Application of Graphene FET Nucleic Acid Biosensor in Human Motion Measurement

Hang Yin

Wuhan Business University
Wuhan 430056, China
E-mail: yinhangyh056@163.com

Received: March 18, 2018 **Accepted: September 7, 2018**

Published: December 31, 2018

Abstract: *In order to explore the application of graphene field effect transistor (FET) nucleic acid biosensor, the sensitivity and selectivity of nano FET biosensor detection are improved by the large physical surface ratio, high electron mobility, excellent thermo conductivity and high mechanical strength. The nano-material graphene is applied to the field effect transistor biosensor, and the FET biosensor based on graphene is constructed. In addition, target molecules with specific probes are immobilized on the surface of graphene. The purpose of detecting nucleic acid molecules with high sensitivity and high selectivity can be achieved by detecting the change of electrical signals before and after hybridization and using nucleic acid – nucleic acid molecules hybridization. At the same time, the DNA biosensor can be reused. The reduced graphene oxide (R-GO) field effect transistor DNA biosensor constructed has high sensitivity, high selectivity and reusability. The research results showed that it has a potential application prospect in human motion measurement as a detection tool. To sum up, graphene FET nucleic acid biosensor is suitable for being used in human motion measurement.*

Keywords: *Graphene FET nucleic acid biosensor, Human motion measurement, Sensitivity.*

Introduction

Biosensor is a device that combines biometric elements with appropriate signal transduction elements to invert and selectively detect the concentration or activity of biochemical substances in various types of samples [6]. Biosensor is a special device based on biometrics, such as nucleic acid, antibody, enzyme, cell and so on, which is highly sensitive to target. The signal transduction element is used to convert the biological signals into signals that can be detected by electricity, sound or light, and the detection of target objects is realized. According to the detection signal, the biosensors can be divided into electronic, acoustic, optical, calorie, etc. At present, the most commonly used are the two types of electronic and optical, and they have been used for nucleic acid detection. The main types are the fluorescent biosensor [1], the electrochemical biosensor [5] and the field effect transistor biosensor [10]. Among them, field-effect transistor biosensor is superior to fluorescence and electrochemical biosensors because it does not require fluorescence labeling and electrochemical indicator, and has higher sensitivity and selectivity. Field effect transistor (FET) biosensor is a new kind of biosensor based on nano-materials developed in recent years. Due to the unique advantages of nano-materials, the sensitivity and selectivity of nano-scale FET biosensors have been greatly improved. Nano-materials are usually defined as the size of the material between 1-100 nanometers [2], which is the transition zone between the cluster and the macroscopic object. It has unique physical and chemical properties, such as surface effect, microsize effect, quantum effect and macroscopic quantum tunneling effect, etc. [9]. The common materials do not have these properties. Therefore, compared with the traditional detection technology, nano FET biosensor has the characteristics of high sensitivity and selectivity, fast analysis speed, no label, simple operation and less reagent consumption, which is very suitable for the detection of biomolecules. It can be foreseen that it will play a more and more important role in medical

testing. In recent years, many nano-materials have been used to prepare nano FET biosensors for nucleic acid detection, such as Sinanowire (SiNW), carbon nanotube (CNT), graphene and so on. Graphene is a two-dimensional nanomaterial with a single layer of carbon atoms. It has the characteristics of high electron mobility, bipolar field effect and large body surface ratio. Compared to one dimensional nano-materials such as silicon nanowires and carbon nanotubes, it is more suitable for FET biosensors. The unique properties of graphene can be used to improve the sensitivity and selectivity of nano FET biosensor detection. Graphene was prepared by chemical oxidation reduction method. On this basis, a field effect transistor biosensor based on graphene is constructed to detect nucleic acid molecules with high sensitivity and selectivity. A nucleic acid probe that complements the target molecules on the surface of the graphene FET biosensor is immobilized on the surface of the graphene, and then the target molecules are hybridized with the probe. The nucleic acid molecules were detected by detecting the electrical signal changes of the FET biosensor.

DNA biosensor based on PNA modified graphene field effect transistor

Preparation of field effect transistors

First, the photolithography layout is drawn according to the needs of the experiment, and the mask plate is made. Then, a photoresist 5350 (positive resist) was coated at a spin speed of 4000 r/min on a silicon wafer surface of 285 nm thick SiO_2, and baked at 110 °C for 3 min. It was then exposed for 2.6 seconds using a deep UV exposure machine. After exposure, it was developed for 17 seconds in developer 300-26. Finally, it is fixed in water and dried under nitrogen. The source and drain electrodes were fabricated by vapor deposition of 5 nm Ti and 45 nm Au on the exposed film by electron beam evaporation. The film is peeled off in acetone and the metal in the unexposed area is removed. The metal in the exposed area is left as the metal electrode to complete the fabrication of the FET.

