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Biosensor based on nanocomposite material for pathogenic virus detection

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ABSTRACT

This paper introduces a DNA biosensor based on a DNA/chitosan/multi-walled carbon nanotube nanocomposite for pathogenic virus detection. An easy, cost-effective approach to the immobilization of probe DNA sequences on the sensor surface was performed. Cyclic voltammograms were used to characterize the probe DNA sequence immobilization. Complementary sequence hybridization was examined by electrochemical impedance spectroscopy. Results revealed that the developed DNA sensor can detect a target DNA concentration as low as 0.01×10^{-12} M. The sensitivity of the prepared sensor was 52.57 kΩ/fM. The reusability and storage stability of the DNA sensor were also investigated. Results showed that the electron-transfer resistance decreased to approximately 35% after 8 weeks and to approximately 80% after 12 weeks of storage.

1. Introduction

The detection of specific DNA sequences is important in numerous applications in modern life science, including clinical diagnosis [1,2], environmental analysis [3,4], and food quality control [5]. A number of approaches (e.g., traditional approaches) have been used for this purpose, including polymerase chain reaction (PCR) [6], real-time PCR [7]. However, these methods are complex, costly, time consuming, and impossible to carry out in on-site or in-field tests. Therefore, the development of an inexpensive, reliable, rapid-detection device remains a challenge for academic and industrial scientists. DNA biosensors are considered as promising complementary tools to traditional methods. In principle, DNA biosensors are fabricated by attaching probe DNA sequences onto the surface of a suitable transducer that can convert a biological response into an electrical [8], optical [9], or mechanical [2,5] signal.

With the development of nanotechnology, the applications of nanomaterials for biosensors are remarkably progressing. Various nanomaterials such as nanowires [10], nanorods [11], nanoparticles [12], carbon nanotubes [13], hybrid materials [14], and nanocomposite materials [15,16] are used for biosensor application. Among them, nanocomposite materials are attracting increased attention because of their considerably flexible chemical structure and unique physical properties useful for the development of novel DNA biosensors with the capability of rapid electron transfer on their surface. Current applications of nanocomposite materials in DNA biosensors have been reported by various research groups [17–19]. Kang et al. [20] constructed a biosensor based on the immobilization of glucose oxidase in chitosan (CHIT) on a glassy carbon electrode modified with gold platinum alloy nanoparticles/multi-walled carbon nanotubes (MWCNTs). It found that biosensor has a detection limit of 0.2 μM, a fast response time of <5 s, and a wide linear range from 0.001 mM to 7.0 mM. The biosensor also has good reproducibility, stability, and selectivity. Wang et al. [21] developed a biosensor based on the covalent attachment of probe DNA onto CHIT/MWCNTs nanocomposite material using glutaraldehyde as an arm linker. In this work, CHIT/MWCNTs material was dropped onto the sensor surface, and the sensor was then immersed in a glutaraldehyde solution and incubated in a DNA solution. Results show that the biosensor has good stability within the linear range of 1.0×10^{-13} M to 5.0×10^{-10} M and a low detection limit of 8.5×10^{-14} M. Recently, Erdema et al. [22] fabricated a disposable sensor using carbon nanotube (CNT)/CHIT modified disposable pencil graphite electrodes for the electrochemical determination of DNA sequence hybridization without using an external indicator. They further performed electrochemical characterizations by electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry. Results show a sensitive detection limit of 13.25 μg/mL within the linear concentration

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Table 1
Strands of DNA used for the DNA sensor.

Oligonucleotides	Sequences
Probe sequence	5′-AACGCCGATACCATTACTTA-3′
Control sequence	5′-AACGCCGATACCATTACTTA-3′
Complementary sequence	3′-TTGCGGCTATGGTAATGAAT-5′

range of the target DNA from 10 μg/mL to 80 μg/mL. In another study, Wang et al. [23] studied a DNA sensor based on the immobilization of probe DNA sequences on an electrode surface modified with CHIT/CNT composite, and used a copper complex as a new redox hybridization indicator. Electrochemical characterization experiments indicate that the oxidation peak currents have a linear relationship with the target DNA concentration ranging from 5.0×10^{-10} M to 1.0×10^{-8} M and a detection limit of 5.0×10^{-10} M for the target NA.

