

Development and Functionalization of Electrolyte-Gated Graphene Field-Effect Transistor for Biomarker Detection

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Abstract

In the current study, graphene and its derivatives have been investigated and used for many applications, including electronics, sensing, energy storage, and photocatalysis. Synthesis and fabrication of high quality, good uniformity, and low defects graphene are critical for high-performance and highly sensitive devices. Among many synthesis methods, chemical vapor deposition (CVD), considered a leading approach to manufacture graphene, can control the number of graphene layers and yield high-quality graphene. CVD graphene needs to be transferred from the metal substrates on which it is grown onto insulating substrates for practical applications. However, separation and transferring of graphene onto new substrates are challenging for a uniform layer without damaging or affecting graphene's structures and properties. Additionally, electrolyte-gated graphene field-effect transistor (EGGFET) has been demonstrated for its wide applications in various biomolecular detections because of its high sensitivity and standard device configuration. In this article, poly (methyl methacrylate) (PMMA)-assisted graphene transferring approach, fabrication of graphene field-effect transistor (GFET), and biomarker immunoglobulin G (IgG) detection are demonstrated. Raman spectroscopy and atomic force microscopy were applied to characterize the transferred graphene. The method is shown to be a practical approach for transferring clean and residue-free graphene while preserving the underlying graphene lattice onto an insulating substrate for electronics or biosensing applications.

Introduction

Graphene and its derivatives have been investigated and used for many applications, including electronics^{1,2}, sensing^{3,4,5}, energy storage^{6,7}, and photocatalysis^{1,6,8}. Synthesis and fabrication of high quality, good uniformity,

and low defects graphene are critical for high-performance and highly sensitive devices. Since the development of Chemical vapor deposition (CVD) in 2009, it has shown colossal promise and set its place as an essential member

of the graphene family^{9,10,11,12,13}. It is grown on a metal substrate and, later for practical uses, is transferred onto insulating substrates¹⁴. Several transferring methods have been used to transfer CVD graphene recently. The poly (methyl methacrylate) (PMMA) assisted method is the most used among the different techniques. This method is particularly well-suited for industrial usage because of its large-scale capability, lower cost, and high quality of the transferred graphene^{14,15}. The critical aspect of this method is getting rid of the PMMA residue for CVD graphene's applications because the residues can cause declination of the electronic properties of graphene^{14,15,16}, cause an effect on biosensors' sensitivity and performance^{17,18}, and create significant device-to-device variations¹⁹.

Nanomaterials-based biosensors have been significantly investigated over the past decades, including silicon nanowire (SiNW), carbon nanotube (CNT), and graphene²⁰. Because of its single-atom-layer structure and distinctive properties, graphene demonstrates superior electronic characteristics, good biocompatibility, and facile functionalization, making it an attractive material for developing biosensors^{14,21,22,23}. Due to field-effect transistors (FET) characteristics such as high sensitivity, standard configuration, and cost-effective mass producibility^{21,24}, FET is more preferred in portable and point-of-care implementations than other electronics-based biosensing devices. The electrolyte-gated graphene field-effect transistor (EGGFET) biosensors are examples of the stated FETs^{21,24}. EGGFET can detect various targeting analytes such as nucleic acids²⁵, proteins^{24,26}, metabolites²⁷, and other biologically relevant analytes²⁸. The technique mentioned here ensures the implementation of CVD graphene in a label-free biosensing nanoelectronics

device which offers higher sensitivity and accurate time detection over other biosensing devices²⁹.

In this work, an overall process for developing an EGGFET biosensor and functionalizing it for biomarker detection, including transferring CVD graphene onto an insulating substrate, Raman, and AFM characterizations of the transferred graphene, are demonstrated. Furthermore, fabrication of EGGFET and integration with a polydimethylsiloxane (PDMS) sample delivery well, bioreceptor functionalization, and successful detection of human immunoglobulin G (IgG) from serum by spike-and-recovery experiments are also discussed here.