Preparation of graphene

Graphene is prepared by a chemical redox method. The reduced graphene oxide (R-GO) solution was prepared using the previously reported method [8]. First, a modified Hummer's method was used to prepare graphene oxide (GO) [11]. Then, 15 mg of GO tablets were weighed into 10 mL of 98% hydrazine and sonicated for 10 min to give a black R-GO solution. The solution was placed in a refrigerator for one week to allow GO to be as thoroughly reduced as possible and to prevent R-GO from agglomerating. The R-GO solution obtained after one week can be used. The solution can be stable for several months without agglomeration.

Preparation of R-GO field-effect transistor biosensor

Field-effect transistor biosensors are prepared by standard semiconductor technologies. The gold electrode is assembled on a silicon wafer by conventional micro/nano processing techniques to make it a source/drain. The entire chip size is 6×4.5 mm. The source and drain electrodes have a thickness of 50 nm and the distance between the channels is 4 μm. The prepared R-GO solution was diluted and applied dropwise on the electrode, and then the hydrazine was completely removed by annealing at 150 °C. The device was immersed in a piranha lotion (concentrated sulfuric acid and 35% H_2O_2 volume ratio of 7:3 preparation) ultrasound 30 seconds. Then, it was rinsed thoroughly with deionized water and blown dry with nitrogen.

PNA probe immobilization

First, 5 mmol/LPASE (dissolved in dimethylsulfoxide) was added dropwise to the prepared R-GO field-effect transistor, and the reaction was carried out at room temperature for 1 hour and then washed with dimethylsulfoxide, ethanol and deionized water respectively. After reacting with 10 μmol/L probe PNA for 2 hours at room temperature, it was washed with 1×PBS containing 0.2% SDS, 1×PBS and deionized water to remove excess unreacted probe PNA. The chip was then blocked with 100 mmol/L ethanolamine for 1 hour and then rinsed with deionized water to avoid DNA nonspecific adsorption on graphene surface.

PNA-DNA hybridization

The different concentrations of target DNA were added to the sensing interface and the probe PNA hybridization for 1 hour. Then, the unreacted DNA was washed with 1×PBS containing 0.2% SDS, 1×PBS and deionized water, respectively, and then dried with nitrogen. Selective test: The same concentration of target DNA, single base mismatched DNA and non-complementary DNA were added to the sensing interface and the probe PNA hybridization for 1 hour. Then, the unreacted DNA was washed with 1×PBS containing 0.2% SDS, 1×PBS and deionized water, respectively, and then dried with nitrogen. Blank test: 1×PBS was added to the sensing interface with the probe PNA reaction for 1 hour. Then, they were respectively washed with 1×PBS containing 0.2% SDS, 1×PBS and deionized water, and then dried with nitrogen.

Detection of electrical signals

10 μL of 0.01×PBS was added dropwise to the sensing interface. A liquid crystal gate R-GO field effect transistor biosensor was obtained. Silver wire is selected as the gate. Then, the biosensors were measured and analyzed for performance using a Keithley4200-SCS semiconductor analyzer and probe station. When measuring I_{ds}-V_g curves (I_{ds} is the source-drain current, and V_g is the gate voltage), V_{ds} (source-drain voltage) is set to atmospheric pressure 0.1 V. The I_{ds}-V_{ds} curve is then measured at different gate voltages.

Characterization atlas

The DXR confocal Raman spectrometer was used to perform Raman analysis of the prepared R-GO. The Zeiss Ultra Plus-43-13 field emission scanning electron microscope was used to analyze the morphology of R-GO in the channel between the two electrodes. DI Nanoscope IV Atomic force microscope was used to analyze the thickness of the R-GO of the channel between the two electrodes.

Testing principle of experiment

The principle of detecting DNA by the R-GO field effect transistor biosensor is shown in Fig. 1. First, field effect transistors are fabricated on silicon using conventional micro/nano processing techniques. Second, the R-GO field effect transistor biosensor is fabricated on a sensing channel by dripping R-GO. The R-GO solution was reduced by 98% hydrazine. Subsequently, PASE (linker molecule) is immobilized on the surface of R-GO by a π-π stacking force acting on the 6-membered ring of the pyrenyl group at one end thereof and the graphene surface. The PNA is immobilized on the surface of graphene by the covalent attachment of the amino group at one end to the amide bond at the other end of the PASE. Ethanolamine solution was used to prevent possible non-specific adsorption of DNA on the graphene surface. Finally, DNA is added to hybridize with the PNA probe. Next, a silver wire is used as the gate to conduct electrical signal detection on the modified biosensor. According to previous studies, it has been shown that the hybridization of target DNA to PNA will play a n-type dopant effect on graphene. When n-type doping of graphene occurs, the measured characteristic curve shifts to the left.

