Electrochemical DNA Biosensor Based on IL-Modified MWNTs Electrode Prepared by Radiation-Induced Graft Polymerization

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ABSTRACT: Electrochemical DNA (E-DNA) biosensors were fabricated by the physical immobilization of probe DNA, 5′-GGA GCT GCT GGC ATT ATT GAA-3′, on ionic-liquid-multiwalled carbon nanotubes (IL-MWNTs) modified with indium tin oxide (ITO) electrodes to detect *Salmonella typhi* (*S. typhi*). IL-MWNTs were prepared by the introduction of 1-butylimidazole bromide onto an epoxy group on poly(GMA)-grafted MWNTs, which were synthesized by radiation-induced graft polymerization of glycidyl methacrylate (GMA) onto MWNTs in aqueous solution. Subsequently, IL-MWNTs were coated onto the ITO electrode surface, and then the physical immobilization of the probe DNA performed in probe DNA solution at room temperature for 1 h. The IL-MWNTs were characterized by elemental analysis, XPS, and TGA. The electron transfer resistance (R_{et}) of the E-DNA biosensor was evaluated after hybridization of the probe DNA and target DNA using electrochemical impedance spectroscopy. The R_{et} increased after the hybridization of probe DNA and target DNA. The DNA used was complementary DNA: 5′-TTC AAT AAT GCC AGC AGC TCC-3′, single-base mismatch DNA: 5′-TTC AAT AAT GGC AGC AGC TCC-3′ and three-base mismatch DNA: 5′-TTC ATT AAT GGC AGC ACG TCC-3′. The dynamic detection range for the sequence-specific DNA of target DNA was from 1.0×10^{-13} to 1.0×10^{-10} mol L^{-1} with a regression equation R_{et} (Ω) = 18.6 C + 128 and regression coefficient (γ) of 0.996. The detection limit was determined to be 3.1×10^{-14} mol L^{-1}. The results demonstrated that the sensitivity of this impedance-based DNA sensor was sufficient for the target DNA sequence detection.
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Key words: E-DNA biosensor; radiation-induced graft polymerization; hybridization; *Salmonella typhi*

INTRODUCTION

The detection of DNA is currently an area of tremendous interest as it plays a major role in clinical, forensic, and pharmaceutical applications. Electrochemical transducers offer many attractive advantages, such as high sensitivity, accurate specificity, simplicity and low cost, for converting nucleic acid hybridization events into useful analytical signals. Nowadays, E-DNA biosensors have been popularly developed for DNA sequence analysis with immobilization of single-stranded DNA (ssDNA) probes on different electrode surfaces.

A large number of articles on carbon nanotube (CNT)-based biosensors have been published over the past several years mainly because CNTs have the following advantages for electrochemical biosensor applications: (1) small size with a large surface area, (2) high sensitivity, (3) fast response time, (4) enhanced electron transfer, and (5) easy protein immobilization on CNT-modified electrodes, coupled with the fact that several methods have been developed.[1–3]

In recent years, direct electrochemistry of biologically important biomolecules in ionic liquids has been studied in both theoretical and practical terms because ionic liquids, with their high polarity, noncoordinating power, high selectivity, fast rates, and great biomolecule stability are considered to be suitable media for supporting biocatalytic processes.[4,5] Biomolecules are usually active and protein refolding is improved in ionic liquids.[6] Zeng and coworkers fabricated a modified GC electrode by entrapping glucose oxidase (GOx) in a nano-Au particle-ionic liquid-*N*,*N*-dimethylformamide composite film.[7] Sun et al. described the fabrication of a modified imidazolium-based carbon ionic liquid electrode (CILE) by entrapping Hb into a sodium alginate hydrogel film.[8] Safavi et al. constructed a modified