Protocol

1. Transferring chemical vapor deposition of graphene

1. Cut the graphene sheet on a copper substrate in half (2.5 cm x 5 cm) using scissors. Apply heat resistive tape to fix the four corners of the graphene square on a spinner gasket (see **Table of Materials**).
- NOTE:** The purchased graphene has a dimension of 5 cm x 5 cm (see **Table of Materials**).
2. Spin-coat the sheet of the graphene with a thin layer (100-200 nm) of PMMA 495K A4 spinning at 500 rpm for 10 s and then 2000 rpm for 50 s. Then bake the sample at 150 °C for 5 min.
 3. Remove the backside of the graphene with oxygen plasma (see **Table of Materials**) at 30 W, 15 sccm for 5 min.
 4. Cut the plasma-treated graphene square into smaller dimensions (1 cm x 2 cm) for device fabrication.

5. Cut the pre-cleaned substrate (SiO_2) into small pieces with an approximate dimension of 2.5 cm x 2 cm.
 6. Etch the copper off using the graphene commercial etchant (Ferric chloride) (see **Table of Materials**). Do not dilute the etchant. Float the sample with the copper side down and the PMMA side up on the liquid etchant.
 7. After copper etching, lift the graphene film slowly using the plasma-treated substrate.
 8. Air-dry the transferred graphene for 2 h and then bake at 80 °C for 15 min.
 9. Remove the PMMA following the steps below.
 1. Warm up the sample with acetone vapor at 70 °C. Keep the sample at ~2 cm above acetone vapor for 4 min with the PMMA side facing down. Then immerse the sample in acetone for 5 min.
 2. Wash the sample with DI water cautiously and observe the transferred graphene under a microscope. Finally, gently blow-dry the sample with N_2 .
 3. Perform Atomic force microscopy (AFM) observation to ensure PMMA residue-free graphene. If PMMA residue is visible in the image, perform the acetone vapor cleaning and immersion once again.
 10. Perform Raman and AFM characterization to confirm the monolayer of graphene transferring and observe the surface properties (**Figure 1A,B**).
 2. Using the E-beam evaporator³⁰ (see **Table of Materials**), deposit 5 nm nickel and 45 nm gold on the graphene sample (**Figure 2B**).
 3. Apply the first photolithography³⁰ process using mask A (**Supplementary Figure 1**) for the patterning of the electrodes (**Figure 2C**).
 4. Spin a positive photoresist (AZ 5214E, see **Table of Materials**) on the sample (2000 rpm for 45 s) and cure the sample at 120 °C for 1 min.
 5. Place the sample in the UV flood exposure system and expose it for ~10 s under 200 mJ/cm².
 6. Develop the sample with a photoresist developer (AZ300 MIF, see **Table of Materials**) for ~2 min, and then rinse with DI water.
 7. Immerse the sample in a gold etchant to etch the gold layer for 10 s; rinse with DI water and remove the remaining photoresist layer by immersing in acetone for 10 min (**Figure 2C**).
 8. Using acetone, IPA, and DI water, wash the sample; bake on a hot plate at 75 °C for 30 min. Then apply the second photolithography process using mask B (**Supplementary Figure 1**) to pattern the graphene channels.
- NOTE:** Use the same process parameters as the first one (step 2.4-2.6), except the UV exposure system in the mask aligner (**Figure 2D**).
9. Immerse the sample in nickel etchant at 60 °C to etch the nickel layer for 10 s; rinse with DI water; blow dry using N_2 (**Figure 2D**).
 10. Place the sample in the plasma asher and remove the exposed graphene using oxygen plasma (100 W for 90 s with oxygen flow at 49 sccm); after that, remove the

2. Fabrication of Graphene Field Effect Transistor (GFET)

1. Wash the substrate with the transferred graphene using acetone, IPA, and DI water; then bake the substrate on a hot plate at 75 °C for 30 min (**Figure 2A**).

photoresist layer by immersing in acetone for 10 min (**Figure 2E**).

