RNA Detection Based on Graphene Field-Effect Transistor Biosensor

Meng Tian,1,2 Shicai Xu,1 Junye Zhang,1 Xiaoxin Wang,1 Zhenhua Li,1 Huilan Liu,1 Ruihong Song,1,2 Ziheng Yu,1 and Jihua Wang1

1Shandong Provincial Key Laboratory of Biophysics, College of Physics and Electronic Information, Dezhou University, Dezhou 253023, China
2School of Life Science, Shandong Normal University, Jinan 250014, China

Correspondence should be addressed to Shicai Xu; shicaixu@dzu.edu.cn and Jihua Wang; jhw25336@126.com

Received 8 December 2017; Revised 17 March 2018; Accepted 24 April 2018; Published 3 June 2018

1. Introduction

The detection of RNA can provide very useful information for molecular biology research [1], genetic disease diagnosis [2], and environmental monitoring [3], which is of great significance in life science. To date, many methods for RNA detection have been developed, such as northern blotting [4], gel electrophoresis [5], and real-time quantitative polymerase chain reaction (QRT-PCR) [6]. However, these methods suffer from many limitations, such as low throughput analysis, expensive reagent, complex operation, time consuming, and poor sensitivity. Therefore, it is necessary to develop a highly efficient, sensitive, fast, and low-cost platform for RNA detection.

In recent years, the significance of biological sensors using nanomaterials, such as silicon nanowires [7, 8], carbon nanotubes [9, 10], and graphene [11, 12], has drawn significant attention to scientific research owing to its unique physical and chemical properties, such as surface effect and microsize effect. It is expected that these biosensors can be used as next-generation diagnostic chips for the future, which will play an increasingly important role in life science research and medical testing. Among the nanomaterials, graphene is a two-dimensional planar film and has many unique properties, such as high carrier mobility and ease of functionalization [13, 14]. These unique properties of graphene have unique electrical advantages in building field-effect transistor biosensors for detecting biomolecules.

In this paper, we developed a graphene field-effect transistor (G-FET) biosensor using graphene as an electric channel. The single strand DNA as probe was immobilized on the surface of graphene by using 1-pyrenebutanoic acid succinimidyl ester (PBASE) as a linker. The target RNA is introduced onto the biosensor sample cell to hybridize with probe DNA. The sensor shows high sensitivity for RNA detection. Significant sensor response was achieved for detecting trace amounts of RNA in range from 0.1fM to 1pM. Moreover, the sensing strategy also shows a high selectivity by performing the contrast experiment. Compared with the traditional RNA detection technology, the G-FET RNA biosensor not only provides fast analysis, simple operation and low cost, but also shows
high sensitivity and selectivity. This kind of biosensor may provide a new way for RNA biosensing applications.

2. Materials and Methods

2.1. Materials. Glass substrate (30 × 30 × 1.1 mm) with indium tin oxides (ITO) electroconductive film was purchased from Hu Nan Xiang Cheng (Shenzhen, China) Ltd. Phosphate buffered saline (PBS) (E607008-0500, pH = 7.2–7.4) was purchased from Sangon Biotech (Shanghai, China) Co., Ltd. Ag/AgCl electrode with analytical measuring electrode was purchased from Yancheng Arduino Analytical Instruments (Jiangsu Province, China) Co., Ltd. RNaseZap was purchased from Sigma-Aldrich. Both DNA probes and RNAs are synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. PBS buffer was purchased from Yancheng Arduino Analytical Instruments. Glass substrate (30 × 3.1 mm) with indium tin oxide (ITO) was purchased from Sangon Biotech (Shanghai, China) Co., Ltd. Both DNA probes and RNAs were composed of 15 bp, which are dissolved and diluted by 0.1x PBS buffer. The sequence of single strand probe DNA, full-complementary RNA (FC-RNA), and noncomplementary RNA buffer was added to the sample cell at room temperature for 1h to make PBASE attach on graphene. Then, the G-FET device was washed with DMF and 0.1x PBS buffer sequentially. After that, 100 nM DNA was introduced to the sample cell at room temperature for 18 h to serve as probe for RNA detection. Finally, the excess unreacted DNA was rinsed with a 0.1x PBS buffer.

