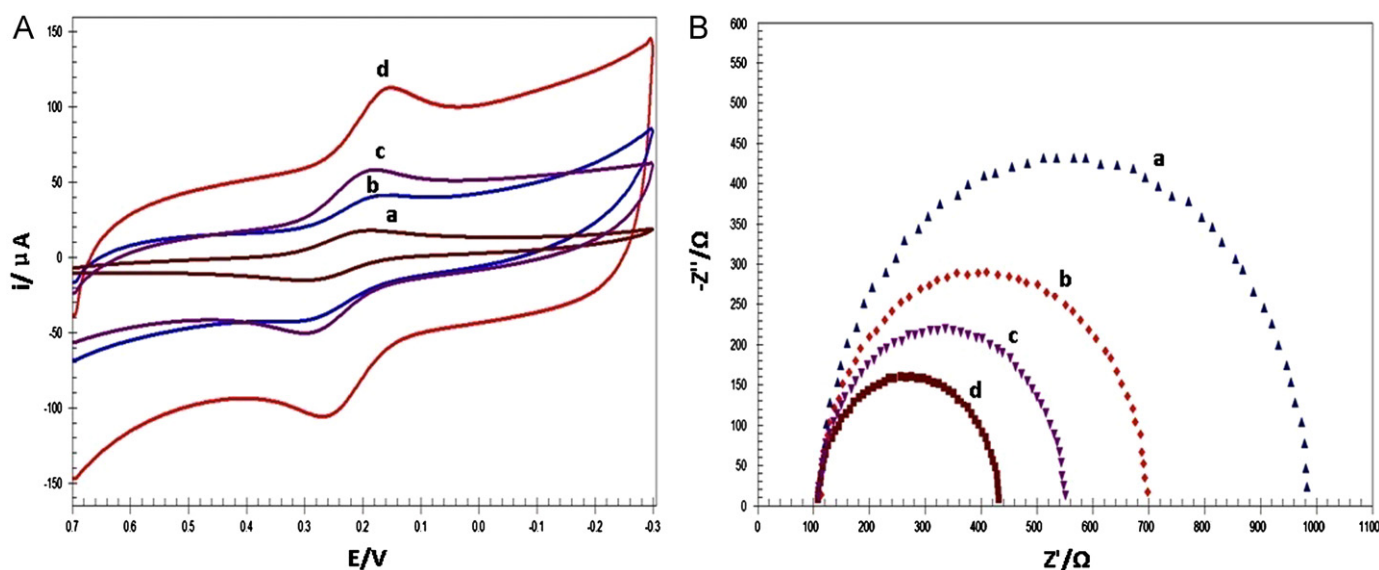


Fig. 2. Cyclic voltammograms (A) and Nyquist diagrams (B) of 1.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 mol/L KCl recorded at (a) bare GCE, (b) FePt/GCE, (c) CNTs/GCE and (d) FePt/CNTs/GCE.

