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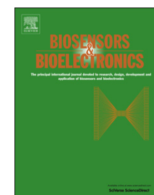

Graphene sheets, polyaniline and AuNPs based DNA sensor for electrochemical determination of BCR/ABL fusion gene with functional hairpin probe

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ABSTRACT

A sensitive and selective electrochemical DNA sensor was developed for the detection of BCR/ABL fusion gene in chronic myelogenous leukemia (CML). Firstly, graphene sheets (GS) suspension was prepared with the aid of chitosan (CS) solution and then fabricated onto the glassy carbon electrode (GCE), followed by the electro-polymerization of aniline to form the PANI layer, then, Au nanoparticles (AuNPs) were electro-deposited onto the modified GCE to immobilize the capture probes. The capture probe employed a hairpin structure and dually labeled with a 5′-SH and a 3′-biotin. After hybridization with the target DNA, hairpin structure was compelled to open and 3′-biotin was forced to stay away from the electrode surface. As a result, streptavidin-alkaline phosphatase (SA-AP) was covalently binded to the capture probe via biotin–avidin system. Reduction currents were then generated after catalyzing the hydrolysis of the electroinactive 1-naphthyl phosphate (1-NP) to 1-naphthol and monitored by differential pulse voltammetry (DPV). Under optimum conditions, the amperometric signals increased linearly with the target DNA concentrations (10 pM to 1000 pM), and the DNA sensor exhibited a detection limit as low as 2.11 pM ($S/N=3$) with an excellent differentiation ability, and the proposed method showed acceptable stability and reproducibility. It has been applied for assay of BCR/ABL fusion gene from real samples with satisfactory results.

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1. Introduction

Chronic myelogenous leukemia (CML) is a clonal neoplastic disorder of hematopoietic stem cells caused by expression of the chimeric BCR/ABL fusion oncogene (Champlin and Golde, 1985; Fialkow et al., 1967; Kantarjian et al., 1993), which is the product of the t (9; 22) Philadelphia (Ph') translocation and proved to be the most characteristic feature of CML (abnormality occurs in more than 95% patients) (Bartley et al., 2010; Kawasaki et al., 1988). With that consideration, determination of BCR/ABL is of great importance for an early diagnosis, a better prognosis and an improvement for detecting minimal residual leukemia cells in CML patients. The existing methods for detecting BCR/ABL fusion gene are mainly about the flow cytometry (FCM) (D'Alessio et al., 2011), real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) (Bennour et al., 2012), chromosome analysis (Soverini et al., 2011), fluorescence in situ hybridization (FISH) (Corbin et al., 2011) and so on.

In last few years, electrochemical DNA sensor has been well recognized as a promising method for the sequence-specific detection of DNA hybridization due to the facts that electrochemical signaling methods are simple, sensitive, specific and cost-effective (Drummond et al., 2003; Li et al., 2011). Significantly, hairpin structures based electrochemical DNA sensors have showed exceptional promise due to their conformational constraints (Kjallman et al., 2008). These kinds of sensors exploit the hybridization-induced conformational changes which can be electrochemically interrogated onto the basis of the distance-dependent electron transfer (Fan et al., 2003). Lin's group has prepared an electrochemical biosensor based on nanogold-modified poly-eriochrome black T film for BCR/ABL fusion gene assay, in which they introduced a method of immobilizing hairpin structure capture probe with nanogold bound, and then, methylene blue was accumulated for DNA hybridization detection (Lin et al., 2010). However, the most hairpin structure designs are

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signal-off sensors, which are easily susceptible to false-positives (Xiao et al., 2007). To eliminate the possible false-positive signals and further improve the sensitivity, enzyme based signal-on strategy has been brought into the hairpin structure based DNA sensor systems (Miranda-Castro et al., 2007).

Recently, graphene sheets (GS), a flat monolayer of one-atom-thick carbon atoms tightly packed into a two-dimensional honeycomb lattice (Brownson and Banks, 2010), are considered to have a range of unusual properties, which exhibit the very large specific surface area, high electrical conductivity and fracture strength (Brownson and Banks, 2011a, 2011b; Brownson et al., 2011). These favorable properties make graphene a promising additive and a supporting component for potential applications in many technological fields (Pumera, 2010; Shao et al., 2010). Ai's group has constructed a highly sensitive biosensor based on the substrate electrode of graphene sheets and dendritic gold nanostructure which has gained satisfactory results for miRNA-21 detection (Yin et al., 2012). However, GS are generally insoluble in water and may leak out upon immobilization onto the electrode surface (Saha et al., 2010). Chitosan (CS) is a biological cationic macromolecule with primary amines, it could be adopted to improve the dispersion and homogeneity of graphene which was functionalized with carboxylic acid groups, as well as enhance the stability of the DNA sensor (Qian and Yang, 2006). Meanwhile, fascinating materials like conductive electroactive polymers (CEPs) have been much investigated (Guiseppi-Elie, 2010; Wallace et al., 1999). Among them, polyaniline (PANI) is considered as one of the most popular and promising CEPs primarily due to its excellent environmental stability and high chemical durability, which also has a superb solution processability (Bo et al., 2011; Dhand et al., 2011; Hu et al., 2012). These special features have made PANI widely used in various kinds of electrochemical applications such as enzyme based biosensor for detection of water pollution, DNA sensor for measurement of sanguinarine and gas sensor for determination of NO_2 gas (Wu and Yin, 2011; Xie et al., 2010; Yang et al., 2013). Huang et al. have prepared the carbon nanotube/polyaniline/Au composite for H_2O_2 detection with a detection limit of 1.4 μM (Feng et al., 2011). Furthermore, metal nanoparticles have been increasingly adopted in the fields of electrochemical DNA sensors owing to their extraordinary electrocatalytic activity (Luo et al., 2006; Wang, 2005). Au nanoparticles (AuNPs) are mostly recommended owing to the fact that they can greatly increase the current response of the modified sensor with a good conductive ability. Meanwhile, they can also largely immobilize the DNA probes via Au-S bonds (Spain et al., 2013). Niu et al. have developed a novel glucose biosensor based on nanocomposites of graphene and gold nanoparticles with a high detection sensitivity (Shan et al., 2010).