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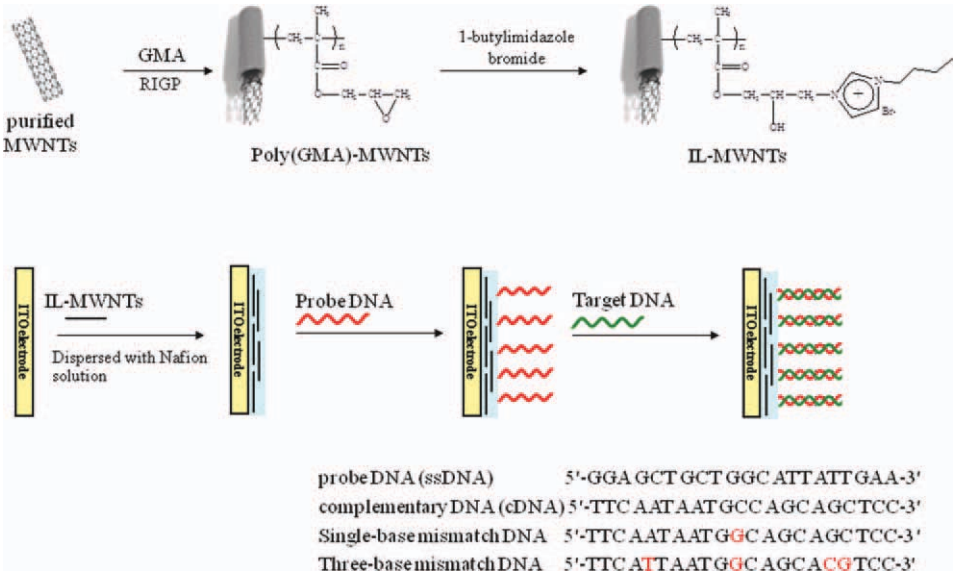

Scheme 1 Preparation procedure of E-DNA sensor based on ionic liquid (IL)-MWNTs electrode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

pyridium-based CILE using octylpyridinium chloride ([OcPy][Cl]) to immobilize direct hemoglobin (Hb) on a CILE.[9] However, to the best of our knowledge, no articles on CNT ion liquid electrodes for the immobilization of enzymes have been published until now, because it is very difficult for the ion liquids to be introduced onto a CNT surface.

Radiation-induced graft polymerization (RIGP) is a useful method for the introduction of functional groups into different polymer matrixes using specially selected monomers. There have been several reports on RIGP of polar monomers onto polymer substrates with hydrophobic properties to obtain hydrophilic properties for versatile applications.[10–12] The RIGP method can be easily modified for the surface of multiwalled carbon nanotubes (MWNTs). In a previous article,[13] an MWNT was modified by various vinyl monomers in an aqueous solution at room temperature by RIGP. In particular, the epoxy groups of poly(glycidyl methacrylate) [poly(GMA)] could be modified to alcohols,[14] amines,[15] phosphonic acids,[16] sulfonic acids,[17] etc.[18] However, little has been reported about the introduction of ionic liquids onto an epoxy group of grafted poly(GMA) chains.

In this study, we synthesized poly(GMA)-grafted MWNTs via RIGP of GMA in an aqueous solution to introduce imidazole bromide. Subsequently, the e-DNA biosensor was fabricated by physical immobilization of the probe DNA onto the IL-MWNTs modified electrode, which was prepared by hand casting of IL-MWNTs onto an indium tin oxide (ITO) electrode surface. The sensing efficiency of the prepared E-DNA biosensor for *Salmonella typhi* (*S. typhi*) was evaluated by electrochemical impedance spectroscopy (EIS).

EXPERIMENT DETAILS

Chemicals

GMA, 1-butylimidazole bromide, and Nafion (per-fluorinated ion-exchange resin, 5% (w/v) in a solution of 90% aliphatic alcohol/10% water mixture) were purchased from the Sigma-Aldrich Co. St. Louis, MO, USA. MWNTs (CM-95) were supplied by Hanwha Nanotech Co. (Korea). Solutions for the experiments were prepared with water purified in a Milli-Q plus water purification system (Millipore Co.; the final resistance of water was 18.2 MΩ cm^{-1}) and solutions were degassed prior to each measurement.

The 21-base oligonucleotides probe DNA (ssDNA), its complementary sequence DNA (cDNA, target DNA, namely a 21-base fragment of *Salmonella typhi* gene sequence), single-base mismatch DNA, and three-base mismatch DNA were purchased from the Bionics Company (Korea), with the following base sequences: Probe DNA (ssDNA): 5′-GGA GCT GCT GGC ATT ATT GAA-3′, complementary DNA (cDNA): 5′-TTC AAT AAT GCC AGC AGC TCC-3′, single-base mismatch DNA: 5′-TTC AAT AAT GGC AGC AGC TCC-3′, and three-base mismatch DNA: 5′-TTC ATT AAT GGC AGC ACG TCC-3′.