2.5. Detection of RNAs. In order to reduce the degradation of RNAs, all the experiments involved in RNA were carried out in RNase-free environment. All buffers and deionized water were disposed with 0.1% DEPC overnight and autoclaved. G-FET device was treated with RNase Zap reagent before adding RNAs. The target RNAs with different concentrations were added to the sample cell to hybridize with probe DNA at room temperature for 5 h. The target RNAs were rinsed with 0.1x PBS buffer to avoid nonspecific binding. The transfer curve of G-FET device was measured in a semiconductor parameter analyzer (PDA FS360) coupled with a probe station (PEH-4). In the measurement, the G-FET device was provided with a varying gate voltage $V_g$ from −0.6 to 0.6 V with the scanning step of 0.025 V by an Ag/AgCl electrode and a constant voltage of 0.01 V was provided between drain and source electrodes.

2.6. Characterizations. The transferred graphene on glass and the functionalization step were characterized by Raman spectra. The Raman spectra of the graphene were performed using a Raman spectrometer (Horiba HR-800) with laser excitation at 532 nm (2.33 eV). The excitation laser spot is about 0.5 μm. A field emission-scanning electron microscope (FE-SEM) (ZEISS, SUPRA) with accelerating voltage of 20 kV was used to analyze the morphology of the as-grown graphene on Cu foil and the transferred graphene on glass.

3. Results and Discussion

Figure 2(a) shows SEM image of as-grown graphene film on copper foil. There are a few light ridges observed on the surface of the sample. The ridges were caused by the difference of thermal expansion coefficient between Cu and graphene [19]. After the graphene was transferred onto the glass substrate, no ridges were observed as shown in Figure 2(b). It could be that the graphene film is spread out in the transfer process. The films are found to be flat and continuous without any holes and tears, indicating the well transfer of graphene.

Figure 2(c) shows the Raman spectrum of graphene films after transferred on glass substrate. The D, G, and 2D bands associated with graphene are clearly seen at ~1348 cm$^{-1}$, ~1585 cm$^{-1}$, and ~2750 cm$^{-1}$, respectively. Among them, the G band is caused by the first-order scattering of the in-plane optical phonon $E_{2g}$ mode, and the 2D band is the result of a second-order process involving two phonons with opposite...
Advances in Condensed Matter Physics

Figure 1: Schematic of the graphene transfer and G-FET fabrication. (a)–(e) Graphene film transferred onto the glass substrate with two ITO electrodes. (f) Photo of the fabricated G-FET.

Figure 2: (a) SEM image of the as-grown graphene on the copper foil. (b) SEM image of graphene on glass substrate. (c) Raman spectrum of the graphene on glass substrate. (d) Raman spectra of graphene in the process of functionalization.
momentum, which are regarded as characteristic bands of the graphene structure [20]. The ratio of the intensity 2D band and G band ($I_{2D}/I_G$) is about 2-3, indicating the grown graphene is monolayer. The D band is due to the out-of-plane breathing mode of the sp² atoms and is related to the structural defects [21]. Raman spectra record the functionalization step of graphene as shown in Figure 2(d). After introduction PBASE molecules into graphene, several new bands were observed. The bands at 1332.7 and 1383.6 cm⁻¹ can be assigned to the introduction of disorder arising from orbital hybridization of the molecule with the graphene plane, and the bands at 1611.3 cm⁻¹ are due to the pyrene group resonance [22]. After the probe DNA was further immobilized on the graphene/PBASE layer, more bands appeared at 1476.4, 1502.5, and 1655.8 cm⁻¹ which can be assigned to the vibrational modes of DNA molecules [23].

