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A sensitive impedimetric DNA biosensor for the determination of the HIV gene based on graphene-Nafion composite film

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ABSTRACT

An impedimetric HIV-1 gene biosensor has been developed based on graphene-Nafion composite film. The biosensor was fabricated by adsorbing the single-stranded DNA (ssDNA) on graphene-Nafion modified on the surface of glassy carbon electrode via the π–π^* stacking interactions. As the negative ssDNA and the steric hindrance, the electron transfer resistance of the electrodes toward the $[Fe(CN)_6]^{3-/4}$ redox couple was difficult, the electron transfer resistance value increased. In the measurement of HIV gene, ssDNA probe with the target DNA to form double-stranded DNA (dsDNA), the formation of helix induced dsDNA to release from the surface of the biosensor. The decrease in the electron transfer resistance was in logarithmically direct proportion to the concentration of HIV-1 gene over a range from 1.0×10^{-13} to 1.0×10^{-10} M. The detection limit of this sensor was 2.3×10^{-14} M. It was found that Nafion could not only stabilize graphene but also increase the dispersion of graphene. The results demonstrate that this graphene-Nafion biosensor possesses good selectivity, acceptable stability and reproducibility for HIV-1 gene detection.

1. Introduction

DNA biosensors are important in diagnostic tests for mutation and early cancer detection, analysis of gene sequences, forensic investigation, and assessment of medical treatment (Joseph, 2002). Traditional methods for the detection of DNA on the basis of base-pair hybridization are slow and labor-intensive. DNA hybridization biosensors offer considerable promise for obtaining sequence-specific information in a simpler, faster and cheaper manner compared to traditional hybridization assays. Various DNA biosensors have been established including fluorescence (Murphy et al., 2009), electrochemical luminescence (Su et al., 2010), surface plasma resonance spectroscopy (Wang et al., 2010) and electrochemical (Pohlmann and Sprinzl, 2010) and so on. Although a DNA biosensor using optical readout has many advantages such as high sensitivity, it needs much expensive instrumentation for optical imaging, laser light and labeled probes. Compared to an optical biosensor, an electrochemical biosensor has many advantages such as inexpensive instrumentation, low detection limit and simplicity due to the ease of obtaining an electrical signal (Zhang et al., 2010).

Graphene, a monolayer of sp^2 hybridized carbon atoms packed into a dense honeycomb crystal structure, has attracted considerable attention in recent years due to its high specific surface area and outstanding electrical conductivity (Novoselov et al., 2004). These characteristic properties have attracted considerable attention in electrochemical sensors field (Deng et al., 2013; Guo and Dong, 2011). Concerning this, versatile graphene/bio interfaces have been realized in various nano-biological fields such as biological bioassays, including DNA (Omid et al., 2014), protein (He et al., 2016), pathogen sensors and cyto-toxicity application (Chen et al., 2013). The chemical reduction of graphene oxide (GO) is an effective method for preparing graphene. Researchers have extensively used chemical reducing agents such as hydrazine, methyl hydrazine, sodium borohydride, hydroquinone etc. But the reducing agents used are toxic and dangerous. In addition, graphene agglomerate easily due to van der Waals force, which limits the application of graphene materials (Yin et al., 2010). Therefore, it is necessary to develop an environmentally friendly synthetic method to prepare graphene and its derivatives, and to improve the dispersion of graphene. Graphene-based composites have been deeply investigated as novel electrode modification materials for the improvement of sensing sensitivity (Huang et al., 2012). Nafion has been widely used for effective dispersion of graphene in aqueous solution and can enhance the stability of graphene modified electrodes (Li et al., 2014; Yin et al., 2010).

