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High sensitivity surface plasmon resonance biosensor for detection of microRNA and small molecule based on graphene oxide-gold nanoparticles composites

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ABSTRACT

A versatile and sensitive surface plasmon resonance (SPR) biosensor based on two layers of graphene oxide-gold nanoparticles (GO-AuNPs) composites was designed for the detection of microRNA (miRNA) and small molecule adenosine. The bottom layer, which acted as a functionalized substrate on the sensor chip, provided a high specific surface area convenient for the immobilization of capture DNA molecules. The upper layer served as a signal-amplification element. By employing these two layers of GO-AuNPs composites, the dual amplification strategy was achieved so that a measurement of miRNA-141 with a detection limit of 0.1 fM was obtained. Moreover, the developed SPR biosensor showed decent selectivity toward miRNA-200 family members. Especially, the SPR biosensor demonstrated its applicability for the detection of miRNA-141 in cancer cell extractions, and the results obtained were consistent with those obtained by qRT-PCR. Interestingly, small molecule adenosine could also be detected using this SPR biosensor in combination with a split aptamer. Considering the superior sensitivity, selectivity and generality, this work promised much potential for the detection of various biomolecules.

1. Introduction

Graphene-nanoparticle composites, which graphene sheets are decorated with nanoparticles, exhibit not only unique physicochemical properties of the nanoparticles and graphene, but also additional advantages and synergistic properties [1]. Thus, it greatly augments their potential for the application in bioanalysis and biosensing. Since noble metal nanoparticles, especially gold nanoparticles (AuNPs), show unique optical and electronic properties and high biocompatibility [2], they are often used to decorate on graphene sheets, resulting in a formation of graphene oxide-gold nanoparticles (GO-AuNPs) composites [3]. Recently, it was reported that GO-AuNPs composites have been particularly well-suited for constructing various sensors, such as electrochemical sensors [4,5], fluorescence sensors [6], surface enhanced Raman scattering sensors (SERs) [7], resonance Rayleigh scattering [8] and surface plasmon resonance sensors (SPR) [9,10]. Generally, the function of GO-AuNPs composites in these sensors can be categorized into two classes. The first one, existing as a sensor substrate, could provide good biocompatibility and high surface area to significantly increase the immobilization of capture probes, resulting in a subsequent increase in sensitivity [11]. In addition, GO-AuNPs composites as a sensing interface could accelerate electron transfer [12]. Thus, they have been often successfully used in various electrochemical and optical biosensors [7,13,14]. The second one, noted as a signal amplification element, also markedly increased the sensitivity of the biosensor. Zhou et al. reported a novel sandwich-type electrochemiluminescence (ECL) immunosensor for HIV-1 p24 antigen detection using GO-AuNPs and Ru(bpy)32+-dopped silica nanoparticles [15]. The GO-AuNPs composite improved the loading of ECL molecules, resulting in an increase of ECL response and a high sensitivity. We also used GO-AuNPs composites as the signal amplification element to develop a simple and sensitive SPR biosensor [9]. Whether GO-AuNPs composite acted as the sensing substrate or the signal element, it could improve the sensitivity of the biosensor. Therefore, it was speculated that the combination of these two factors can further build a high sensitivity sensor.

On the basis of our previous work, a novel and sensitive SPR biosensor based on two layers of GO-AuNPs composites was developed. As far as we known, this was the first report in which GO-AuNPs composites acted as not only the sensing substrate but also the signal amplification element. This approach was conducted in two simple steps as shown in Fig. 1. First, thiolated capture DNA was covalently

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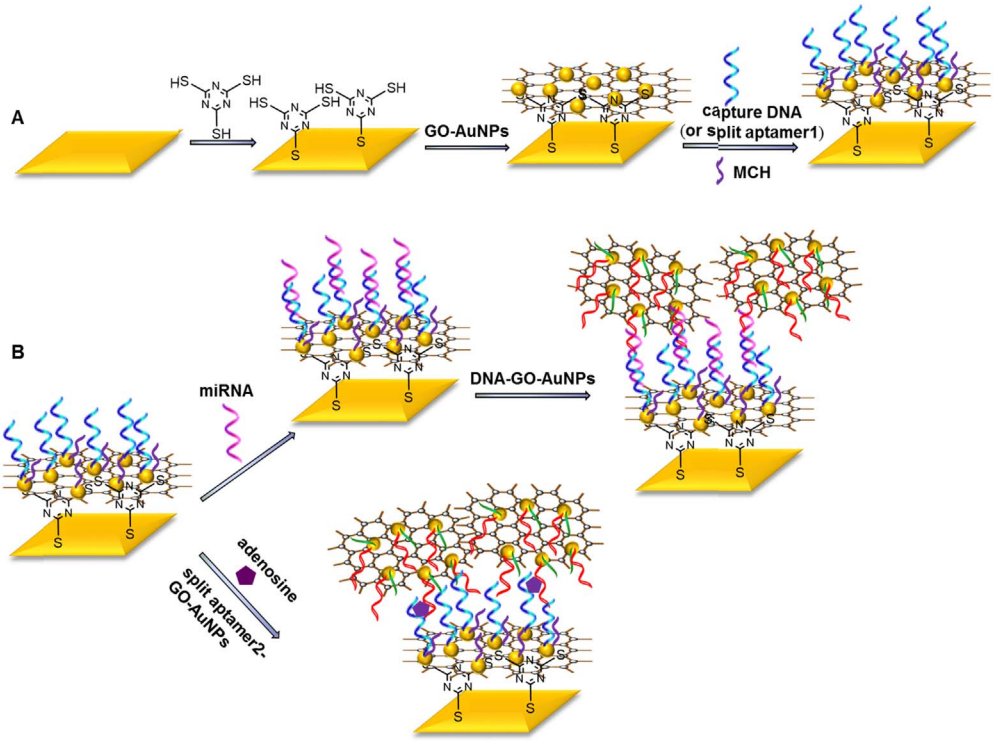