All oligonucleotides stock solutions of 21-base oligomers (1.0×10^{-10} mol L^{-1}) were prepared using 0.1*M* PBS (0.1*M* phosphate, pH 7.0, and 0.1*M* NaCl), which were stored at 4°C. More dilute solutions were obtained via diluting aliquots of the stock solution with ultrapure water prior to use.

Synthesis of the poly(GMA)-MWNTs by RIGP

The MWNTs were purified to remove the catalyst and noncrystallized carbon impurities. Briefly, the MWNTs

TABLE I
Elemental Analysis of the Purified MWNTs, Poly(GMA)-MWNTs, and IL-MWNTs

Sample	N (%)	C (%)	H (%)	O (%)
Purified MWNTs		76.14	0.74	11.81
Poly(GMA)-MWNTs		66.78	4.80	22.25
Ionic liquid-MWNTs	5.35	55.79	4.63	18.58

were treated with a 3 : 1 (vol %) mixture of H_2SO_4/HNO_3 and in the process, the MWNTs were cut into shorter segments. The purified and cut MWNTs were used as the supporting materials for grafting with GMA. The MWNTs (2.0 g) and GMA (2.0 g) were mixed in an aqueous solution (70 mL). Nitrogen gas was bubbled through the solution for 30 min to remove oxygen gas, and the solution was irradiated by γ-rays from a Co-60 source under atmospheric pressure and ambient temperature. A total irradiation dose of 30 kGy (a dose rate = 1.0×10^4 Gy/h) was used. The poly(GMA)-MWNTs were dried in a vacuum oven at 50°C for 8 h. IL-MWNTs were synthesized using poly (GMA)-MWNTs and 1-butylimidazole bromide in acetonitrile at 60°C for 16 h. The obtained product was placed into Et_2O and then crystallized in a freezer for 2 h. The solid state product was obtained by decantation and dried in a vacuum oven at room temperature.

Preparation of the E-DNA biosensor based on IL-MWNTs

Scheme 1 shows the fabrication procedure of the IL-MWNTs modified electrode based on an epoxy group-modified MWNTs prepared by RIGP. IL-MWNTs (5.0 mg) were well-dispersed in a 0.5% Nafion solution (0.1 mL). Subsequently, the solution (10 μL) was coated by the hand-cast method on the surface of a precleaned ITO electrode. The prepared electrode was dried for 3 h at room temperature.

Immobilization of the probe DNA onto IL-modified electrode and its hybridization for target DNA

Immobilization of DNA probes was performed by immersing the IL-MWNTs modified electrode into 0.1*M* PBS (pH 7.0) solution containing 1.0×10^{-11} mol L^{-1} probe DNA for 1 h at room temperature, followed by washing the electrode with 0.2% sodium dodecyl sulfate (SDS) solution and ultrapure water, respectively, for 5 min each to remove the unbound probe DNA. The probe DNA/IL-MWNTs modified electrode was immersed in a hybridization solution containing the target DNA (*S. typhi*) and hybridized for 1 h at room temperature, followed by washing the electrode with ultrapure water to remove the unhybridized DNA.

Instrumentation

Electrochemical experiments were performed with an electrochemical impedance spectroscometer model IM6eX (ZAHNER, Germany). All experiments were carried out with a conventional three-electrode system. The working electrode was ITO (working area 0.7×1.1 cm^2) coated with the composite films, counter electrode was the platinum wire, and reference electrode was an Ag/AgCl (saturated KCl). The X-ray photoelectron spectra of the samples were obtained using a MultiLab ESCA2000 (Thermo Fisher Scientific, USA). The thermal gravimetric analysis (TGA) was conducted on a Scinco TGA S-1000 (Seoul, Korea) under a N_2 flow from 25 to 700°C at a heating rate of 20°C/min.

To determine the sensitivity for the prepared E-DNA biosensor, the EIS measurements for E-DNA biosensor were performed using an IM6eX. The AC voltage amplitude was 5 mV and the voltage frequencies ranged from 10^5 to 0.01 Hz in the EIS measurements. The supporting electrolyte solution was 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ and 1.0 mmol L^{-1} $K_4[Fe(CN)_6]$ (1 : 1) solution containing 0.1 mol L^{-1} KCl.

