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Review on Graphene FET and its Application in Biosensing

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ABSTRACT

Graphene, after its first production in 2004 have received lots of attentions from researchers because of its unique properties. High mobility, high sensitivity, high selectivity and high surface area make graphene excellent choice for bio application. One of promising graphene base device that has amazingly high sensitivity is graphene field-effect transistor (GFET). This review selectively summarizes the recent progress in fabrication and application of GFET for various biosensors. This article begins with short description and history of ion-sensitive field-effect transistor (ISFET). After that, advantages of graphene ISFET will be summarized. Then, GFET fabrication process including graphene sheet growth different methods, drain/source electrode deposition and lithography and passivation will be discussed. Finally, different application of GFET in detection of Deoxyribonucleic acid (DNA), pH and protein will be present and quality of GFET biosensor will be examined.

Keyword: Graphene; ISFET; GFET; DNA; Protein; Bioapplication.

1. INTRODUCTION

Nowadays, the technologies that are applicable to detect protein, DNA (Deoxyribonucleic acid), antigen-antibody and etc are time consuming, complex and expensive. For example, detecting DNA sequence with modern techniques needs several processing step. Biomolecule concentration amplification by PCR (Polymerase Chain Reaction) and labeling are first step. Microarrays of different cells utilize to detect different type of solute biomolecule. After that, expensive microarray laser beams are used to read cells [1]. In the past few years, researchers have introduces new electrical signal to eliminate these complex procedures.

Electrical detection methods exhibit highly sensitive detection of chemical and biological species because the surface analyte or analyte-analyte bindings occur very close to the channel. ISFET (Ion-Sensitive Field Effect Transistor) has received lots of attention due to their cheap price, small size, fast answer and the possibilities for mass production [2, 3]. ISFET needs no optical reading and able to sense biomolecules without the need of PCR Therefore ISFET microarrays can be used outdoors to control the spread of diseases and environmental pollution. Compatibility of ISFET with Modern microelectronics makes it possible to use am-

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plifying and analyzing circuits with ISFET die on the same chip without extra effort [4].

The principle of an ion sensitive field effect transistor (ISFET) was first introduced by Bergveld [5] in 1970. The Structure of ISFET was based on ion sensitive gate metal-oxide-semiconductor-field-effect-transistor (MOSFET). However the gate metal connection was intentionally removed exposing gate oxide. Different experiments by Bergveld illustrated that concentration of sodium chloride (NaCl) in solution results in variation of current between drain and source. In next works, Matuso et al. found the MOSFET native silicon dioxide (SiO_2) used as the ion-sensing membrane to be sensitive to concentration of sodium (Na^+) as well as hydrogen (H^+) ions in the analyte solution [6].

Silicon transistor, because of their simplicity and well known fabrication process primarily used as ISFET until recently that new low-dimensional structures such as graphene nanoribbon, Si nanowires [7] and carbon nanotubes [8], have been applied. This is because of their size, large surface to volume ratio, and potentially higher sensitivity. One of promising alternative for silicone ISFET is graphene based ISFET that has really unique characteristics [9]. Since, graphene sheet electrical characteristics are sensitive to surface conditions; graphene field-effect transistors (GFETs) have been reported in many research as biosensor for different analyte such as H^+ ions, small molecules, proteins, DNA, viruses and cells [9]. Detection process takes place in ambient conditions where the analyte is in its reference condition. The analyte changes some properties in recognition layer

that is detectable with transducer. Graphene ISFET first introduced by Das et al. [10] in 2008. Later, the performance of such a device was studied by Ang et al. [11] and Ohno et al. [12].