The detection of DNA can be measured by moving the I_{ds}-V_g curve of the monitoring device to the left. As shown in Fig. 1, the addition of non-complementary DNA to PNA does not cause a change in the electrical signal.

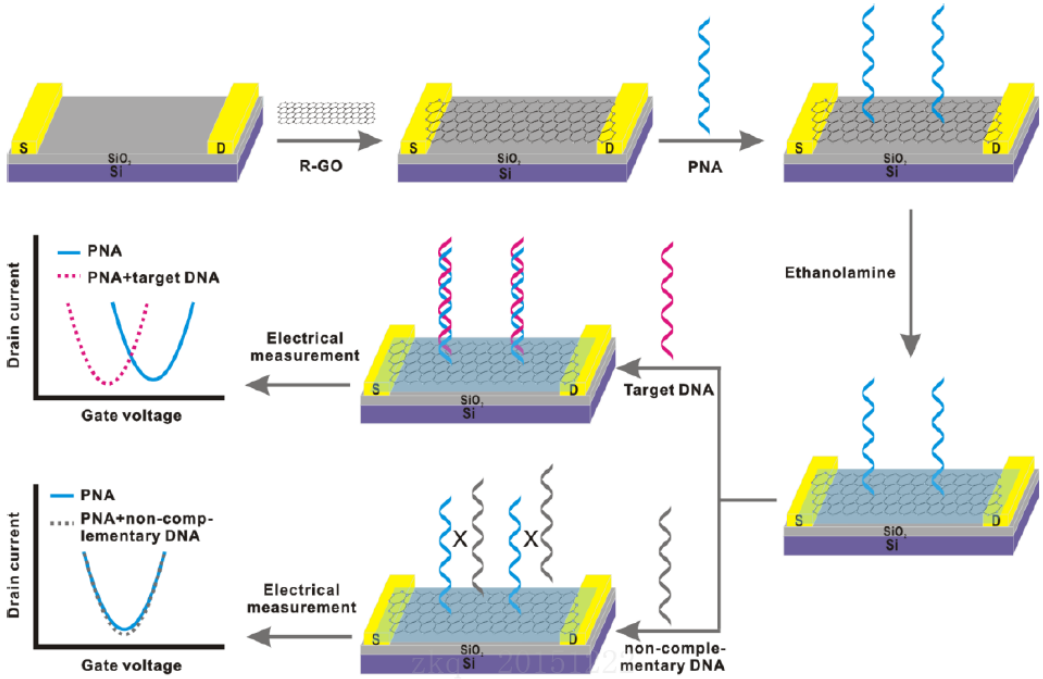

Fig. 1 A schematic diagram of the DNA detection principle of R-GO field effect transistor biosensor

MiRNA biosensor based on gold nanoparticles modified graphene field effect transistor

Modification of AuNPs on the surface of R-GO

$HAuCl_4$ solution was prepared from an equal volume of deionized water and ethanol to a concentration of 10 mmol/L. Then, the R-GO field-effect transistor biosensor was immersed in the $HAuCl_4$ solution for 30 min. After the reaction is completed, it is rinsed with deionized water and finally dried with nitrogen.

PNA probe and AuNPs surface

First, a 10 mmol/L mercaptoethylamine solution was added to AuNPs-modified graphene FETs. It is covalently bound by the Au-S bond and washed overnight at room temperature. Then, 2.5% glutaraldehyde was added to 1H with mercapto ethylamine. After the reaction is completed, it is washed. After adding 10 μmol/L probe PNA for 2 hours at room temperature, it was washed with 1×PBS, 1×PBS and 1% PBS containing 0.2% SDS and deionized water to remove excess unreacted probe PNA. The chip was blocked with 100 mmol/L ethanolamine for 1 hour and then washed in order to avoid nonspecific adsorption of graphene surface as much as possible.