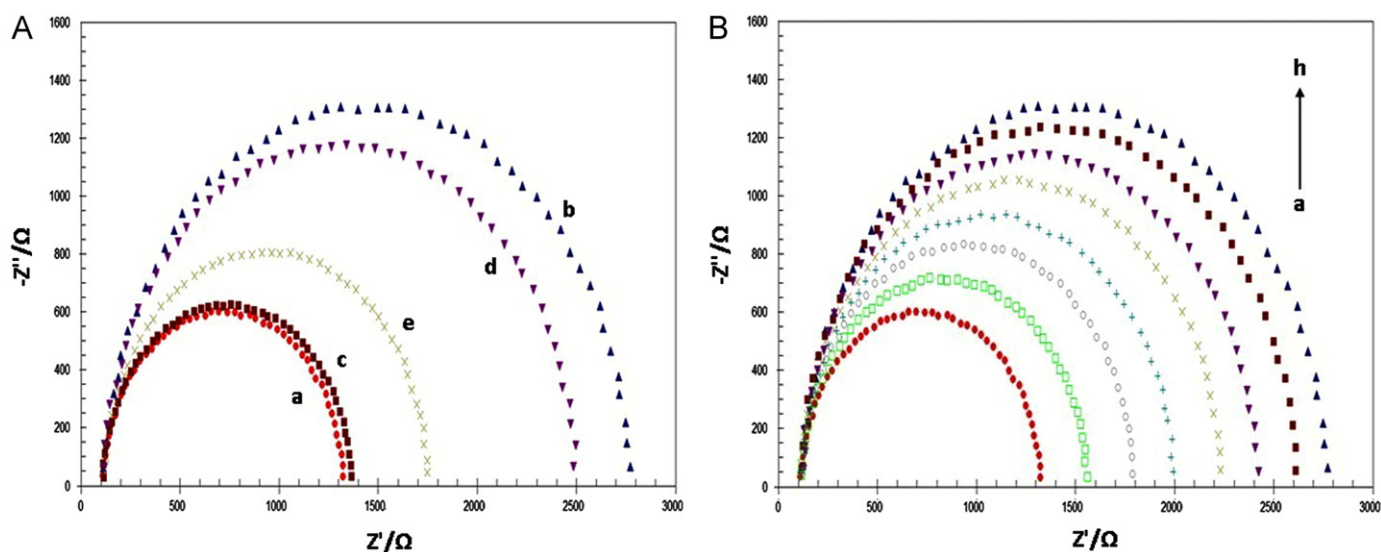


Fig. 3. (A) Nyquist diagrams of 1.0 mmol/L [Fe(CN)₆]^{3-/4-} in 0.1 mol/L KCl recorded at (a) ssDNA/FePt/CNTs/GCE, (b) dsDNA/FePt/CNTs/GCE (hybridization with cDNA), (c) the electrode hybridized with ncDNA, (d) the electrode hybridized with single-base mismatched DNA and (e) the electrode hybridized with double-base mismatched DNA. (B) Nyquist diagrams of 1.0 mmol/L [Fe(CN)₆]^{3-/4-} in 0.1 mol/L KCl recorded at (a) ssDNA/FePt/CNTs/GCE and after hybridization reaction with different concentrations of PML/RARA fusion gene target sequence: (b) 1.0×10^{-12} ; (c) 1.0×10^{-11} ; (d) 1.0×10^{-10} ; (e) 1.0×10^{-9} ; (f) 1.0×10^{-8} ; (g) 1.0×10^{-7} and (h) 1.0×10^{-6} mol/L.

Nyquist diagram was shown in curve b. The R_{et} value increased obviously, suggesting that hybrids (dsDNA) were formed at the electrode. The increase of the R_{et} value was negligible after the ssDNA probe was hybridized with the ncDNA (curve c), indicating that the hybridization reaction was not achieved. After the ssDNA probe was hybridized with the single-base mismatched DNA sequence (curve d) or double-base mismatched DNA sequence (curve e), the increase of the R_{et} value was much smaller than that obtained from the hybridization with the cDNA (curve b). And the single-base mismatched and double-base mismatched DNA sequence could also be recognized via comparing the increase of the R_{et} value. The results demonstrated that this DNA biosensor displayed high selectivity for the hybridization detection.