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Various electrochemical DNA biosensor based on graphene or its derivatives (Niu et al., 2011). An electrochemical DNA biosensor was developed using carminic acid modified screen-printed carbon electrode (nafion-graphene/SPCE) with a detection limit of 5×10^{-9} M (Li et al., 2014). The DNA biosensor was designed based on gold nanoparticles and graphene modified electrode (Zhang and Jiang, 2012). A sensitive electrochemical DNA biosensor was reported by thionine-graphene nanocomposite modified electrode with the detection limit of 1.26×10^{-13} M (Zhu et al., 2012). A novel biosensor for uracil-DNA glycosylase activity was developed based on the electrochemical oxidation of guanine bases at the graphene modified electrode (Jiao et al., 2016). An ultrasensitive electrochemical DNA biosensor was also reported based on graphene/Au nanorod/polythionine for human papilloma virus DNA detection (Huang et al., 2015). Li et al. 2015 developed a graphene-rGO double-layer electrochemical biosensor for the detection of DNA sequence with a detect limit of 1.58×10^{-13} M. Though these methods have high sensitivity, hybridization indicators or labeled of DNA probes are generally needed. To overcome these limitations, increasing researches have been made to develop label-free electrochemical DNA biosensor. The EIS sensor has high sensitivity and selectivity without labeling or hybridization indicator, the weak damage to the film of modified electrode. A novel gold nanoparticles-ionic liquid/3,4,9,10-perylene tetracarboxylic acid/graphene platform was reported and was used for label-free EIS sensing of DNA (Hu et al., 2011). Our group synthesized electrochemically reduced GO and constructed a label-free impedance sensor for HIV-1 gene (Gong et al., 2015).

Acquired immune deficiency syndrome (AIDS) is a severe communicable immune deficiency disease caused by the human immune deficiency virus (HIV), mostly HIV-1 (Zheng et al., 2012). The World Health Organization (WHO)/Joint United Nations Program on HIV/AIDS (UNAIDS) estimates that 40 million people are living with HIV/AIDS infection to date (McMichael, 2006). Thus, testing for HIV-1 infection is greatly desirable to identify unknown infected individuals, scale up treatment service and protect the healthy population. To date, various strategies have been developed, such as the reverse transcriptase-polymerase chain reaction (Gibellini et al., 2004), nucleic acid sequence-based amplification (Mohammadi et al., 2012), and a signal amplification methodology termed the branched-chain DNA technique. All of these tests check for the presence of antibodies to HIV have the following disadvantages: high assay cost, relatively long analysis time, and reliance on expensive, dedicated equipment (Anny et al., 2011). Therefore, highly specific, rapid, and convenient HIV detection systems are required to facilitate the identification of infected individuals, monitoring the disease progression of infected individuals, and investigating clinical trials of new drugs or vaccine candidates (Yin et al., 2010).

In this article, we describe a simple, sensitive impedimetric DNA biosensor for the determination of the HIV-1 gene, based on graphene as a sensing platform. The environmentally friendly L-cysteine was used as reducing agent, and GO was reduced to graphene, which could overcome the environmental hazard in graphene preparation. Nafion was used to effectively disperse graphene in aqueous solution and to enhance the stability the modified electrodes. A schematic representation of the DNA biosensor, with fabrication steps and performance is shown in Fig. 1.

2. Experimental

2.1. Synthesis of graphene

Graphene oxide (GO) was synthesized from natural graphite according to modified Hummers' method (Hummers and Offeman, 1958; Kovtyukhova et al., 1999). 50 mg of GO was added into 50 mL of deionized water and ultrasonic for 30 min to obtain GO suspension (1 g/L). 0.3 g of L-cysteine was added to the above solution and reluxed at 95 °C for 10 h. The dispersion was filtered with a nylon membrane (0.22 μm), then dried at vacuum drying oven (60 °C) for 48 h to obtain pure graphene.

2.2. Fabrication of the DNA biosensor and hybridization

The glassy carbon electrode (GCE) was sequentially polished with 0.3 μm and 0.05 μm alumina powder and washed ultrasonically in water and ethanol for a few minutes. 2 mL 0.2 wt% Nafion was added into 1 mL 1 g/L graphene suspension and sonicated for 30 min to give a homogeneous solution. 5 μL of the graphene-Nafion suspension was drop-coated onto the surface of the pretreated GCE and dried in air (graphene-Nafion/GCE).