PNA-miRNA hybridization

Before adding miRNA, RNaseZap reagent must be used to desensitize the chip, and then follow-up experiments are carried out. Sensitivity test: The target miRNA of different concentrations was added to the sensing interface and hybridization with the probe PNA for 30 min. Then, the unreacted miRNA was cleaned and removed with 1×PBS, 1×PBS and deionized water containing 0.2% SDS, and it was dried by nitrogen. Selective test: The same

concentration of target miRNA, single base mismatched miRNA and non-complementary miRNA were added to the sensing interface respectively, and then hybridized with the probe PNA for 30 min. Then, 1×PBS, 1×PBS and deionized water containing 0.2% SDS were used to clean the unreacted miRNA, and it was dried by nitrogen. Blank test: 1×PBS was added to the sensing interface to react with the probe PNA for 30 min. Then, 1×PBS, 1×PBS and deionized water containing 0.2% SDS were used to clean the unreacted miRNA, and it was dried by nitrogen.

Detection of electrical signals

The measurement and performance analysis of the electrical signal of the biosensor was carried out with the Keithley 4200-SCS semiconductor analyzer and the probe table. When measuring I_{ds}-V_g curves, V_{ds} is set to atmospheric pressure 0.1 V.

Characterization atlas

The Zeiss UltraPlus-43-13 field emission scanning electron microscope was used to analyze the morphology of R-GO in the channel between the two electrodes. DI Nanoscope IV atomic force microscope is used to analyze the thickness of R-GO.

Principle of test detection

Fig. 2 is a schematic diagram of the working principle of the AuNPs modified graphene FET biosensor for the detection of miRNA. The traditional micro nano processing technology was used to prepare FET, and the R-GO solution was reduced by the same chemical method [7]. Then, the R-GO drops are applied to the sensing channel as a conductive material. The graphene FET device is immersed in the HAuCl4 solution for a period of time. AuNPs is deposited on the surface of graphene by instant deposition. The PNA probe is covalently bonded to the AuNPs surface via a connector. Mercaptoethylamine is bonded to AuNPs through the Au-S bond. With the addition of glutaraldehyde, the aldehyde group at one end reacts with the amino group of thiamethylamine and the other is reacted with the amino group on the PNA, thus binding the covalent PNA to the AuNPs surface. Ethanolamine is added. Finally, the miRNA and PNA probes were added to produce a hybrid reaction. The electron detection was used to monitor the hybridization reaction, and the miRNA was detected by the change of the electrical signal. In theory, the target miRNA molecules can cause n type doping in the device. The miRNA contains the base of electrons, which can be doped with graphene. The miRNA can be detected by monitoring the left shift of the I_{ds}-V_g curve of the device before and after hybridization.

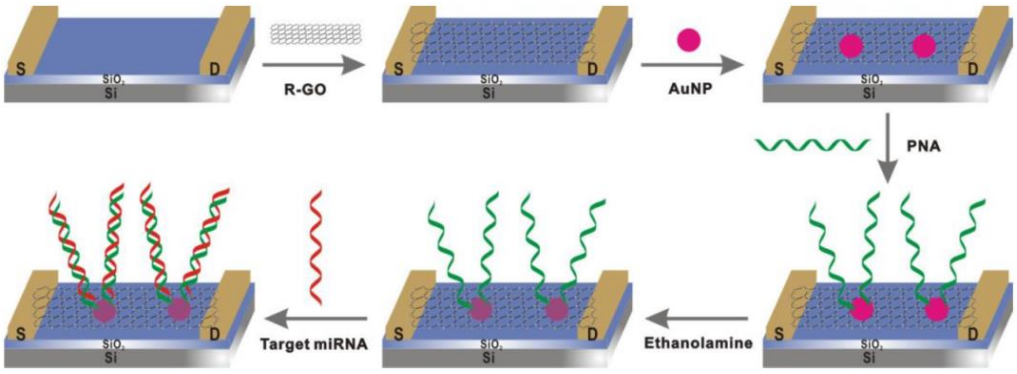

Fig. 2 Schematic diagram of the principle of miRNA detection for AuNPs modified FET biosensor

Results and analysis

DNA biosensor based on PNA modified graphene field effect transistor

In order to study the electrical performance of R-GO field-effect transistor biosensor, the graphene transfer and output characteristic curves were measured in this paper, that is, I_{ds}-V_g and I_{ds}-V_{ds}. It can clearly be seen from Fig. 3 that the R-GO field effect transistor biosensor has significant bipolar characteristics over a small range of gate voltages (-0.4 to 0.4 V). The VCNP (the gate voltage corresponding to the minimum conductivity) should theoretically be zero. In fact, it shifted slightly to the right. This indicates that some substances in the surrounding environment are adsorbed on the R-GO to cause P-type doping. To further verify the electrical performance of the R-GO FET, Fig. 4 shows the output characteristic of R-GO. As the gate voltage decreases slightly, the source and drain currents also decrease. This shows that the device is very sensitive to changes in gate voltage. The device is sensitive to the analyte.

