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Graphene-based portable SPR sensor for the detection of *Mycobacterium tuberculosis* DNA strain

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Abstract

In this study, we presented the novel concept of graphene utilization for the detection of *Mycobacterium tuberculosis* DNA (deoxyribonucleic acid) hybridization in surface plasmon resonance (SPR) biosensor. The DNA sequences were obtained from DNA fragment IS6110, which was proven as the stable biomarker for *Mycobacterium tuberculosis* complex (MTBC). A few graphene layers on top of SPR sensing chip were deposited by simple drop casting method from its dispersion solution. The presence of graphene layers plays the major role for single strain DNA immobilization. The single strain DNA (ssDNA) was covalently bond with the gold nano urchin (GNu) as the sensing probe (ssDNA-GNu). The binding mechanism between graphene layers and ssDNA probe is mainly due to the π-π stacking force. Furthermore, hydrogen bond influences the hybridization mechanism of the complementary single strain DNA (cssDNA) and the ssDNA; which has the higher energy compared to the π-π stacking force. Consequently, the presence of the cssDNA target in the reaction chamber disrupts the ssDNA-GNu from the few graphene layers. The experimental results demonstrated the detection limit of this method was achieved around 28 fM of cssDNA target in the salt buffer.

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Keywords: Biosensor; DNA; Mycobacterium tuberculosis; Graphene; Surface plasmon resonance; Gold nano urchin.

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1. Introduction

Graphene is a monolayer hexagonal pattern (honeycombs) of pure carbon atoms in the two-dimensional region. Since the first fabrication in 2004 by Novoselov et al.[1,2], this material becomes more promising for modern electronics breakthrough due to its particular material properties. Subsequently, the optical properties of graphene attract much attention as well in wide research areas including optics, materials, medicines, and sensors [3–5]. The plasmonic in graphene material was reported can be tunable to the similar range of the plasmonic behaviour in the thin metal film [6]. Based on this finding, the integration of graphene layer in the thin metal sensing is an interesting approach to improve the sensing and biosensing mechanism and its performance[7].

Wang et al. presented for the first time the graphene-based SPR sensor with the aptamer binding to monitor the a-thrombin in the detection chamber. Amine or sulfide group tagged the aptamer and absorbed by graphene sheets due to the π-π stacking mechanism. Subsequently, when the a-thrombin presences in the detection chamber, it attracts the tagged-aptamer due to strong covalent bonding. Therefore, the aptamers were released from the sensing surface because the covalent bonding energy larger than π-π stacking energy[8].

The graphene-based method leads a new concept of DNA strain detection since the conventional DNA hybridization direct detection format with the limit of detection in the nanomolar and subnanomolar range [9]. One particular obstacle in this research is the transfer process of a graphene sheet on the thin gold layer. Song et. al., suggested the crystalline Au {111} film to improve the absorption energy of graphene layer on the Au layer[10]. In addition, a report claimed that the self-assembly sheet of graphene oxide in the gold layer can be achieved[11].

Several scientists presented the signal detection enhancement. They proposed the utilization of gold nanoparticle as the probe label of ssDNA[11–13]. The unyielding plasmonic field in the nanoparticle-induced the signal amplification especially for the optical-based sensor such as SPR biosensor[14]. Not only merely that, but the gold nanoparticle has also been utilized in the sensor and biosensor platform for the signal amplifications[15].

Mycobacterium tuberculosis complex (MTBC), a pathogen responsible for tuberculosis, is the most deadly bacteria in underdeveloped country, worldwide regarding the World Health Organization (WHO) report. The rapid spreading during an outbreak is due to airborne transferability of these bacteria and makes an effortful prevention and isolation to control the spreading. Therefore, an effective and efficient method using portable platform for detecting these pathogenic bacteria is required.

In this study, we presented the rapid detection of the MTBC DNA fragment IS6110 [16] by portable SPR biosensor. The complementary ssDNA (cssDNA) sequence was configured as the probe labeled GNu and immobilized in the few graphene layers. A few graphene layers in this study were prepared by the straightforward and low-cost solution process. Next, the target ssDNA sequence can be hybridized and detected by the custom-modular SPR biosensor. The very low detection limit around 24 fM of target ssDNA MTBC was achieved in our study.

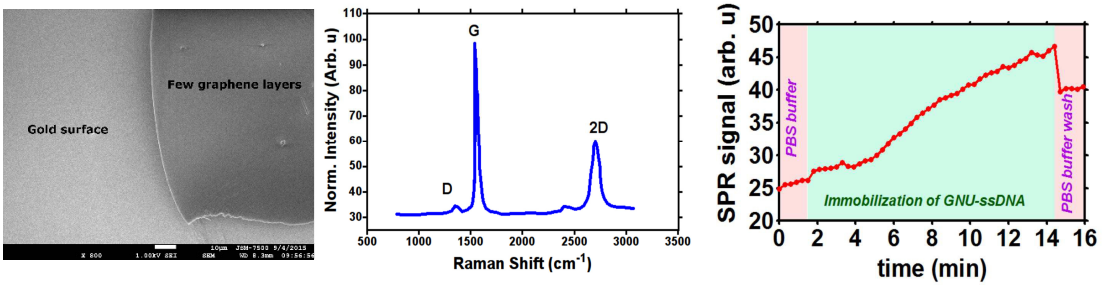

Fig. 1. (a) FESEM image of few graphene layers on gold sensing with drop cast method. (b) Raman spectra of few layers graphene by drop casting method in our sensing chip. (c) The SPR signal of probe GNu-ssDNA immobilization in the graphene layers.