11. Wash the sample using acetone, IPA, and DI water; bake on a hot plate at 75 °C for 30 min and apply the third photolithography process using mask C (**Supplementary Figure 1**) for the patterning of the passivation photoresist layer to protect the underlying graphene on the substrate. Use the same process parameters as the first one (step 2.4-2.6), except the UV exposure system in the mask aligner (**Figure 2F**).
12. After the third photolithography process, immerse the sample in nickel etchant at 60 °C for 10 s to remove the remaining nickel layer; then rinse with DI water and blow dry using N₂ (**Figure 2G**). Finally, bake the sample on a hotplate at 120 °C for 30 min (**Figure 2H**).

3. Functionalization of GFET for IgG Detection

1. Assemble the sample-delivery channel.
 1. Fabricate the sample-delivery channel in PDMS using soft lithography techniques³¹.
 2. Immerse the graphene device in 0.1 M of NaOH solution for 30 s; rinse with DI water and leave a thin water layer on the device surface to assist PDMS well's alignment and bonding. Then activate the PDMS well's surface using oxygen plasma.
 3. Align the sample delivery channel and the graphene device under a microscope; place the aligned device in a 60 °C oven for 3 h to allow the bonding. The assembled device is shown in **Figure 3A**.
2. Functionalize the GFET.
 1. Functionalize the graphene surface with IgG aptamer (see **Table of Materials**). Use pipettes to

load and remove each reagent or buffer from the PDMS well. The schematic process is shown in **Figure 4**.

NOTE: The following steps were operated at room temperature.

2. After rinsing the graphene surface with DMSO three times, apply 1-pyrene butyric acid N-hydroxysuccinimide ester (PBASE, 10 mM dissolved in DMSO, see **Table of Materials**) and keep for 2 h.
3. After rinsing with DMSO, apply 5' amino-modified IgG aptamer (20 µM in 1x PBS), incubate for 3 h, and rinse with 1x PBS three times.
4. Apply bovine serum albumin (BSA, 10% w/v in 1x PBS) on graphene for 1 h and rinse with 1x PBS three times.

4. IgG detection

1. Rinse the device with 0.01x PBS three times. Fill the PDMS well with 0.01x PBS (detection buffer) (**Figure 3A,B**).
2. Connect the electrodes with a high-performance parameter analyzer (see **Table of Materials**). Connect the source electrode to the ground, the drain, and the gate electrodes to Source Measurement Units (SMU 1 and SMU 2) equipped with the parameter analyzer, respectively (**Figure 3C**).
3. Set up the measurement parameters and turn on the sampling process.
4. Test the response of the EGGFET to IgG by continuously monitoring the drain current. Dissolve IgG in 0.01x PBS with different concentrations, add the solution into the detection chamber, and monitor the drain current continuously. Save the data.

Representative Results

The representative results show the transferred CVD graphene characterized by Raman and AFM, respectively. The G peak and the 2D peaks of the Raman image give comprehensive information regarding the existence and the quality of the transferred monolayer graphene³² (**Figure 1**). Standard lithography processes^{30,31} were applied for fabricating the GFET device, as shown in **Figure 2**. **Figure 3** shows the fabricated GFET with assembled PDMS sample delivery wells and the experimental setup. The PDMS was mixed at a weight ratio of 10:1 and cast into a Petri dish. Then the whole dish with PDMS mixture was baked in an oven at 60 °C for 3 h. The cured PDMS was peeled off the dish and trimmed to a cube (1 cm x 1 cm x 1 cm). The well (6 mm diameter) was then created by punching the PDMS cube with a puncher.

Schematic functionalization processes for IgG detection by EGGFET are shown in **Figure 4**, and **Figure 5** shows the IgG detection under different electrolyte conditions²⁴. PBASE, a

widely used functionalization reagent for graphene, can be adsorbed on graphene surface through a π - π interaction²⁴ without damaging graphene's electrical properties (**Figure 4A**). A 5' amino-modified IgG aptamer is conjugated with PBASE by the amide bond linkages between the reactive *N*-hydroxysuccinimide (NHS) ester in PBASE and the amine group on the 5' end of the IgG aptamer (**Figure 4B**). Bovine serum albumin (BSA) incubation, a standard approach for biosensor detection, was used to block the remaining unconjugated sites after rinsing the device with 1x PBS (**Figure 4C**). A more detailed discussion can be found in our previously published work²⁴. The Ag/AgCl reference electrode was applied to define the gate potential during the detection. The detection range, the concentration range that a sensor can reliably measure, is determined to be around ~2-50 nM for the EGGFET device. More detailed discussions for chemical and measurement principles involved in IgG detection and EGGFET's sensitivity and detection limit were reported previously²⁴.