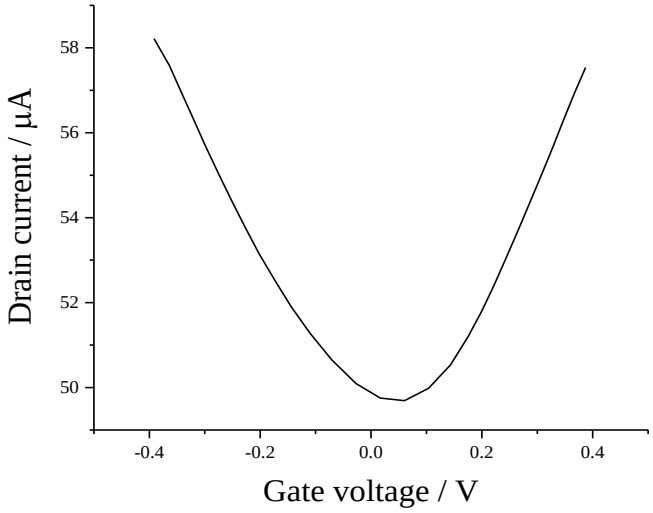

Fig. 3 I_{ds}-V_g curve of unmodified R-GO FET biosensor (V_{ds} = 0.1 V)

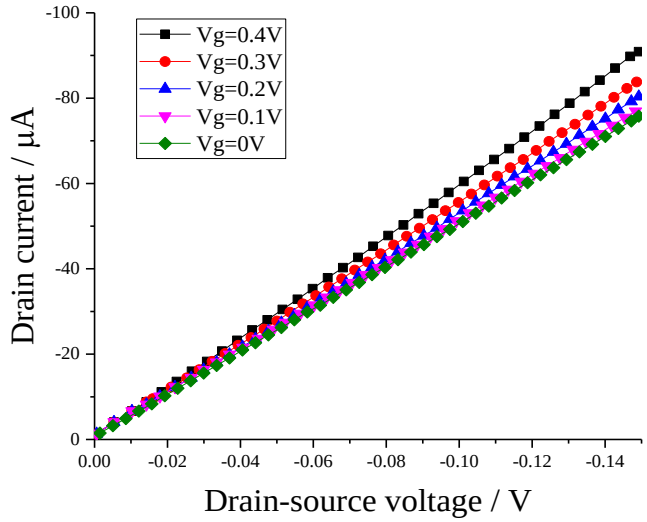

Fig. 4 I_{ds}-V_{ds} under different gate voltage output curve

In order to investigate the selectivity of the biosensor for the DNA to be tested, the same concentration of non-complementary DNA, single base mismatched DNA, and complementary DNA were added to PNA-modified R-GOFET biosensors, respectively. 1×PBS was used

instead of DNA. It was added to a PNA modified R-GOFET biosensor as a blank control test. Fig. 5 shows the transfer curves of PNA-modified R-GOFET biosensors after reaction with 1×PBS, 1 nmol/L non-complementary DNA, 1 nmol/L single-base mismatched DNA and 1 nmol/L complementary DNA, respectively. As shown in the Fig. 5, the VCNPs of the complementary DNA shift to the left much more than the shifts of 1×PBS, non-complementary DNA and single-base mismatched DNA. The above experiments show that R-GO field-effect transistor biosensors can well distinguish between complementary DNA, single base mismatched DNA and non-complementary DNA. The PNA modified R-GO field effect transistor biosensor has high selectivity.

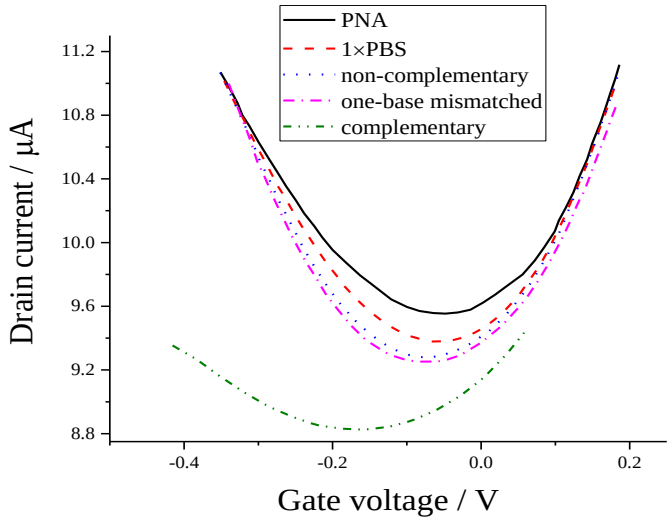

Fig. 5 The transfer curves of PNA-modified R-GOFET biosensors after reaction with 1×PBS, 1 nmol/L non-complementary DNA, 1 nmol/L single-base mismatched DNA and 1 nmol/L complementary DNA