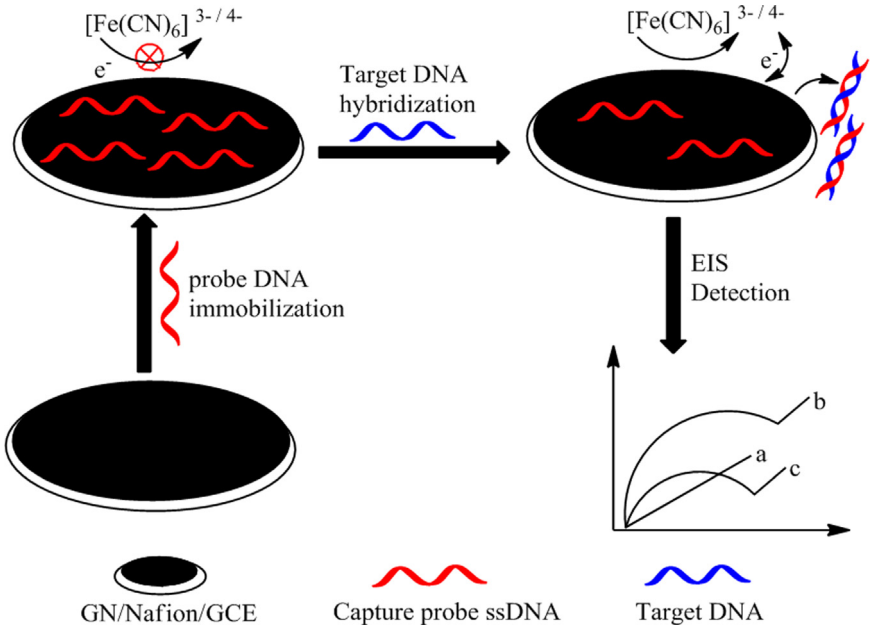

Fig. 1. Schematic diagram of the fabrication of the impedimetric DNA biosensor and the detection of target.

5 μL of 1×10^{-6} M the capture probe was dropped on the graphene-Nafion/GCE for 1 h to obtain ssDNA/graphene-Nafion/GCE. Finally, the obtained biosensor was rinsed and stored at 4 °C before use.

The hybridization reaction was conducted by dropping 5 μL binding buffer contained different concentration of HIV-1 gene or other mismatched DNA sequences onto the surface of the DNA biosensor for 60 min. Then the electrode was rinsed with DNA washing buffer to remove un-hybridized DNA.

2.3. Electrochemical measurements

The DNA sensor was immersed in placed into an electrochemical cell containing 5 mL of 10 mM PBS (pH 7.40), 5.0 mM $K_4[Fe(CN)_6]$-$K_3[Fe(CN)_6]$ and 0.10 M KCl. The concentration of HIV-1 was quantified by an increase of the electron transfer resistance ΔR_{et} ($\Delta R_{et}=R_{et,i}-R_{et,0}$), where $R_{et,0}$ is the electron transfer resistance in the absence of HIV-1 and $R_{et,i}$ is the electron transfer resistance in the presence of HIV-1.

3. Results and discussion

The detection mechanism of the EIS strategy for HIV gene was illustrated in Fig. 1. Graphene-Nafion composite was used as the sensing platform to immobilized the capture probe ssDNA. The capture probe was adsorbed on the surface of graphene via π–π stack interaction between hexagonal cells of graphene and DNA base rings. In the presence of HIV gene oligonucleotide, probe hybridizes with it to form double helix DNA, which 'stands' on graphene-Nafion/GCE and 'leave' the sensing surface. Hence, DNA dybridization induces graphene-Nafion interfacial property changes, including negative charge and conformational transition from 'lying' ssDNA to 'standing' dsDNA and 'leaving'. These changes are monitored by EIS and adopted as the analytical signal.