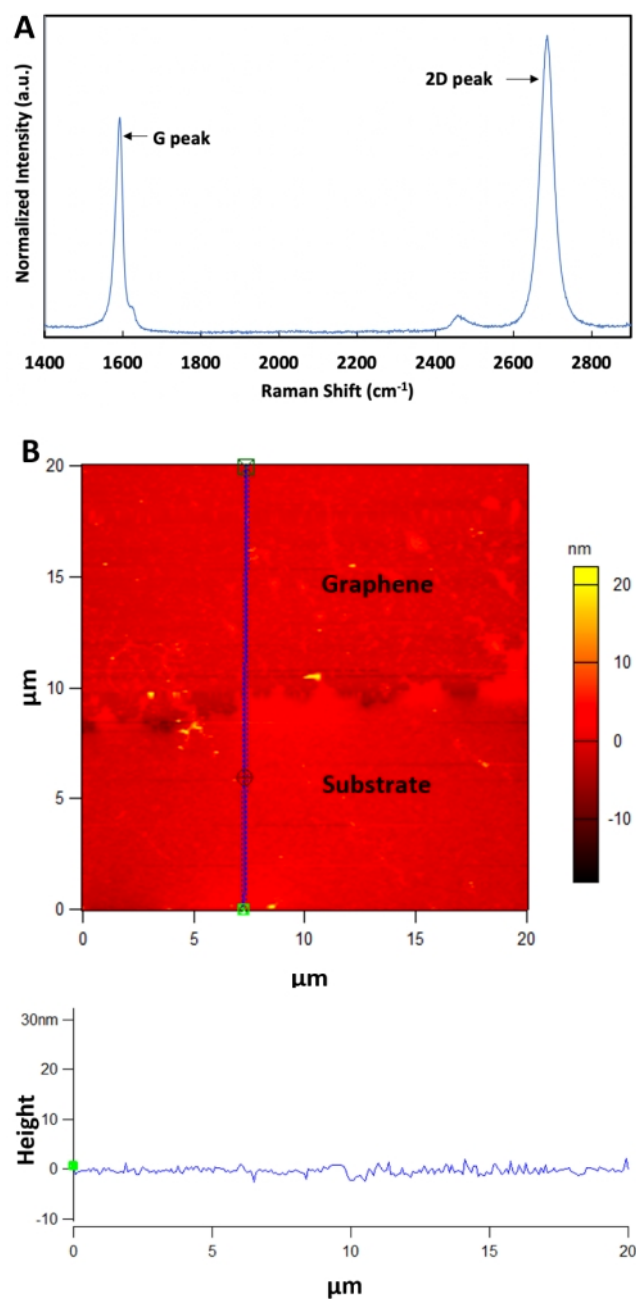


Figure 1: CVD graphene is characterized by Raman and AFM spectroscopy. (A) Representative Raman spectrum of the transferred graphene. The G peak and the 2D peaks are the predominant peaks of pristine graphene. (B) Representative AFM image of the graphene. The corresponding height profiles in the AFM image are shown in the bottom panel along the blue dashed line. [Please click here to view a larger version of this figure.](#)