RESULTS AND DISCUSSION

The functionalization of MWNTs is one of the most active fields in nanotubes research, and it is an effective tool to broaden the electrochemical application spectrum of MWNTs. On irradiation with γ-rays of a

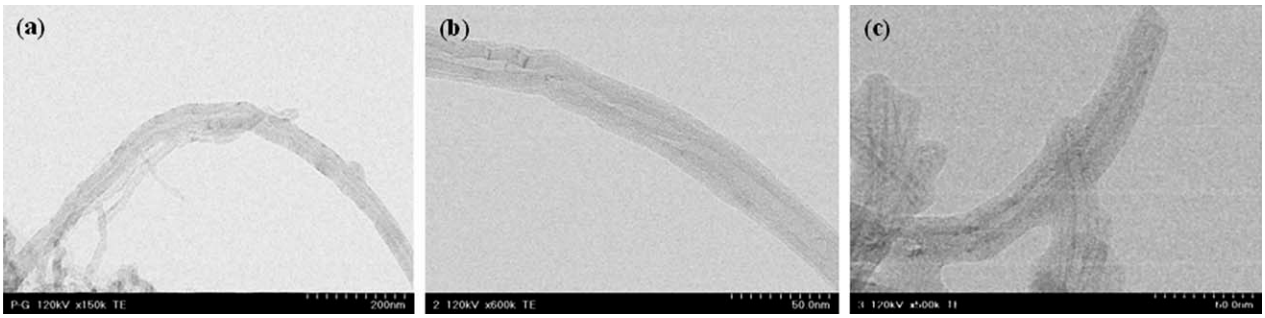

Figure 1 TEM images of purified MWNTs (a), poly(GMA)-MWNTs (b), and IL-MWNTs (c).

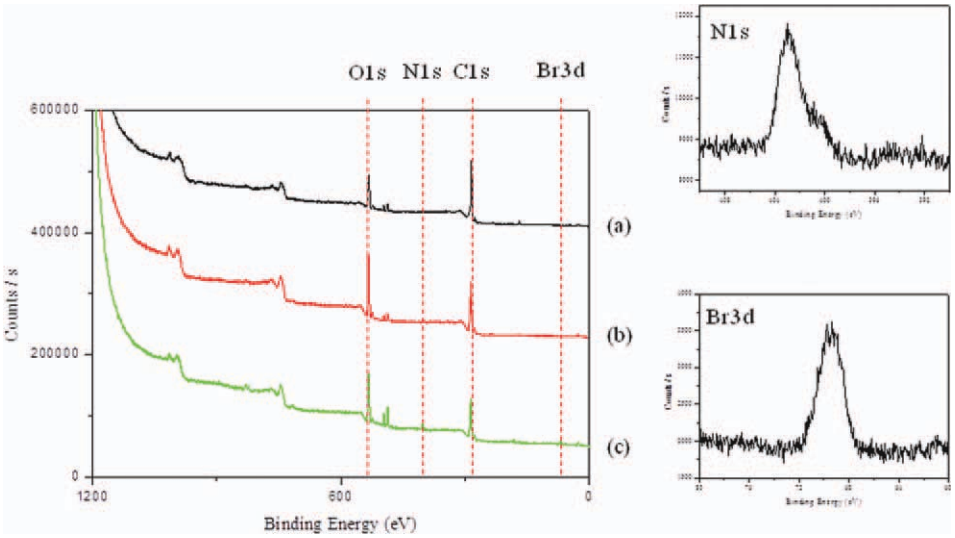

Figure 2 XPS survey scan spectra of purified MWNTs (a), poly(GMA)-MWNTs (b), and IL-MWNTs (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mixture of vinyl monomers containing functional groups and MWNTs, the radical polymerization of the vinyl monomers occurred on the surface of the MWNTs. As a result, tubular-type vinyl polymer-grafted MWNTs with functional groups were successfully obtained in a one-step reaction. The ionic functional groups on the MWNTs surface can readily immobilize DNA by physical adsorption. MWNTs can be used as an electron transfer material to increase the sensitivity in E-DNA biosensor. We performed RIGP of GMA monomer in the presence of MWNTs in an aqueous solution at room temperature to introduce the epoxy group. The epoxy group can be converted to an ionic liquid group such as the imidazolium cation for the purpose of enhancing electron transfer and affinity with DNA. In our previous article, the morphology, physical, and chemical properties of the vinyl polymer-grafted MWNTs prepared by RIGP were evaluated.[13] To enhance electron transfer and the affinity with DNA, we have introduced butyl imidazolium bromide according to Scheme 1 and carried out the corresponding elemental analysis (Table I). The content of imidazolium ion was estimated to be $\sim$ 11.0% by the elemental analysis. This indicates that butyl imidazolium cations were successfully introduced onto the epoxy groups of poly(GMA)-MWNTs.