2. Material and Method

Basic platform of the portable SPR biosensor utilizing organic light source in this experiment was described in our previous reports[17,18]. The DNA probe and target sequence were obtained from MTBC DNA fragment IS6110. The DNA probe sequence was SH-CGTGCGGCTA TTACGAGGAC TCCACGCTGG (30 mers). The sequence of ssDNA target was CCGAT AATGCTCCTG AGGTGCGACC (25 mers). While the sequence of mismatched single strain DNA (mssDNA) utilized in the specificity test was ACAGC ATTGCGCGTT CAGACACCGC (25 mers).

A gold sensing chip was prepared and produced based on the protocol in our previous report[18]. Next, the chip was annealed in the baking chamber 300 °C for 30 min, following by immediate quenching process for improving Au {111} crystallization[10,19]. Subsequently, the graphene powder was dispersed in the 1% of dispersant solution until reach the concentration 0.1 mg/mL. Next, the graphene powder solution was dropped on the sensing surface, and was baked at 80 °C.

The GNu was diluted into PBS solution in the concentration value of 100 nM, mixed with the GNu solution with the ssDNA in PBS buffer with a similar concentration of 100 nM. Later, the mixture solution was stored at room temperature for 20-24 hrs to obtain covalently binding between the GNu and the ssDNA probe. For target cssDNA, the series dilution on salt buffer was prepared from 100 fM to 100 nM. Salt buffer for DNA hybridization environment was prepared by 0.1 M of NaCl diluted in the mixture of 1 M of K_2HPO_4 and 1 M of KH_2PO_4.

3. Results and Discussion

A few-layer graphene deposition on the gold chip sensing by drop cast method can be achieved successfully. We measured the sensing chip by Raman spectroscopy to obtain graphene layers profile. The FESEM and Raman measurement result are depicted in Fig. 1 (a) and (b), respectively.

In (a) **(b)** **(c)**

Fig. 1(b) the 2D spectrum shows a lower intensity compared to the G-spectra, it indicates the multilayer graphene were obtained in our sensing [20]. This method leads to simple, less time consuming and low-cost production of few-layer graphene-based SPR sensing chip.

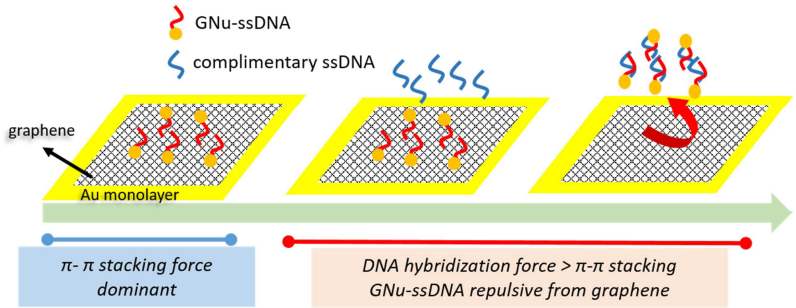

Fig. 2. Detection mechanism of cssDNA targets in this study. Probe immobilized to the graphene layers due to the stacking force, following the DNA hybridization, the probes were released from graphene layers due to the hydrogen bond.

Annealing and quenching step of sensing chip before graphene solution deposition was an attempt to enhance Au {111} crystallization [19]. Higher temperature annealing was suggested to obtain very high order Au {111} crystallization. In the sensing chip, the probes were immobilized successfully in the graphene layer due to π-π stacking force between graphene and ssDNA (Fig 1(c)).

The schematic flowchart can describe the mechanism of the cssDNA detection in Fig. 2. The probe of GNu-ssDNA was gradually absorbed to the graphene surface due to the π-π stacking force between ssDNA probe and the graphene layer. The GNu in this study plays the major role in signal amplification. Thus, the probe immobilization can be detected significantly in the SPR sensor measurement, as depicted in Fig. 1(c).

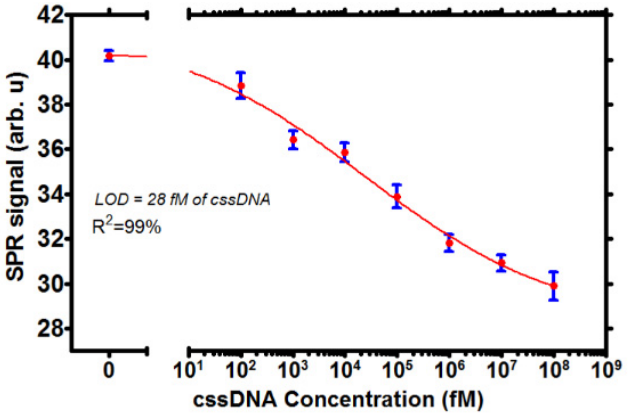

Fig. 3. The trend line of delta SPR signal from the measurements of series concentrations DNA target cssDNA.

From the level signal after PBS wash protocols in the series concentration measurement, the trend line of SPR signal differences were plotted in Fig. 3. The trend line with a correlation coefficient of 99% was achieved. Finally, the detection limit calculation was estimated around 28 fM of cssDNA target.

4. Conclusion

In this study, we successfully presented the proof of concept of detection single strain DNA target from *Mycobacterium tuberculosis* using graphene-based portable SPR sensor. The sensing layer using graphene by drop cast method leads to simple, low cost, and time efficient sensing production. Furthermore, the limit of detection reached the very low concentration of the target DNA, around 28 fM in the salt buffer solution.

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