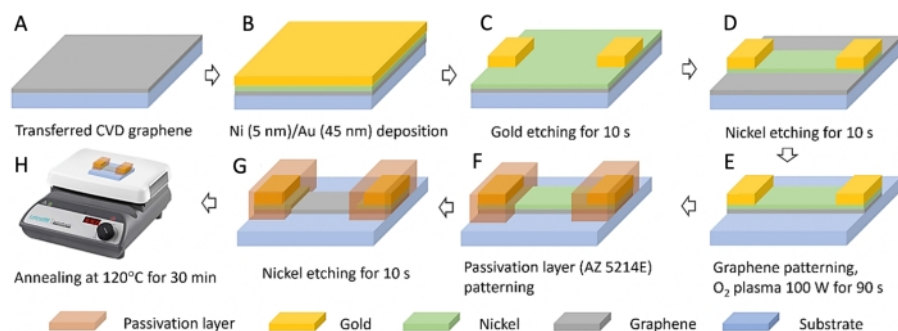


Figure 2: Schematic fabrication of graphene field-effect transistor. (A) Monolayer graphene transferred onto silicon dioxide substrates. (B) Nickel and Gold deposited on transferred graphene. (C) Gold etched after the first photolithography process. (D) Nickel etched after the second photolithography process. (E) Removing unprotected graphene using oxygen plasma. (F) Coating the pattern with photoresist for passivation layering and performing the third photolithography process. (G) Nickel etched after the third photolithography process. (H) Annealing after etching nickel. [Please click here to view a larger version of this figure.](#)

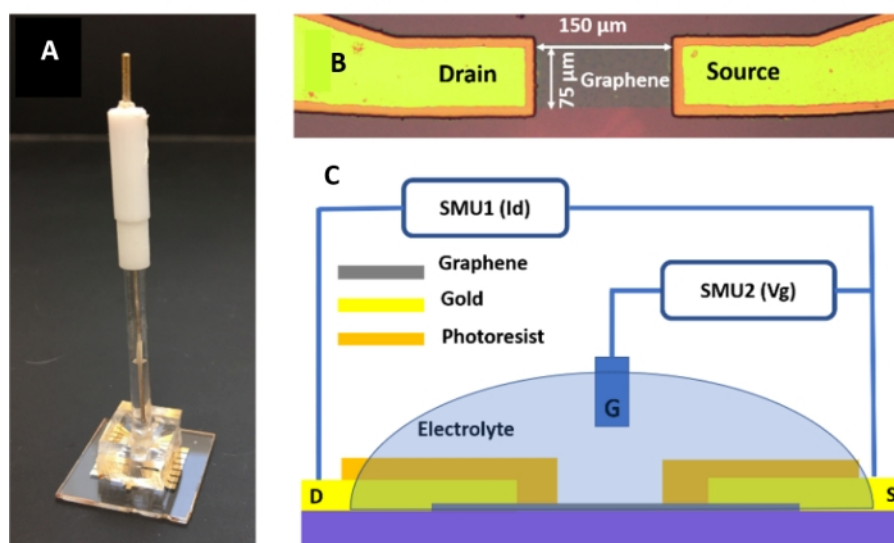


Figure 3: Device and experimental setup for IgG detection. (A) The EGGFET biosensor integrated with a standard Ag/AgCl reference electrode and a PDMS well for containing the sample. (B) The enlarged view of the graphene channel. (C) The schematic diagram of the circuit connection for detecting IgG using the EGGFET biosensor. [Please click here to view a larger version of this figure.](#)