Graphene is a single layer of carbon atoms with hexagonal lattice. Electrons in graphene act like relativistic particles without mass, which contribute to very unique thermal and electrical properties. Its properties like high mobility, high saturation velocity for electrons and holes, good mechanical strength, high thermal conductance and ballistic transport made graphene attractive research area since its discovery in 2004 [14]. Recently, because of its good biocompatibility, graphene based biosensors received lots of attentions from researchers hoping to create devices that are smaller in size, cheaper in price and more reliable than other systems using current technology. Mechanism of this kind of sensors is that graphene channel conductance changes with different biological or chemical species adsorption on surface of channel. Change in conductance results change in I-V characteristics. Biomolecules on the surface of the graphene act as electron donors or acceptors. The long electronic mean-free-path in graphene [15] (order of micrometers) implies that electrons can travel large distances through a device without being restricted. These properties can be very useful in biosensors to make very sensitive devices. Although graphene ISFET possess high performances, there is lots of work needs to be done to conquest practical issues such as nanostructures uniformity and stability in fabrication process [15].

In this paper, first, basic principles of graphene ISFET

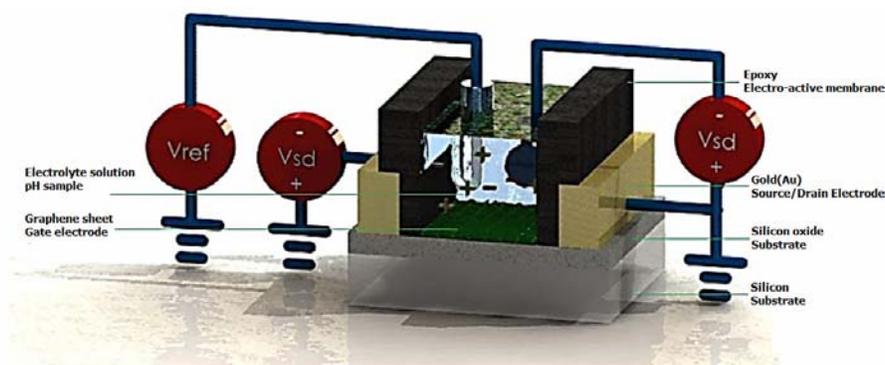


Figure 1: Schematic diagram of a graphene based ISFET. Positive (or negative depending on the applied liquid-gate voltage) ions near the surface of the graphene make up the Debye layer [13].

performance and fabrication process has been explained. After that, interface between electrolyte/graphene is investigated in detail. At the end, graphene ISFET application in wide range of biosensors like DNA, cell and protein sensors has been reviewed.

2. GRAPHENE FET PERFORMANCE AND CHARACTERIZATION

Graphene ISFET is like silicon ones with little difference that has huge effect on characteristics of transistor. The difference is graphene channel that makes device faster and more sensitive than typical silicon ISFET. In the graphene ISFET configuration, regulation of the channel conductance is controlled by gate voltage from a reference electrode placed on top of the channel, across an electrolyte and electrolyte acts as the dielectric [16]. The gate voltage compels ions at the graphene/electrolyte interface, which in turn derive charge carriers by capacitive charging of the ideally polarizable interface. In particular, it has been shown that, because of the ambipolar nature of graphene, adsorbed hydroxide (OH^-) and hydronium (H_3O^+) ions are able to regulate the channel conductance by inducing holes and electrons, respectively. Figure 2 shows a typical conductivity vs. electrochemical gate potential plot obtained in an ionic liquid. The reaction between solution with different pH values and the surface of graphene has a significant effect on the conductivity. It is obvious that device conductivity reaches a minimum value at charge neutral point [16].

Higher conductance graphene FET is because of

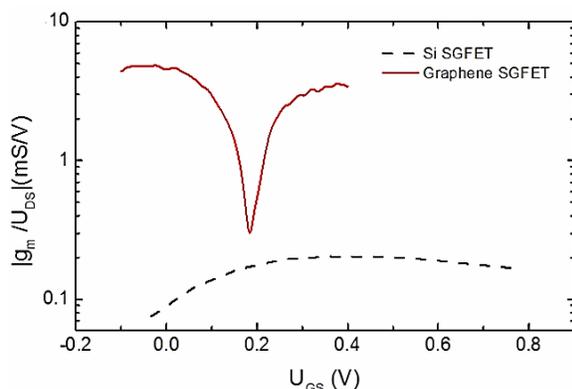


Figure 2: Comparison of the transconductance of graphene and Si ISFET [16].

higher charge carrier mobility (material parameter) and the higher interfacial capacitance (depend on the device design).