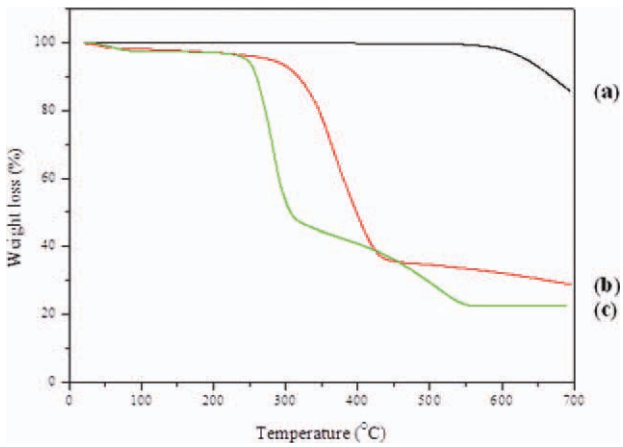

Figure 3 TGA curves of the purified MWNTs (a), poly (GMA)-MWNTs (b), and IL-MWNTs (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

We also evaluated the morphology of MWNTs by TEM, as shown in Figure 1. The diameter of the purified MWNTs was about 40 nm, as shown in Figure 1(a); after RIGP, the diameter increased to 60 nm for the poly(GMA)-MWNTs, as shown in Figure 1(b). When we introduced 1-butylimidazole bromide to an epoxy group of the grafted poly(GMA), the diameter increased to 80 nm, as shown in Figure 1(c). On the other hand, the morphology of the poly (GMA)-MWNT, as shown in Figure 1(b), was shown to be of tubular type. The reason for the tubular-type morphology was considered as follows: to do this, we used as a vinyl monomer GMA, which is composed of hydrophilic >C=O (carbonyl group) and —C(O) —C— (epoxy group) sites and a hydrophobic vinyl group site. The vinyl group of the GMA comes to the surface of the MWNT because of a hydrophobic interaction, while the carbonyl and epoxy group of monomer comes to the surface in an aqueous solution because of a hydrophilic–hydrophilic interaction.

Figure 2 shows an XPS survey scan spectrum of the purified MWNTs (a), poly(GMA)-MWNTs (b), and IL-MWNTs (c). There were significant changes in the XPS data after the introduction of butyl imidazolium bromide onto the poly(GMA)-MWNTs. The