Fig. 1. Schematic illustration of the SPR biosensor based on the GO-AuNPs composites.

attached to the GO-AuNPs functionalized Au film surface (shown in Fig. 1A). In the second step, target miRNA and DNA functionalized GO-AuNPs composites (DNA-GO-AuNPs) were respectively introduced, and then the sandwich structure was formed due to DNA/RNA hybridization (shown in Fig. 1B). In this step, target miRNA was recognized, and the two layers of GO-AuNPs were used to enhance the SPR response. Accordingly, target miRNA was detected sensitively. More importantly, on the basis of split aptamers, another target, small molecule adenosine, was also detected using this approach. An aptamer for adenosine was first designed in two flexible ssDNA pieces. One (i.e. split aptamer1) was tethered on the GO-AuNPs functionalized Au film and the other (i.e. split aptamer2) was modified on the GO-AuNPs composites. In the presence of adenosine, the GO-AuNPs labelled adenosine-aptamer complex was formed since the two split aptamers reassembled into an intact aptamer structure. By employing the GO-AuNPs amplification strategy, target adenosine could also be detected sensitively. Theoretically, this approach was potentially used for various types of target detection, on condition that the appropriate probe can be found and replaced.

2. Experimental

2.1. Materials and reagents

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), trisodium citrate and diethylpyrocarbonatex (DEPC) were obtained from Shanghai Reagent Company (Shanghai, China). Thiocyanuric acid, 6-mercapto-1-hexanol (MCH), adenosine, uridine, cytidine and guanosine were purchased from Sigma (USA). DNA oligonucleotides and RNA oligonucleotides (shown in Table 1) were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and TaKaRa Biotechnology Co. (Dalian, China), respectively. Ultrapure water (18.2 MΩ cm) was prepared using a Millipore system (Millipore, USA). All oligonucleotides were dissolved into 10 mM PBS buffer (pH 7.4) containing 100 mM NaCl. All solutions used were protected from RNase degradation through DEPC treatment.

Table 1
Sequences of all oligonucleotides used.

Name	Sequence
Capture DNA	5′-AGA CAG TGT TAT TTT TTT TTT-SH-3′
Assistant DNA	5′-SH-TTT TTT TTT TTT TCC ATC TTT ACC-3′
Helper DNA	5′-SH-TTT TTT TTT TTT T–3′
miRNA-141	5′-UAACACUGUCUGGUAAAGAUGG-3′
miRNA-429	5′-UAAUACUGUCUGGUAAAACCGU-3′
miRNA-200a	5′-UAACACUGUCUGGUAACGAUGU-3′
miRNA-21	5′-UAGCUUAUCAGACUGAUGUUGA-3′
Random miRNA	5′-N_{22}-3′
Split aptamer1	5′-SH-TTT TTT TTT TAC CTG GGG GAG TAT-3′
Split aptamer2	5′-SH-TTT TTT TTT TTG CGG AGG AAG GT-3′

2.2. Synthetization and modification of graphene oxide (GO)-gold nanoparticles (AuNPs) composites

The GO-AuNPs composites were synthetized as described in the previous work [9,16]. Briefly, after $HAuCl_4$ solution (0.48 mM) was mixed with GO solution (1 mg mL^{-1}) for 30 min at room temperature, the mixture was heated to 80 °C. Next, sodium citrate (1.6 mM) was added dropwise. After incubation for 60 min, the mixture was centrifuged at 5000 rpm for 15 min, and the resultant precipitate was rinsed with water to remove free AuNPs. GO-AuNPs composites were characterized by TEM. It showed that Au nanoparticles with an average size of 18 ± 2 nm was observed at the GO surface (Fig. S1, Supporting information).

DNA functionalized GO-AuNPs composites (DNA-GO-AuNPs) were obtained according to our previous work [9]. In brief, the mixture of assistant DNA (or split aptamer2) and helper DNA was incubated with GO-AuNPs composites solution for 16 h at 4 °C. Next, the solution was aged in 10 mM phosphate buffer (pH 7.0, 100 mM NaCl) for 40 h at 4 °C. After the solution was centrifuged at 13,500 rpm for 20 min, the precipitate was washed twice using 10 mM phosphate buffer (pH 7.0, 100 mM NaCl). Finally, DNA-GO-AuNPs were obtained and dispersed in 10 mM phosphate buffer (pH 7.0, 100 mM NaCl). The DNA-GO-