3.1. Characterization of graphene and the fabrication of the DNA biosensor

Fig. 2 shows the XRD spectrum, Raman spectrum and SEM of graphene to illustrate its structure and morphology. XRD pattern of the as-prepared of graphene are shown in Fig. 2A. XRD pattern shows two peaks at 2θ value of 26.35, 43.08° for as-prepared sample for the planes (002), (101), which was the same as that reported previously (Selvam et al., 2013). The width of graphene peak in the XRD pattern can be attributed to two factors: first, the small sheet size and, second, a relatively short domain order or turbostratic arrangement of graphene stacked sheets, each of which broadens the XRD peak. As indicated in the Raman spectra shown in Fig. 2B, Raman spectra of single layer graphene reveal a G peak at 1574 cm^{-1}, D peak at 1343 cm^{-1}, respectively. The D band of graphene associates with the existence of defects, the lower intensity of D peak, the fewer defects of the graphene layer. It also associates with doping in graphene (Elias et al., 2009). The characteristic morphology of graphene can be observed from the image (Fig. 2C) displaying the featureless basal plane across the majority of the region and the folded sheets at certain locations. Meanwhile, structures with mostly smooth basal planes and sharp edges were also observed.

The morphologies of graphene-Nafion composite dispersed on the GCE surface was studied by SEM (Fig. 2D). The layered structure and a curly morphology consisting of a thin wrinkling

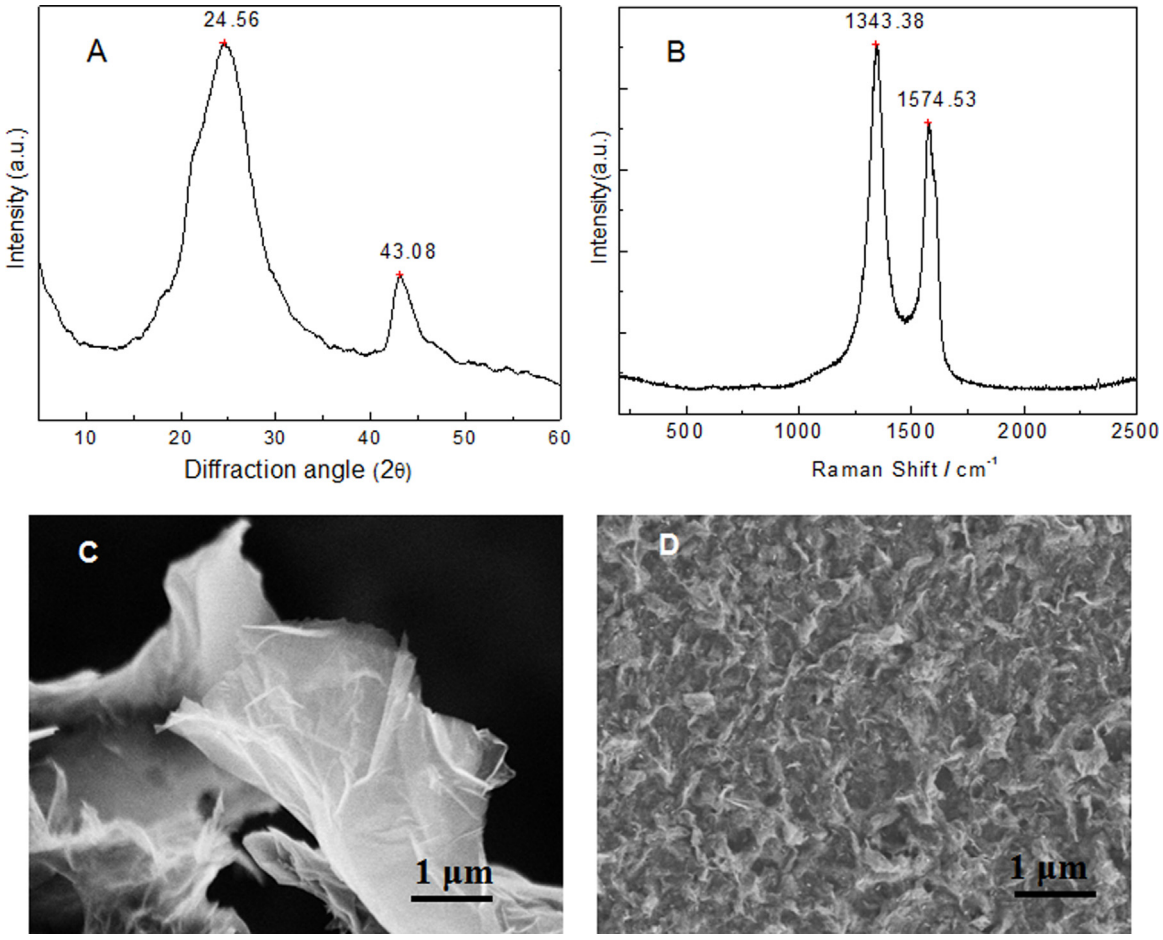

Fig. 2. XRD spectrum (A), Raman spectrum (B) and SEM image of graphene (C), SEM images of graphene-Nafion composites on the surface of GC electrode (D).