In the current work, a DNA biosensor based on DNA/CHIT/CNT nanocomposite materials was fabricated for label-free pathogenic virus detection. An easy method of DNA probe sequence immobilization on the sensor surface was established. The interaction between probe and target DNA strands was examined by EIS. Results revealed that the sensor can detect a target-DNA concentration as low as 0.01×10^{-12} M. The sensitivity of the prepared sensor was 52.57 kΩ/fM. Thus, using the DNA/CHIT/CNT nanocomposite materials was found to effectively increase sensitivity and enhance biosensor performance.

2. Experimental

2.1. Chemical reagents

MWCNTs were purchased from Shenzhen Nanotech Port Co., Ltd. (diameter, <10 nm; length, 5–15 μm; purity, ~95%; amorphous carbon content, <3%). DNA sequences (Table 1) were provided by Invitrogen Co. The stock solution (10 μM) of all oligonucleotides was prepared with Tris–EDTA (TE) buffer solution. TE buffer solution, CHIT (deacetylation degree ≥ 90%), and 65% nitric acid (HNO_3) were purchased from Sigma–Aldrich. Potassium ferrocyanide and potassium ferricyanide were purchased from Beijing Chemical Reagent (China). All other reagents were commercially obtained. All aqueous solutions were prepared using Milli-Q water.

2.2. Electrode fabrication

A sensor based on interdigitated electrodes (IDEs) was designed and fabricated. A dual electrode with a finger width of 10 μm and a gap size of 10 μm was fabricated using the conventional photolithographic method. Interdigitated finger electrodes were fabricated by sputtering 10 nm Ti and 200 nm Pt on a ~100 nm-thick silicon dioxide layer thermally grown on top of silicon wafer. Fig. 1 presents the schematic of IDEs.

2.3. Preparation of DNA/CHIT/MWCNT-modified sensor

The DNA/CHIT/MWCNT mixture was prepared as follows. First, 10 mg of MWCNTs was denatured in 50 mL of concentrated HNO_3 (15 M) and refluxed for 8 h in a silicone oil bath maintained at 110 °C to form carboxylic groups. Second, the denatured MWCNTs were rinsed with distilled water until the solution pH was neutral. Finally, the MWCNTs were dried at 80 °C in a vacuum oven. The denatured MWCNTs (5 mg) were then dispersed in 5 mL of 1% acetic acid solution containing 1.5 mg of CHIT. Ultrasonication was conducted at 125 W for 120 min to form covalent coupling between the carboxylated ends of MWCNTs and the free amine group of CHIT. Before modification, the surface of the electrodes was initially cleaned with KCr_2O_7 in 98% H_2SO_4 followed by electrochemical treatment [cyclic voltammetry (CV) with sweeping potential from −1.5 V to +2.1 V and scan rate of 25 mV/s] in 0.5 M H_2SO_4 to activate the electrode surface. The surface was then rinsed with double-distilled water. Subsequently, 10 μL of CHIT/MWCNT dispersion solution was drop coated onto the sensor surface and dried in a desiccator to form a CHIT/MWCNT nanocomposite film on the sensor surface. Finally, the modified sensor surface was incubated in DNA solution at $T = 37$ °C for 18 h. The sensor was rinsed with Milli-Q water (18 MΩ cm^{-1}) and dried under nitrogen flow.

2.4. CV measurements

All CV measurements were performed using an IM6 impedance analyzer with IM6 THALES software. An IDE serving as a working