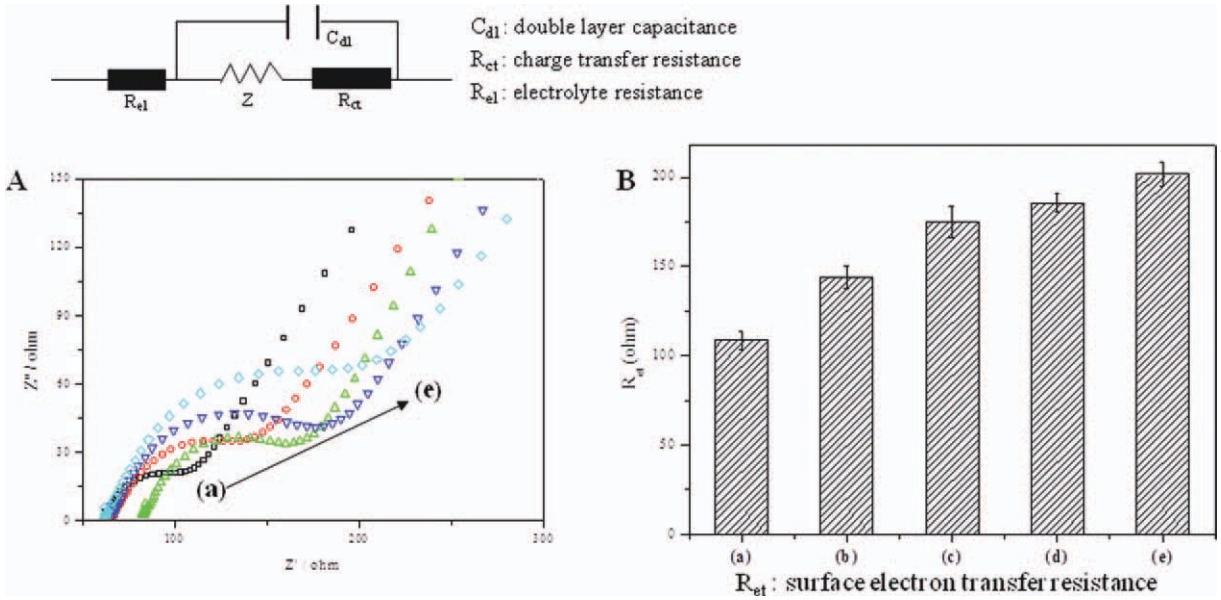

Figure 4 A: Nyquist diagrams recorded. B: Histograms representing the R_{et} values measured at IL-MWNTs (a), ssDNA/IL-MWNT (b), the electrode hybridized with three-base mismatched DNA (c), the electrode hybridized with single-base mismatched DNA (d), and dsDNA/IL-MWNTs (hybridized with cDNA) (e) modified electrode. Supporting electrolyte solution is 1.0 m*M* K4Fe(CN)6/K3Fe(CN)6 (1 : 1) containing 0.1*M* KCl. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

characteristic Br 3d peak at 70 eV and the N 1s peak at 399 eV appeared after introduction of butyl imidazolium bromide onto the poly(GMA)-MWNTs. This illustrates that the IL-MWNTs were successfully prepared by the RIGP method.

Figure 3 shows the TGA curves of the purified MWNTs (a), poly(GMA)-MWNTs (b), and IL-MWNTs (c) prepared by RIGP. As shown in Figure 3, the first weight loss (%) from 50 to 250°C for the poly(GMA)-MWNTs and IL-MWNTs was due to loss of moisture due to the hydrophilic properties of the grafted poly(GMA). The second weight loss appeared in a range of 250–600°C and was due to the loss of grafted poly(GMA). As a result, the graft yield was determined to be ~ 40% after RIGP of the GMA monomer. From these results, we confirmed the successful preparation of poly(GMA)-MWNTs. However, 20% of MWNTs remained after introduction of the imidazolium cations, as shown in Figure 3(c). As a result, it can be concluded that the IL-MWNTs via RIGP were successfully prepared.

As mentioned above, IL-MWNTs can be used as supports for biomolecule immobilization due to their positive charge. E-DNA biosensors were successfully