In this work, we developed an electrochemical DNA sensor adopting functional hairpin structure probe for the detection of BCR/ABL fusion gene of CML. Firstly, GS suspension was prepared with the aid of CS solution (Shan et al., 2010), and then modified onto the electrode to increase the effective area and enhance the current response of the designed DNA sensor, and CS-GS was also employed as a sensitive material for the electro-polymerization of aniline to form the PANI layer (Wang et al., 2009) which could improve the stability of the developed DNA sensor. Moreover, PANI could further increase the effective electrode surface area and thus lead to plenty AuNPs modified onto the surface, which possessed the ability to anchor more DNA probes to amplify the signals. Finally, electro-deposition of AuNPs was carried out to superbly strengthen the current response and to serve as the immobilizing material for DNA probes via Au–S bonds. The capture probe employed a hairpin structure and dually labeled with a 5′-SH and a 3′-biotin. The biotin terminal acted as an affinity tag for the enzyme binding, which was shielded by the atretic hairpin structure from being approached by SA-AP. Hybridization of target DNA forced the probe to open and compelled the biotin to be away from the electrode. Hence, the biotin label became accessible by SA-AP, and the catalytic signal was observed by using the 1-NP as the enzymatic substrate. As a result, hybridization event was sensitively transduced to the enzymatically amplified electrochemical current signals. The detection strategy was shown in Scheme 1. Taking advantages of AuNPs/PANI/CS-GS, biotin–avidin signaling amplification and alkaline phosphatase, the DNA sensor showed a high detection sensitivity, and it had been applied for assay of longer DNA chain at about 600 bp in PCR real samples with satisfactory results.

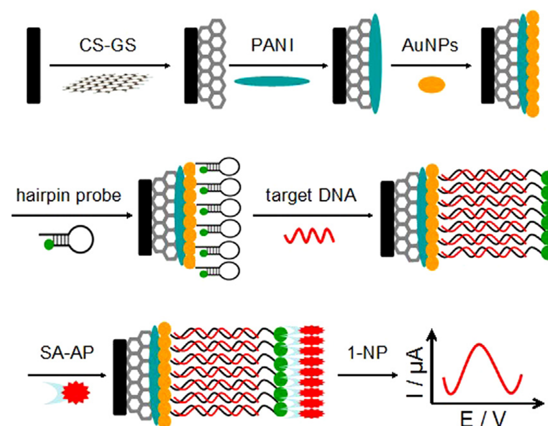
2. Experimental

2.1. Reagents and materials

All oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China), and their sequences were illustrated in Table S1. 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. Graphene sheets (GS) were purchased from Pioneer Nanotechnology Co. (Nanjing, China). 6-mercapto-1-hexanol (MCH), bovine serum albumin (BSA; 96–99%), gold chloride ($HAuCl_4$), Streptavidin-Alkaline Phosphatase (SA-AP) and 1-Naphthyl phosphate (1-NP; 99%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Aniline (99%+) were bought from Adamas Reagent Co. Ltd. (Brussels, Switzerland). Two pairs of primer were purchased from BGI Inc. (Beijing, China) for the PCR amplification of BCR/ABL fusion gene sample, their base sequences were also showed in Table S1. Premix Taq Version 2.0, DL2000 DNA Marker and agarose were purchased from Takara (Dalian, China). All other reagents were analytical grade and used as received. Water (resistivity, 18.2 MΩ) was purified using the Millipore-Q water purification system. A 20 mM Tris–HCl buffer (pH 7.40) containing 0.10 M NaCl, 5.0 mM $MgCl_2$ and 0.005% Tween-20 was used as washing buffer. A DEA buffer (pH 9.60) containing 0.10 M diethanol amine, 1.0 M $MgCl_2$ and 10 mM KCl was used as the working buffer. A 2 × SSC buffer (pH 7.50) containing 30 mM sodium citrate and 300 mM NaCl was used as hybridization solution.

2.2. Apparatus and measurements

Cyclic voltammetric (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetric (DPV) were performed on a



Scheme 1. Schematic illustration of the electrochemical DNA sensor construction process.

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