The sensitivity of the R-GO field effect transistor biosensor was investigated. The PNA modified R-GO FET device was hybridized with a variety of different concentrations of complementary DNA, as shown in Fig. 6. From Fig. 6, the VCNP of the device is gradually shifted to the left with the complementary DNA concentration from 10 fmol/L to 1 nmol/L. Signal to noise ratio must be greater than or equal to 3. Finally, the limit of detection is 100 fmol/L instead of 10 fmol/L. Dong et al. [4] used a CVD grown graphene to make a liquid-gate transistor for DNA testing and achieved a sensitivity of 10 pmol/L. Chen et al. [3] prepared a CVD-grown graphene field-effect transistor biosensor for the DNA-free detection. The detection limit is 1 pmol/L (10-12 mol/L). Yin et al. [12] assembled a platinum nanoparticle-modified R-GO field-effect transistor biosensor to detect DNA. Sensitivity is 2.4 nmol/L. Table 1 lists the sensitivity of several graphene FET biosensors for detecting DNA. It can be seen from the table that the prepared R-GO field-effect transistor biosensor has the highest sensitivity for detecting DNA. The probe used in this article is PNA instead of DNA. PNA improves the sensitivity of the device.

Finally, the reusable performance of the R-GO field effect transistor biosensor was investigated. The method used was to hybridize PNA-DNA after crossing, and then cross to cross again, so repeated 3 times. The method of hybridization is to immerse the hybrid device in the urea solution of 8.3 mol/L and place the 5 min at room temperature. PNA-DNA double chain is unraveled. The deionized water is used to rinse the DNA of the chain, and then it is dried by nitrogen.

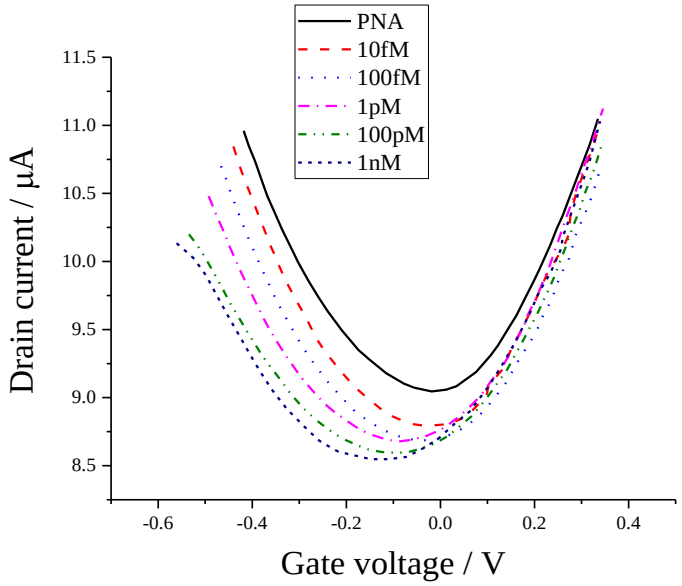

Fig. 6 The transfer curves of PNA modified R-GO FET biosensor and complementary DNA hybridization (fM is an abbreviation of fmol/L)

Table 1. Performance comparison of several DNA biosensors based on graphene FET

Research group	Probe	Sensitivity, (fmol/L)
Chen et al. [3]	ssDNA	1000
Dong et al. [4]	ssDNA	10000
Yin et al. [12]	ssDNA	2400000
this study	PNA	100

On the same device, hybridization is performed again. Hybridization conditions were exactly the same as the first time. After repeated operations for 3 times, signal values after 3 hybridizations were obtained. Compared with the first hybridization, the percentages of the second and third times were 96.67% and 83.33% respectively. It shows that the device has good reusability.

miRNA biosensor based on gold nanoparticles modified graphene field effect transistor

Fig. 7 shows the SEM control of the graphene FET biosensor before and after the modification of AuNPs. Fig. 7a is the graphene FET channel when the AuNPs is not modified. It can be seen from the figure that the few layers of graphene are connected to the source leaks on both sides, and the folds of graphene in the channel can be clearly seen. Fig. 7b is the graphene FET channel after the modification of AuNPs. The small bright spot in the figure is the appearance of AuNPs distributed on the surface of graphene. The illustration of the upper right corner is an enlarged view at the dotted line frame. It is clearer to see a large number of AuNPs. This shows that AuNPs has been successfully modified on the surface of graphene.