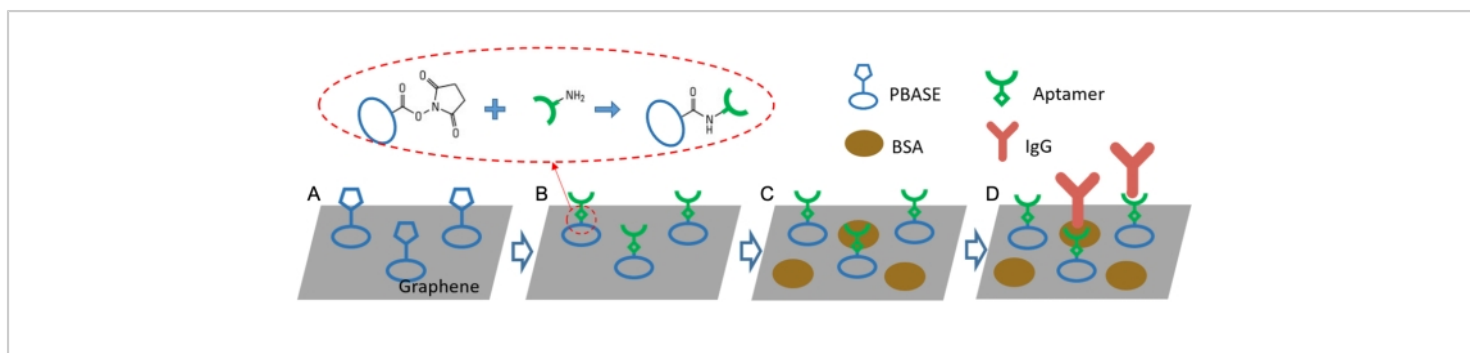


Figure 4: Functionalization of the graphene surface for IgG detection. Reprinted with permission from Reference²⁴.

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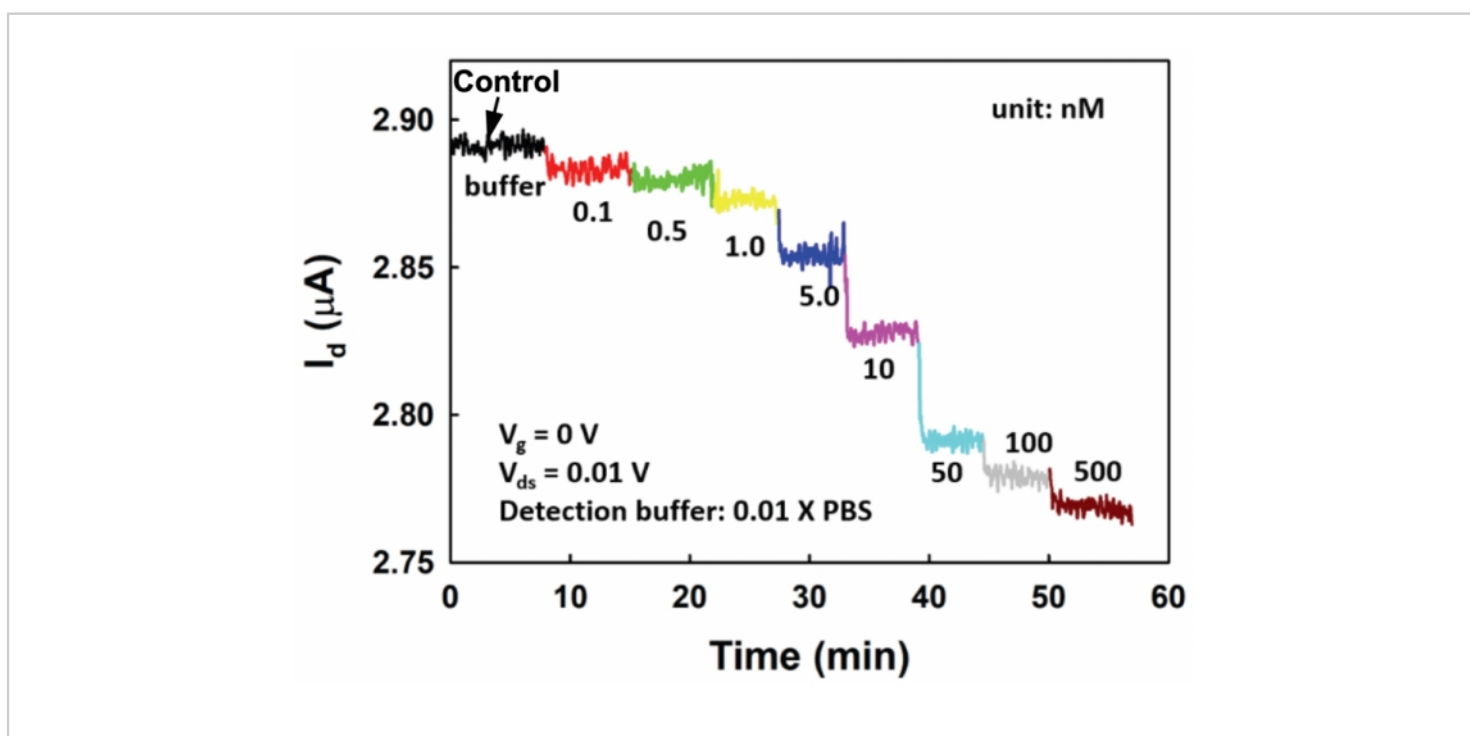