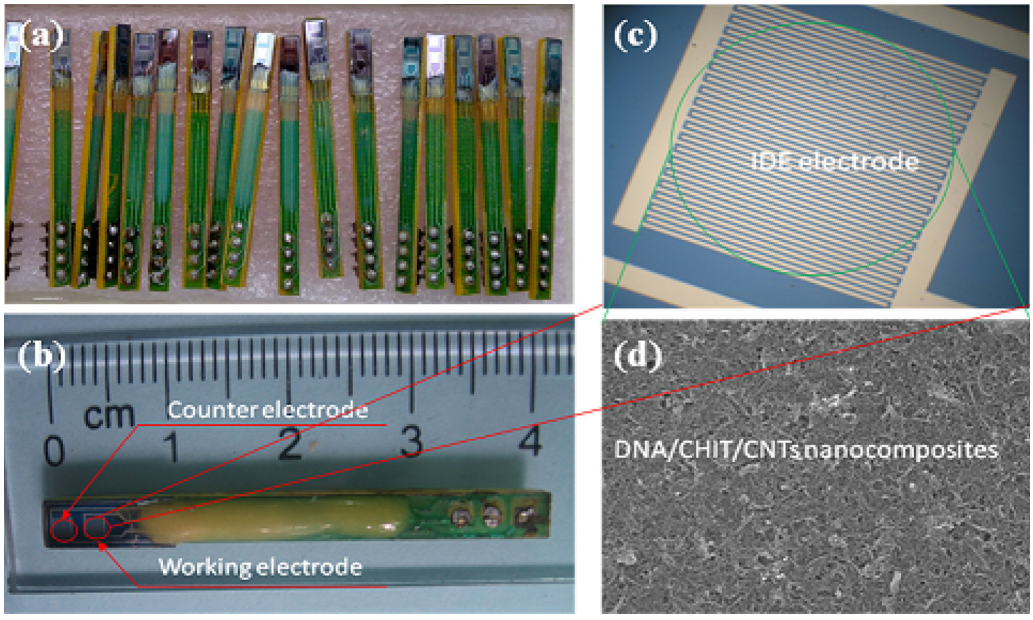

Fig. 1. Schematic of the interdigitated electrodes, electrodes (a), one electrode (b), window (c), and density of CNTs on electrode surface (d).

electrode was immersed in a measuring cell filled with 1 mM PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$ (pH 7.4) connected to test and sense probes. A Pt electrode was connected to the counter electrode on the IM6 impedance analyzer, and the Ag/AgCl electrode was used as a reference electrode. The potential was scanned from −0.1 V to 0.6 V at a scan rate of 100 mV/s.

2.5. Detection of complementary sequence hybridization using the DNA sensor

The probe DNA-modified electrode was used to determine the concentration of the target DNA sequences inside the sample. Measurements were performed with an IM6 impedance analyzer using IM6 THALES software. The probe DNA-modified electrode was immersed in a measuring cell containing 1 mM PBS solution with a defined concentration of the target DNA (pH 7.4) for 30 min at room temperature, and then rinsed with TE buffer to remove non-specifically adsorbed DNA sequences. EIS was used to determine hybridization by dipping the hybridized electrode in 1 mM PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$ as an indicating probe. The hybridized electrode was connected to the test and sense probes. Pt electrode was connected to the counter electrode on the IM6 impedance analyzer, and the Ag/AgCl electrode was used as a reference electrode. All tests were conducted in an open circuit. The tested frequency range was from 1 Hz to 100 kHz with an amplitude of ±5 mV. The Nyquist frequency was recorded. The difference between the electron transfer resistance (R_{et}) before and after hybridization was taken as the signal produced by the interaction reaction between the probe DNA and target DNA sequences.

3. Results and discussion

3.1. CV characterization of DNA immobilization on sensor surface

The immobilization of probe DNA sequences on the sensor surface is a significant step in the development of DNA biosensors. To date, various immobilization methods have been studied, including electrostatic binding [24], physical absorption [25,26], and covalent attachment [13]. In the current work, the physical absorption method was used to immobilize probe DNA sequences on the sensor surface. The preparation process was performed as described in Section 2. After immobilization, the DNA sensor was characterized by CV using $[Fe(CN)_6]^{3-/4-}$ as an electrical probe. Fig. 2 presents the CV data of probe DNA sequence immobilization in PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 10 mV/s versus a Ag/AgCl reference electrode. The CV results showed a reversible redox current of 1.5 mA of $[Fe(CN)_6]^{3-/4-}$ at a potential of 0.28 V on bare IDE. When IDE was coated with a layer pure CHIT film, the decrement of the peak current of IDE was larger than that of bare IDE (curve b) because CHIT film has poor conductivity and can block the electron transfer of the redox probe. The excellent conductivity of the added MWCNT in the CHIT film accelerated the electron transfer between redox probes, resulting in significantly increased current compared with pure CHIT film. When the probe DNA sequences were adsorbed onto the CHIT/CNT film, the peak current of $[Fe(CN)_6]^{3-/4-}$ decreased to 0.45 mA because the probe DNA sequences were immobilized on the sensor surface and formed a thin film that blocked the electron transfer between the redox probe and sensor surface. The decrease in peak current showed that the probe DNA sequences were successfully grafted onto the CHIT/CNT film on the sensor surface. Subsequently, the CV characteristic of the DNA sensor at different probe DNA concentrations (0.01×10^{-12} M to 10×10^{-12} M) was investigated. Thin film formation on the sensor surface increased with increased probe DNA concentration, thereby blocking the electron transfer between the redox probe and sensor surface. Consequently, the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ decreased (data not shown). This result again confirmed that the probe DNA sequences were successfully grafted onto the sensor surface of CHIT/CNT film.