The difference between the R_{et} values (namely ΔR_{et}) of 1.0 mmol/L [Fe(CN)₆]^{3-/4-} containing 0.1 mol/L KCl at the probe ssDNA/FePt/CNTs/GCE and that at the hybridization modified electrode (dsDNA/FePt/CNTs/GCE) was used to be the measurement signal for quantitative analysis of the PML/RARA fusion gene target sequence. The concentration of the PML/RARA fusion gene target sequence in the hybridization solution was changed from 1.0×10^{-6} to 1.0×10^{-12} mol/L, and the results were shown in Fig. 3B. The ΔR_{et} value between before and after hybridization was linear with the logarithm of the PML/RARA fusion gene target sequence concentrations in the range of 1.0×10^{-12} to 1.0×10^{-6} mol/L with the regression equation $\Delta R_{et}(\Omega) = 195.7 \lg C + 2638.5$ and the regression coefficient (R) 0.9957, and a detection limit of 2.1×10^{-13} mol/L could be estimated using 3σ rule (where σ was the relative standard deviation of 11 parallel measurements of the blank solution). The results indicated that the label-free strategy based on this FePt/CNTs/GCE platform exhibited high sensitivity for electrochemical detection of DNA hybridization, which may be ascribed to the following reasons. The high specific surface area and strong adsorption ability of CNTs provided a good candidate for anchoring FePt NPs. Using ethylene glycol as the stabilizer, the FePt NPs possessed small size and long-term stability. The small size effect further increased the surface area of CNTs and the platform's conductivity was enhanced due to the presence of FePt NPs. Importantly, the abundant FePt NPs with fine biocompatibility provided more binding sites for probe ssDNA, which might improve the sensitivity for the target sequence detection.

3.5. Stability, reusability and reproducibility investigation

The stability of oligonucleotide probes on the modified electrode surface is believed to be a crucial factor to achieve high sensitivity. To investigate the stability of the probe ssDNA/FePt/CNTs/GCE, the electrode was incubated in PBS buffer solution (pH 7.0) at room temperature for 12 h, respectively. Then the probe ssDNA modified electrode was tested in 1.0 mmol/L [Fe(CN)₆]^{3-/4-}, and the obtained EIS signals almost remained unchanged compared with the unincubated electrode. The ssDNA/FePt/CNTs/GCE was stored at 4 °C for 2 weeks and the decrease of the impedance response was got as 4.51%. The results demonstrated that the prepared DNA biosensor had good stability and could be applied for further quantitative analysis.

The reusability of the DNA biosensor was also evaluated. The dsDNA on the hybridized electrode was hot denatured by immersing the electrode into boiling water for 10 min, and then cooled down rapidly with an ice salt bath, followed by rinsing the electrode with ultrapure water. The renewed biosensor was investigated using 1.0×10^{-6} mol/L target DNA. The Nyquist diagrams of [Fe(CN)₆]^{3-/4-} at the renewed probe modified electrode and its hybridized electrode were recorded. The results indicated that the two R_{et} values at the two electrodes and their ΔR_{et} value were, respectively, almost the same as those obtained in the first experiment. Repetitive experiments showed that the DNA biosensor could be reused for three times without losing its sensitivity, exhibiting the fine reusability of the proposed DNA biosensor.

The reproducibility of this impedance-based DNA biosensor is highly significant in DNA assay. In our experiment, six parallel-fabricated probe modified electrodes were performed to detect 1.0×10^{-9} mol/L target DNA. Nyquist diagrams of these electrodes before and after hybridization were recorded and a relative standard deviation of 4.25% for the ΔR_{et} value was evaluated. The results suggested that the DNA biosensor was highly reproducible.

4. Conclusions

In summary, a novel FePt/CNTs platform was constructed and used successfully for label-free impedance sensing of DNA hybridization. The decoration of CNTs with FePt NPs integrated

the advantages of CNTs and FePt NPs, owning large surface area, good conductivity, and fine biocompatibility, which provided a favorable interface for DNA biosensing. Hybridization to its cDNA could cause interfacial property changes, which were monitored by the EIS technique and employed as the signal for DNA hybridization detection. The approach does not need oligonucleotide probe or target to be labeled previously, which makes it advantageous in terms of simplicity and noninvasiveness. Furthermore, this electrode modification strategy is also expected for extensive applications in sensing of other biomolecules.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 21205057 and 21101087), the Shandong Province Natural Science Foundation (Nos. ZR2010BQ030 and Y2005B02), and the Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

Appendix A. Supplementary materials

Supplementary materials associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2012.10.052>.

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