Figure 5: The response of the EGGFET biosensor to biomarker IgG under different diluents. Reprinted with permission from Reference²⁴. [Please click here to view a larger version of this figure.](#)

Supplementary Figure 1: Mask designs used for photolithography processes. (A) The mask design used in the first photolithography process. The electrodes are given with dimensions in the enlarged image A1. (B) Mask design used in the second photolithography with dimensions. (C)

Mask design used in the third photolithography process. The electrodes are given with dimensions in the enlarged image C1. (D) The final product of all three photolithography processes and the enlarged image D1 shows the electrode

configurations. The units for the dimensions are in millimeters (mm). [Please click here to download this File.](#)

Discussion

The purchased CVD graphene on copper film needs to be trimmed to the right size for the following fabrication steps. Cutting of the films can cause wrinkling, which needs to be prevented. The parameters provided in the fabrication step can be referred to for plasma etching of graphene, and these numbers could be varied when using different instruments. The etched sample must be closely monitored and inspected to ensure complete graphene etching. Multiple pre-cleaning methods can be applied to clean the substrates, such as sonication in acetone, IPA, and DI water for 5 min, DI water rinsing, and nitrogen gas drying or treatment with O₂ plasma (300 W, at ~100 sccm for 5 min). The copper etching rate is about 1.25-1.67 micron/min while using the commercial ferric chloride copper etchant. Close observation is necessary for the etching process. Following the etching, a sufficient rinsing with DI water is needed.

The acetone cleaning technique mentioned in the protocol is the optimum residue cleaning technique. Plasma cleaning has the risk of harming the monolayer graphene. So, the most graphene layer-friendly technique is acetone cleaning. But removing PMMA residue is also of primary importance as it affects the latter processes. Doing Raman spectroscopy and AFM can give the real-time quality of graphene and the PMMA residue. The instruments and the chemicals used in the protocol are critical as these directly influence the quality of the fabricated device. So, the quality of the instruments and the validity of the chemicals need to be checked and updated.

PBASE needs to be kept dry and stored in -20 °C freezer to avoid hydrolysis for bioreceptor functionalization. The stored vial needs to reach room temperature before opening it;

otherwise, water could condense inside the vial and hydrolyze the PBASE. To make 10 mM of PBASE, 100 mM of PBASE solution needs to be prepared first by dissolving 38.5 mg of PBASE in 1 mL of DMSO and then diluting it by a factor of 10.

Because the reagents and buffers were added or removed by pipetting directly into the PDMS well, the device demonstrated in the manuscript would not allow for an in-site calibration with negative control. A multichannel array integrated with a properly designed microfluidic device would be necessary for this purpose. Further development of the device, such as combining it with a lateral flow platform, would provide great potential for point-of-care applications³³. In addition, the interface between solid and liquid is a topic of great scientific and technological importance³⁴. For example, in the particular case of aqueous media and graphene, it plays a crucial role in many emerging applications of graphene, e.g., analytical chemistry³⁵, energy storage and conversion³⁶, water filtration³⁷, and biosensing³⁸. Unraveling the behavior at the interface has essential scientific and technical significance, especially for an accurate and more in-depth understanding of graphene's properties and practical applications^{39,40}.

In the present work, an in-detail protocol is provided to demonstrate the development of the EGGFET biosensor and its application in biomarker detection. For practical uses of CVD graphene transferred by the PMMA approach, it is critical to remove PMMA residues completely to get a clean surface. The method effectively removes PMMA residues while preserving the underlying graphene lattice. The functional device shows consistent results for detecting human IgG. Interested researchers could use this protocol as a reference to build devices for specific applications, such as

studying interface interactions, biosensing, developing similar devices using other nanomaterials, etc.

Disclosures

The authors have no competing interests or conflicting interests to disclose.

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