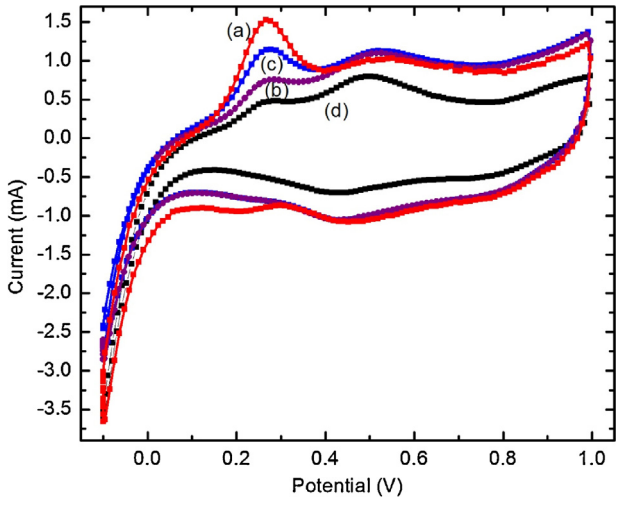

Fig. 2. Cyclic voltammograms of probe DNA immobilization in 1 mM PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 100 mV/s and probe DNA concentration of 1 μM at room temperature: (a) bare interdigitated electrode, (b) CHIT modified interdigitated electrode, (c) CHIT/CNT-modified interdigitated electrode, and (d) DNA/CHIT/CNT-modified interdigitated electrode.

3.2. EIS characterization of DNA sequence hybridization

3.2.1. Complementary sequence hybridization detection

EIS is a powerful technique for the detection of viral pathogens under label-free conditions. In this work, impedance measurements were performed in the presence of 20 mM $[Fe(CN)_6]^{3-/4-}$ as the redox probe to examine the hybridization of complementary strands. Fig. 3 shows a Nyquist plot for bare sensor after probe DNA immobilization and after fully complementary sequence hybridization. The impedance spectrum was a semicircle that represented a measurement of the charge transfer resistance R_{et} on the sensor surface. Fig. 3(a) shows that when the bare sensor was immersed in an electrolyte solution containing the redox probe, the reduction process of the redox probe occurred and electrons were transferred between the two sets of array electrodes through the redox probe. In this case, R_{et} was determined to be 11.93 kΩ. When the probe DNA sequences were immobilized on the sensor surface, a large semicircle corresponding to R_{et} = 14.23 kΩ was found, indicating that the immobilized probe DNA strands inhibited electron transfer on the sensor surface. For the complementary strand hybridization, DNA helix sequences were formed on the sensor surface when target/immobilized DNA matching occurred. This hybridization event created another barrier layer that blocked the access of the redox probe to the sensor surface. Consequently, the diameter of the semicircle increased and R_{et} became 15.21 kΩ. By contrast, no significant signal change was observed for the DNA sensor exposed to the control sequence. Based on these results, an equivalent circuit of the system based on the models of Randles [27] and Yang et al. [28] was simulated and is presented in Fig. 3(b). This equivalent circuit includes the ohmic resistance of the electrolyte solution (R_s) and the Warburg impedance (Z_w), which is the impedance caused by the diffusion of the redox probe to the interface from the electrolyte bulk. These elements were unaffected by the reaction on the sensor surface. The double layer capacitance C_{dl} represents the electrical double layer at the electrode/solution interface, and R_{et} is the electron-transfer resistance. C_{dl} and R_{et} represent the interface properties of the sensor depending on the dielectric and insulating