In order to further prove that AuNPs has been successfully modified on the surface of graphene, and the PNA probe has been successfully immobilized on AuNPs, the transfer curve of graphene FET biosensor before and after modified AuNPs and fixed PNA probe has been tested, as shown in Fig. 8.

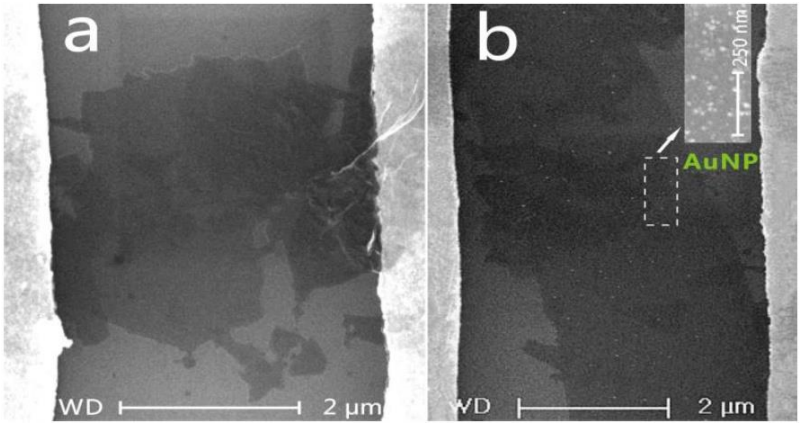

Fig. 7 The SEM diagram of the graphene FET biosensor before AuNPs modification (a) and after modification (b)

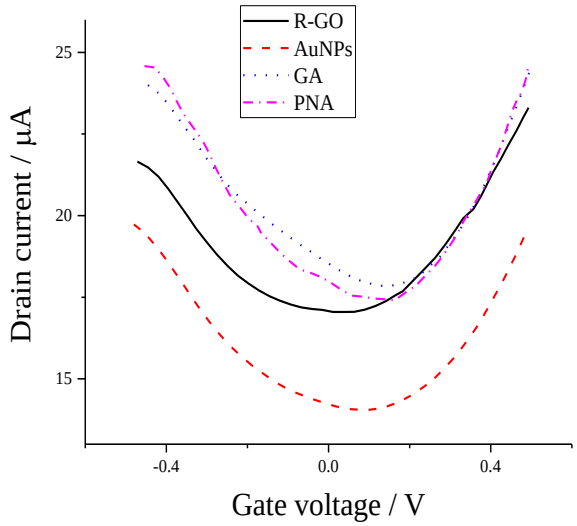

Fig. 8 The transfer curve of the graphene FET biosensor before and after the modification of AuNPs

From the black line in the figure, the bipolar characteristics of graphene can clearly be seen. VCNP (the gate voltage corresponding to the minimum conductivity, the theoretical value should be 0) is slightly shifted to the right. It is indicated that the graphene is doped with P type due to the incorporation of adsorbate in the surrounding environment. The red line in the figure is obtained after the modification of AuNPs, which shifts to the right relative to the black line. This shows that the modification of AuNPs has the P type doping of graphene. The blue line was obtained by the treatment of glutaraldehyde, and it shifted rightward relative to the AuNPs curve. The green curve is shifted to the left relative to the blue line. PNAs are capable of n-type doping of devices as miRNAs. It shows that PNA immobilization is successful. The above results demonstrate that AuNPs have been successfully modified on graphene surface and PNA has been successfully immobilized on the device surface.

The selectivity of AuNPs modified graphene FET biosensor was studied. The non-complementary miRNA, the single base mismatch miRNA, the complementary miRNA and the probe PNA were hybridized respectively. 1×PBS instead of complementary miRNA was added to the device as a blank control test. Fig. 9 shows the transfer curves of AuNPs-modified graphene FET biosensors with PNA as a probe after reaction with 1×PBS, 1 nmol/L non-complementary miRNA, 1 nmol/L single-base mismatched miRNA, and 1 nmol/L

complementary miRNA. It can be seen from Fig. 9 that the curves corresponding to complementary miRNAs shift to the left much more than the other three. Experiments show that PNA and complementary miRNA are specific binding hybridization. The graphene FET biosensor can well distinguish between complementary miRNAs, single base mismatched miRNAs and non-complementary miRNAs. Therefore, the biosensor has good selectivity.