paper-like structure were observed, suggesting that during the solvent evaporation of solution-cast films enhanced alignment of graphene layers parallel to the surface of Nafion fibrils. This can be attributed to the unique di-block morphology of Nafion wherein nano-sized additives such as graphene and Nafion are embedded in the interstitial regions (Jung et al., 2011). A similar observation in graphene-Nafion composite (Jung et al., 2011) and graphene oxide nano-sheets (Ansari et al., 2010) and nano-clay reinforced polymer composites has been reported by various researchers.

The characterization of the fabrication of the DNA biosensor is shown in Fig. S1. The results of cyclic voltammetry and EIS indicated that graphene can effectively increase the electron transfer rate of $Fe(CN)_6^{3-/4-}$ due to its outstanding electrical conductivity.

3.2. Electrochemical characteristics of the DNA biosensor

As to estimate the effect of graphene on the electron transfer of the biosensor CV spectroscopy of different modified electrodes were checked (Fig. S2A). The peak current value increased clearly after the probe DNA hybridized with the target HIV-1 gene compared with probe-DNA absorbed electrode. After hybridization, the strong and stable binding between ssDNA and the complementary DNA induces dsDNA releasing from electrode surface, as the interaction between dsDNA and graphene is weaker than the binding between ssDNA and complementary DNA.

To confirm the fabrication and the detection of the biosensor, the electrochemical behavior of methylene blue (MB) used as the indicator at different electrodes was checked. The ssDNA/graphene-Nafion/GCE shows higher peak current values than the graphene-Nafion/GCE which might ascribed to the electrostatic attraction of MB on the phosphate backbones of ssDNA (Fig. S2B). This is the two electron and one proton transfer oxidation/reduction process between MB and its reduced form, leucomethylene blue (LB) (Hu et al., 2012). Moreover, after the probe DNA hybridized with the target DNA (curve c), the MB/LB redox peak currents obviously decreased. This is attributed to the dsDNA release from the graphene-Nafion/GCE resulted in a decrease in the amount of adsorbed MB (Yan et al., 2005). Comparing with the adsorption of MB on the ssDNA/graphene/GCE, the low amount of electrostatically attracted MB contributed to the low peak currents.

3.3. Performance of the DNA biosensor for HIV-1

The quantitative behavior of the DNA biosensor was assessed by measuring the dependence of ΔR_{et} on the concentration of the HIV-1 gene under optimized conditions. As shown in Fig. 3A, the decreased EIS value was directly related to the logarithmic concentration of HIV-1 in the range of 1.0×10^{-13} M to 1.0×10^{-10} M, The regression equation was ΔR_{et} (Ω) = −713 log C-5283 (c is in units of M) with a regression coefficient of 0.9850. The detection limit was estimated to be 2.3×10^{-14} M HIV-1 gene (S/N=3) (Currie, 1995). The result indicated that the label-free electrochemical strategy exhibited high sensitivity, which maybe ascribed to the high specific surface area of graphene provided a good candidate for ssDNA anchoring. A comparison of this method with some reported genosensors in terms of the detection limit and the signal amplification strategy is provided in Table S1. Although the detection limit obtained by differential pulse voltammetry with a gold nanorod decorated graphene oxide sheet is quite lower than that obtained in our work, it requires a complicated decorating procedure. Therefore, the detection limit obtained is considerably satisfactory.