Figure 3 shows the transfer characteristics (drain-source current ($I_{ds}$) versus gate voltage ($V_{gs}$)) of the PBASE modified G-FET (black curve) and after probe DNA functionalization (red curve). Here, the drain-source voltage was set to be a small value of 0.01 V to reduce the electrical damage to probe DNA. After the probe DNA was modified on the G-FET by PBASE, the charge neutrality point voltages ($V_{cnp}$) were shifted to the positive gate voltage direction. The change of electric potential of graphene can be explained by the negative electrostatic gating effect. As DNA has a negatively charged triphosphate group, it can modulate the Fermi level of graphene by inducing excess hole carriers and in turn shift $V_{cnp}$ to the positive direction [24, 25].

Figure 4(a) shows the transfer characteristics of DNA probe-modified G-FETs before and after addition of target RNAs (full-complementary) with different concentration from 0.1fM to 1pM. With the concentration increase of the target RNAs, $V_{cnp}$ successively shifted to the negative direction. The shift can be explained as an electron transfer effect caused by nonelectrostatic stacking interaction between RNAs and graphene layer [26]. Figure 4(b) shows the $V_{cnp}$ shift as a function of the concentration of target RNAs. The G-FET sensor shows a very high sensitivity. The electrical potential of graphene potential was significantly decreased when the RNA concentration was increased from 0.1fM to 1pM. It is noted that the detection limit of our G-FET device for RNA is as low as 0.1fM. The detection limit is two orders of magnitude lower than that reported in the literature where the reduced graphene oxide is used as electric channel [14]. Here, the high sensitivity of our G-FET device can be attributed to the excellent transfer properties of CVD graphene. To represent the capability of the quantitative detection of RNA and its reproducibility, the linear fit calibration curve ($R^2 = 0.970$) with error bars was also shown in Figure 4(b). The reasonable linear response of G-FET is observed from 0.1fM to 1pM. However, as indicated by error bars, the fluctuation of sensor response was relatively large, especially for lower concentration detection.

Besides sensitivity, selectivity is also a critical parameter to assess the performance of a sensor. In order to verify the selectivity of the G-FET device for RNA detection, identical concentrations of noncomplementary RNA (NC-RNA) and full-complementary RNA (FC-RNA) were introduced into the sample cell, respectively. As shown in Figure 5, a significant shift of $V_{cnp}$ occurs when detecting FC-RNA. In contrast, for NC-RNA detection, the shift of $V_{cnp}$ is negligible. This contrast indicates that only a small amount of NC-RNA binds to the probe DNA by nonspecific adsorption. Therefore, the G-FET biosensor makes a good distinction between target RNA and noncomplementary RNA, indicating the high selectivity of our G-FET RNA sensor.

4. Conclusions

In this work, we developed a G-FET biosensor for detecting trace amounts of RNA. The addition of target RNA affects the electrical potential of graphene and allows label-free detection of RNA by readout of the shift of $V_{cnp}$. Benefiting from the excellent transfer properties of the CVD graphene, the sensing sensitivity of the sensor was significantly enhanced. The detection limit of G-FET biosensor for sensing RNA is as low as 0.1fM, which is two orders of magnitude lower than the previously reports. Moreover, the G-FET biosensor can readily distinguish target RNA from NC-RNA, showing high selectivity for DNA detection. The developed G-FET...
Advances in Condensed Matter Physics

Figure 4: (a) Transfer characteristics of G-FET with addition of different concentrations of target RNA from 0.1 fM to 1 pM. (b) The change of $\Delta V_{cap}$ of the G-FET with addition of the target RNA from 0.1 fM to 1 pM.

Figure 5: The transfer characteristics of the pristine DNA-modified G-FET biosensor (black) and after addition of NC-RNA (red) and FC-RNA (blue).

provides a possible way for label-free and ultrasensitive RNA detection, suggesting a promising future for biosensing applications.

Conflicts of Interest

There are no conflicts of interest related to this paper.

Acknowledgments

The authors are grateful for financial support from National Natural Science Foundation of China (11604040, 61671107, and 11704059), Research Foundation for Advanced Talents of Dezhou University (320061), Project of Shandong Province Higher Educational Science and Technology Program (J15LJ05), Shandong Province Natural Science Foundation (ZR2016FQ08), and Taishan Scholars Program of Shandong Province (Tshw201502045).

References