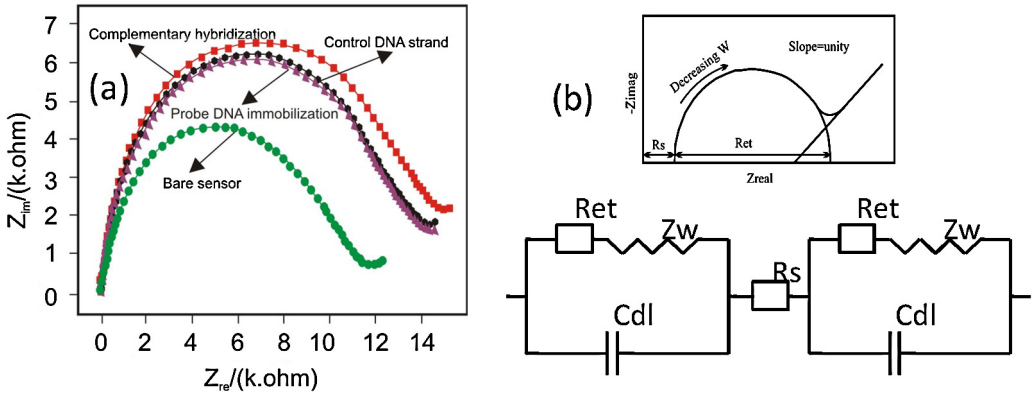

Fig. 3. (a) EIS spectra of complementary strand hybridization between DNA probe and complementary sequences in 1 mM PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$. The DNA target concentration was 10×10^{-12} M, and the hybridization time was 30 min. (b) Equivalent electrical circuit of electrochemical impedance measurement: resistance of electrolyte solution (R_s), Warburg impedance (Z_w; resulting from the diffusion of the redox probe), double layer capacitance (C_{dl}), and electron-transfer resistance (R_{et}).

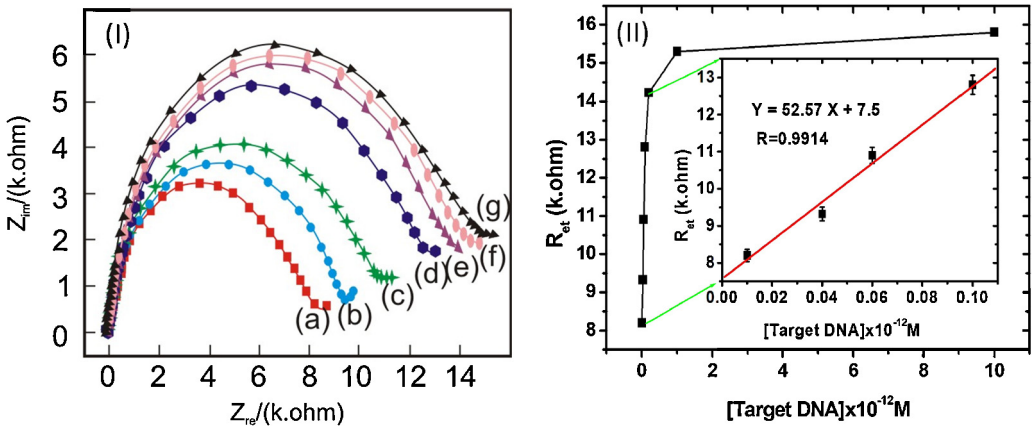

Fig. 4. EIS spectra of complementary strand hybridization in 1 mM PBS using 20 mM $[Fe(CN)_6]^{3-/4-}$ as a redox probe with different target DNA concentrations: (a) 0.01×10^{-12}, (b) 0.04×10^{-12}, (c) 0.06×10^{-12}, (d) 0.1×10^{-12}, (e) 0.2×10^{-12}, (f) 1×10^{-12}, and (g) 10×10^{-12} M. (I) Electron-transfer resistance (R_{et}) as a function of the target DNA concentration (II).

Table 2
Simulated value of elements in the equivalent circuit.

	Zw ($\Omega/s^{1/2}$)	C_{dl} (nF)	R_s[a] (Ω)	R_{et} (Ω)
Bare sensor	1511	1317	900	1470
DNA probe-immobilized	1543	1289	900	1513
Control sequence	1535	1342	900	1573
Complementary strands hybridization	1611	1367	900	1625

[a] R_s was fixed at 900 Ω.

features at the sensor/electrolyte interface [28]. By fitting the electrochemical impedance spectra to the equivalent circuit, the value of each electrical element in the equivalent circuit was obtained, as shown in Table 2.

3.2.2. Effect of target DNA concentration on sensor response

The effect of target DNA concentration on sensor response was next investigated. As shown in Fig. 4(I), the R_{et} values linearly increased with increased target DNA concentration (from 0.01×10^{-12} M to 0.1×10^{-12} M). However, at higher concentrations (>0.1×10^{-12} M), the sensor signal tended to reach the saturation value because of the fully complementary hybridization between the probe DNA and target DNA sequences. The relationship between the electron-transfer resistance and target DNA concentration was plotted, as shown in Fig. 4(II). The sensor had a detection limit of 0.01×10^{-12} M and sensitivity of 52.57 kΩ/fM.