To test whether the bioassay was sufficiently to distinguish target DNA, the sensing interface was incubated with target DNA and three mismatched DNAs (one-base mismatched DNA, two-base mismatched DNA, and three-base mismatched DNA) at the same concentrations, respectively. The difference between the R_{et} before and after hybridization was adopted as the measurement signal. As shown in Fig. 3B, the ΔR_{et} value obtained from complementary DNA hybridization shows the highest difference compared with that from the hybridization of one-base mismatched DNA, two-base mismatched DNA, and three-base mismatched DNA. In principle, hybridization with fully complementary sequences induced much greater interfacial property changes compared to with mismatched sequences due to the higher hybridization efficiency (Hu et al., 2012). Hence, the largest ΔR_{et} value was observed after hybridization with complementary sequences. More mismatched bases exhibited greater interference with hybridization efficiency, resulting in less double helix formation and a lower signal intensity.

The reproducibility of the DNA biosensor was investigated by parallel measurements in PBS buffer. The relative standard deviation was 5.1% (n=7), revealing acceptable reproducibility for the detection of DNA. The stability of the biosensor was also investigated. It was stored in the refrigerator at 4 °C for one week.

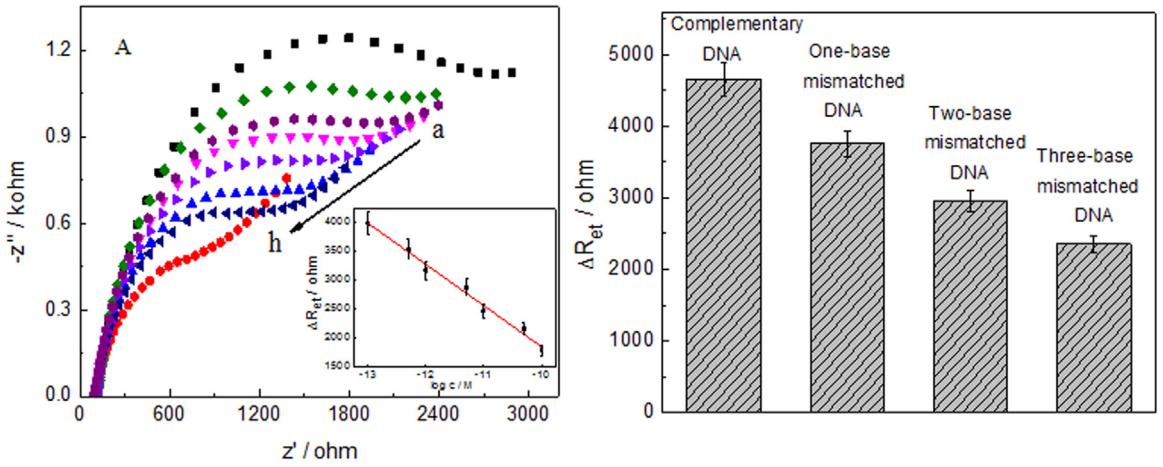

Fig. 3. (A) Nyquist plots of DNA biosensor to different concentrations of HIV-1 gene. Inset: calibration curve of HIV-1 gene. (a) 0; (b) 1.0×10^{-13}; (c) 5.0×10^{-13}; (d) 1.0×10^{-12}; (e) 5.0×10^{-12}; (f) 1.0×10^{-11}; (g) 5.0×10^{-11}; and (h) 1.0×10^{-10} M. (B) ΔR_{et} of the biosensor to different DNA sequences.The concentration was 1.0×10^{-11} M. The illustrated error bars represent the standard deviations of three repeated measurements. EIS measurement conditions were in 10 mM PBS (pH 7.40) containing 5 mM $K_3Fe(CN)_6$-5 mM $K_4Fe(CN)_6$-0.10 M KCl, the frequency was from 100 kHz to 0.1 Hz and the amplitude was 5 mV.

No apparent change of EIS signals was observed before one week, showing that the DNA sensor had good stability.

4. Conclusion

An simple and sensitive impedimetric DNA biosensor for the determination of HIV-1 gene was developed by employing graphene-Nafion composite film modified glassy carbon electrode. Under optimized conditions, the decrease of R_{et} was linearly related to the logarithm of target oligonucleotide with a detection limit of 2.3×10^{-14} M. Compared with the existing methods for DNA detection, the strategy eliminated the requirement for DNA labeling, representing a comparatively simple method. This method may also hold promise for potential applications in DNA biosensing and DNA nanostructure framework construction.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.02.045.

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