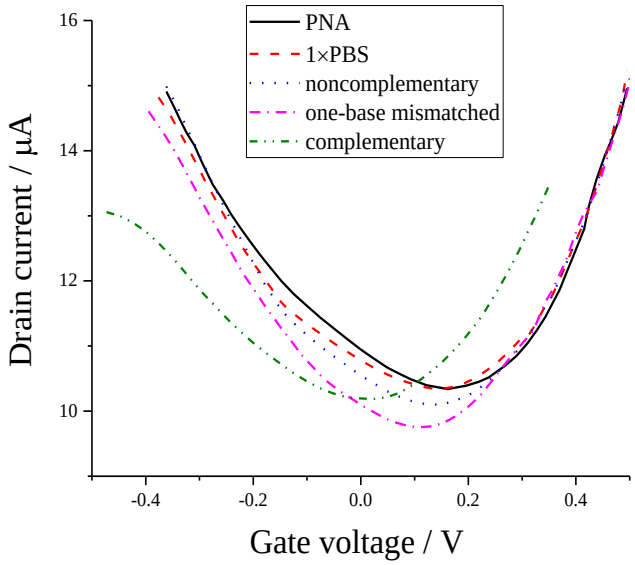

Fig. 9 The transfer curves of AuNPs-modified graphene FET biosensors with PNA as a probe after reaction with 1×PBS, 1 nmol/L non-complementary miRNA, 1 nmol/L single-base mismatched miRNA, and 1 nmol / L complementary miRNA

In order to investigate the sensitivity of AuNPs-modified graphene FET biosensors, different concentrations of complementary miRNAs were individually hybridized to PNAs on the device for experiments. The results are shown in Fig. 10.

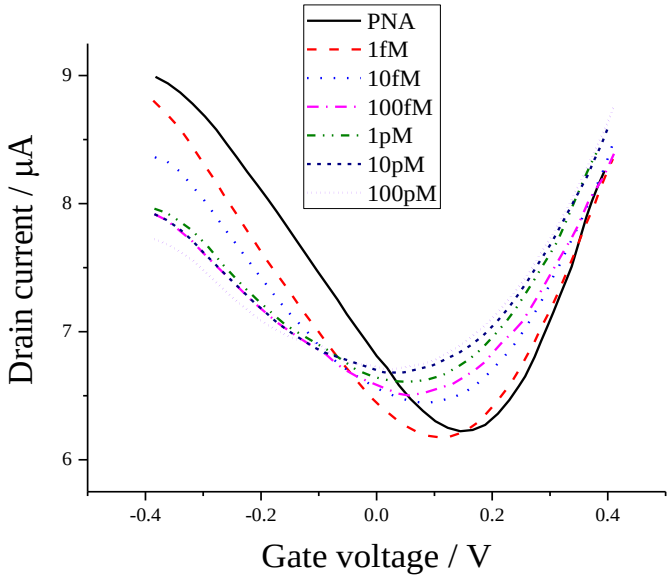

Fig. 10 The transfer curve of PNA-immobilized AuNPs-modified graphene FET biosensor after hybridization with different concentrations of complementary miRNA

It can be seen from Fig. 10 that the VCNP of the device is gradually shifted to the left with the complementary miRNA concentration from 1 fmol/L to 100 pmol/L. Signal to noise ratio must be greater than or equal to 3. Finally, the limit of detection is 10 fmol/L instead of 1 fmol/L. Therefore, the biosensor also has high sensitivity.

Conclusion

A novel graphene FET biosensor was developed to detect DNA by PNA-DNA hybridization. It has the characteristics of no marking, high sensitivity, high selectivity and reuse. As a channel conductive material, R-GO shows obvious bipolar characteristics. It is also particularly sensitive to small changes in gate pressure. The PNA probe has a good sequence specific affinity and stability for target DNA. The detection limit can be as low as 100 fmol/L. This sensitivity is more than one order of magnitude higher than the sensitivity of the DNA-DNA hybrid-based graphene field effect transistor DNA biosensor, which has been reported. AuNPs-modified graphene FET biosensors can detect miRNAs with no labeling, ultra-sensitive and high selectivity. It is confirmed that AuNPs are successfully modified on the surface of graphene. The AuNPs modified graphene FET biosensor has high sensitivity and selectivity. As a detection tool, this highly sensitive and highly selective biosensor has potential applications in human motion measurement.

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Assoc. Prof. Hang Yin
E-mail: yinhangyh056@163.com

Hang Yin was born in 1984. Now she is working as an Associate Professor in Wuhan Business University, Hubei Wuhan, China. She received B.Sc. and M.Sc. degrees in Wuhan University. Her research interests are application of biosensors and biological materials in sports.