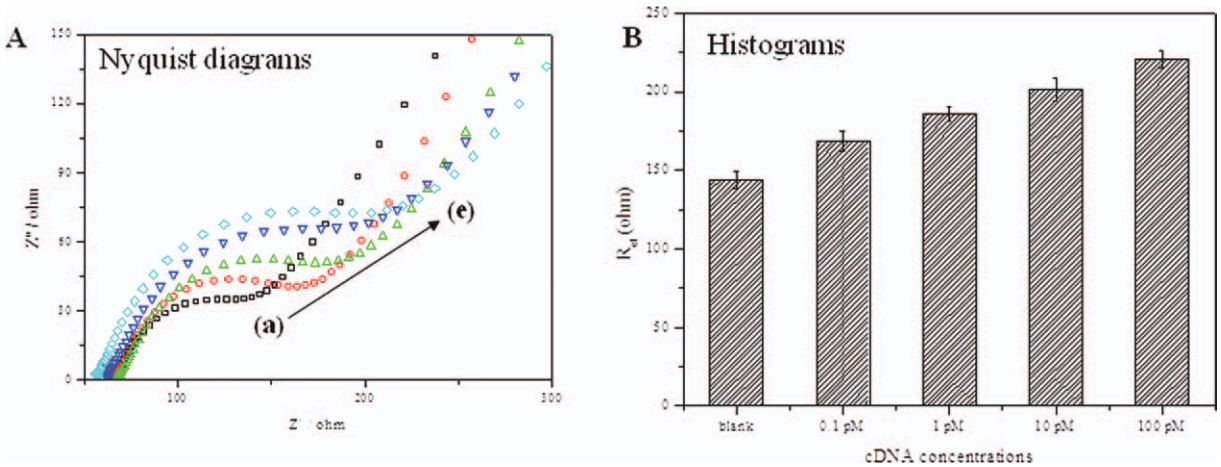

Figure 5 Surface electron transfer resistance (R_{et}) for the prepared E-DNA biosensor in 1.0 m*M* K3Fe(CN)6 solution with 0.1*M* KCl. (a) E-DNA biosensor without target DNA. (b) E-DNA biosensor hybridized with cDNA of 1.0×10^{-13} *M* concentration, (c) 1.0×10^{-12} *M* concentration, (d) 1.0×10^{-11} *M* concentration, and (e) 1.0×10^{-10} *M* concentration. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

prepared by physical adsorption of probe DNA as shown in Scheme 1. The change in the value of the surface electron transfer resistance (R_{et}) of the E-DNA biosensor was expected according to hybridization between probe DNA and matching DNA in electrolyte. Figure 4 shows the surface electron transfer resistance (R_{et}) for the prepared E-DNA biosensor in 1 m*M* $K_3Fe(CN)_6$ solution with 0.1*M* KCl. (a) IL-MWNTs-modified electrode without probe DNA, (b) E-DNA biosensor, (c) E-DNA biosensor hybridized with three-base mismatched DNA, (d) E-DNA biosensor hybridized with single-base mismatched DNA, and (e) E-DNA biosensor hybridized with complementary DNA. In the E-DNA biosensor, the negatively charged phosphate backbone of the probe DNA prevented $[Fe(CN)_6]^{3-/4-}$ from reaching the electrode surface and led to a larger R_{et} value (curve b) than that at the IL-MWNTs modified electrode (curve a). When the probe DNA of the E-DNA biosensor was hybridized with complementary target DNA (cDNA) in electrolyte solution, the R_{et} was further enhanced to a much larger value (curve e). After the probe DNA of the E-DNA biosensor was hybridized with three-base mismatched DNA sequence (curve c) or single-base mismatched DNA sequence (curve d), the increase of the R_{et} value was much smaller than that obtained from the hybridization with the complementary DNA sequence (curve e). In addition the single-base mismatched DNA sequence and the three-base mismatched DNA sequence could also be recognized via comparing the increase of the R_{et} value. These results demonstrate that this DNA biosensor displays very high selectivity for DNA hybridization detection.

Figure 5 shows the surface electron transfer resistance (R_{et}) for the prepared E-DNA biosensor in 1.0 m*M* $K_3Fe(CN)_6$ solution with 0.1*M* KCl. (a) E-DNA biosensor without target DNA, (b) E-DNA biosensor hybridized with cDNA of 1.0×10^{-13} *M* concentration, (c) 1.0×10^{-12} *M* concentration, (d) 1.0×10^{-11} *M* concentration, and (e) 1.0×10^{-10} *M* concentration. The R_{et} value before and after hybridization was linear with the logarithm of the sequence-specific DNA concentrations. The dynamic detection range for the sequence-specific DNA of target DNA was from 1.0×10^{-13} to 1.0×10^{-10} mol L^{-1} with a regression equation R_{et} (Ω) = 18.6 C + 128 and regression coefficient (γ) of 0.996. The detection limit was determined to be 3.1×10^{-14} mol L^{-1}. The results demonstrated that the sensitivity of this impedance-based DNA sensor was sufficient for the target DNA sequence detection.

Three modified electrodes were prepared with the same procedure and used for 1.0×10^{-11} *M* target DNA detection. The results showed an acceptable reproducibility with a RSD of 4.7%. In addition, the sensor was stable for at least 10 days upon storage at 4°C in a dry state, indicating the fine regeneration ability of the biosensor.

CONCLUSION

In this study, we prepared IL-MWNTs by RIGP to prepare E-DNA biosensors. These IL-MWNTs enhanced greatly the immobilization of the probe DNA on the films. The E-DNA biosensor has been used in the detection of DNA sequence-specific of target DNA with high sensitivity, a low detection limit, wide dynamic detection range and could effectively discriminate completely complementary target sequence, single-base mismatched sequence, and three-base mismatched sequence. It can be expected that the new E-DNA biosensor will be widely useful for highly sensitive clinical and other biotechnology applications.

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