3.2.3. Effect of DNA sequence hybridization time on sensor response

Fig. 5 shows the influence of hybridization time on sensor response. The increase in hybridization time from 10 min to 120 min resulted in increased electron-transfer resistance. The increased hybridization time led to increased hybrid performance. As aforementioned, such hybridization created a barrier layer that blocked the access of the redox probe to the sensor surface, which

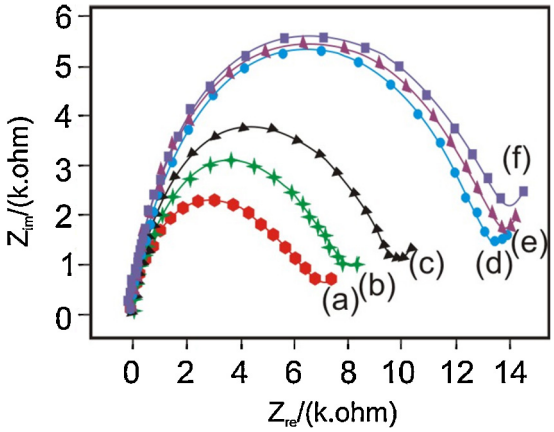

Fig. 5. Effect of different hybridization times on sensor: (a–f, respectively) 10, 15, 20, 40, 80, and 120 min.

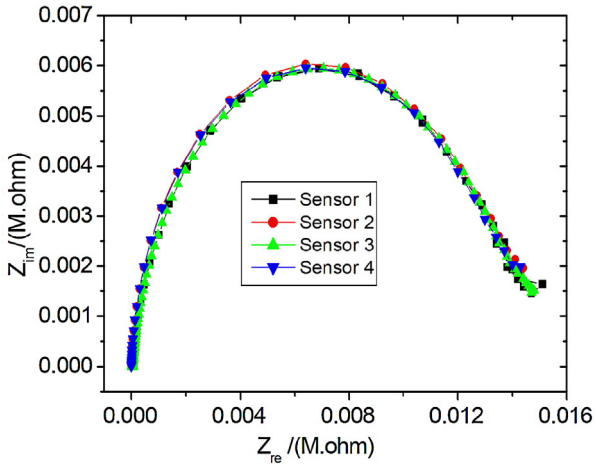

Fig. 6. Reusability of DNA sensor, 20 mM $[Fe(CN)_6]^{3-/4-}$. The DNA target concentration was 10×10^{-12} M, and the hybridization time was 30 min.

resulted in increased diameter of the semicircle. In other words, R_{et} increased. However, R_{et} tended to reach the saturation value when the hybridization time was more than 40 min because the amount of probe DNA that was immobilized on the sensor surface completely hybridized with the target DNA.

3.2.4. Storage stability

The lifetime of the DNA sensor was determined by performing EIS analysis for up to 12 weeks with an interval of 1 week. R_{et} was observed to decrease slowly with time (data not shown). The decrease in R_{et} was about 35% after 8 weeks and about 80% after 12 weeks of storage.

3.2.5. Reusability of DNA sensor

The reusability of a DNA sensor is critical to its application. Thus, the reusability of the prepared DNA sensor was also investigated. After use, the DNA sensor was thermally denatured at 98 °C for 10 min and then quickly frozen in an ice bath for 5 min to obtain single DNA strands. Afterwards, the DNA sensors were immersed in a cell containing target DNA sequences for 30 min at room temperature and then rinsed with TE buffer to remove non-specifically adsorbed DNA sequences. Complementary hybridization was monitored with EIS by dipping the electrode in 1 mM PBS solution containing 20 mM $[Fe(CN)_6]^{3-/4-}$ as an indicator probe. Fig. 6 presents the electrochemical spectroscopy of the DNA sensor after fist DNA hybridization process (sensor 1) and the denatured sensor (second DNA hybridization-sensors 2–4). The output signal of the DNA sensor after fist DNA hybridization process was unchanged compared with that of the denatured sensor. The average value of the electron-transfer resistance R_{et} was 0.0151 MΩ, which corresponded to the relative standard deviation of 2.95% ($n = 4$). In this work, each measurement was plotted corresponds to the three times of the mean value. These results suggested that the DNA sensor was reproducible and reusable.

4. Conclusion

An easy, cost-effective approach to the immobilization of probe DNA sequences on a sensor surface was established. A DNA sensor based on DNA/CHIT/CNT nanocomposite was also developed for label-free pathogen virus detection. Results showed that the DNA sensor can detect a DNA target as low as 1.2×10^{-12} M. The sensor sensitivity obtained was 52.57 kΩ/fM. The reusability and storage stability of the DNA sensor were further investigated. R_{et} was found to decrease by about 35% after 8 weeks and about 80% after 12 weeks of storage. These results can serve as a foundation for the development of an array sensor that can simultaneously determine various targets.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2013.11.016.

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