3. GRAPHENE FET FABRICATION

Typically, the procedure of transistors fabrication is complicated, including preparation of substrate grown graphene, fabrication of electrode through photolithography and lift-off processes and deposition of source, drain and gate electrode. The most important part of graphene FET fabrication among several steps, is graphene sheet. Quality of 2-dimensional layer has huge effect on sensitivity and performance of biosensor. Fabrication of high-quality graphene in large volumes is vital for high performance and commercial devices. There are several major processes to achieve high quality graphene layer such as chemical vapor deposition (CVD) [17], mechanical exfoliation [18], thermal decomposition [19] and unzipping carbon nanotubes [20].

At its initial discovery, graphene was made with simple scotch tape. In this mechanical exfoliation method, a flake of graphite was put on a piece of tape and then the tape was stuck together many times, spreading the flake over the surface of the tape [14]. Since bulk graphite is simply many layers of the hexagonal graphene lattice, when it is spread out over a large area, some spots will be one atom thick and exhibit the properties of graphene. This method produces best quality graphene layer. The primary disadvantage of this method is weak control on layer and small area ($<1\text{mm}^2$) production that makes it inappropriate method for mass production [18].

Thermal decomposition of silicon carbide (SiC), although more costly than exfoliation methods, but can make large area graphene layer. As described in recent reviews, one approach is epitaxial growth of graphene layers on the basal faces of single-crystal silicon carbide heated to above 1200°C in an ultrahigh vacuum. The resulting graphene layers, which grow as silicon evaporates from the crystal, tend to show various defects such as substrate-induced corrugations [19].

For mass production, full sheet of graphene is necessary. It is achievable by CVD method. This process

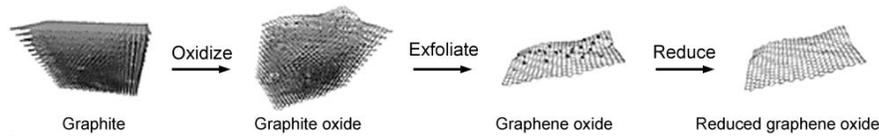


Figure 3: Graphene oxide reduction process steps [21].

Table 1: Main methods of producing graphene.

Method	Pioneers	Description
Peeling/Drawing	2004, Noveselof et al. [14]	Mechanical exfoliation (repeated peeling) of 3D graphite crystals with Scotch tape.
Epitaxial Growth	2004, Berger et al. [19]	Thermal decomposition of SiC in high vacuum and at high temperature
CVD	2008, Li et al. [20]	Using the atomic structure of a metal substrate to seed the growth of the graphene
Chemical Method	2009, Choucair et al. [21]	A direct chemical synthesis of graphene Nano sheets in a bottom-up approach based on ethanol and sodium reagents.
Unzipping Nanotube	2009, Koshynkin et al. [22]	Unzipping multiwall carbon nanotubes by plasma etching

catalyzes a reaction between substrate and gases under certain conditions [16, 17]. First of all, copper foil loads to containing chamber that is pumped down to pressure in order of 30 mTorr. After that, small amount of hydrogen gas enters the vacuum chamber at 1000°C to remove copper oxide (150 mTorr).

In the next step, methane gas enters vacuum chamber at higher pressure (6 mTorr). With help of hydrogen gas as a catalyzer, decomposed carbon from methane deposits on copper foil. This reaction begins in isolated areas on the surface of the copper, and constructs nucleation sites. Lattice structure is not perfect at these points. Carbon starts to assemble itself hexagonally from these sites until two domains are encountered. Reaction between methane and copper foil can no longer occur as soon as the lattice of carbon covers a section of copper. The result is a one-atom thick layer of carbon, which has automatically structured itself into the hexagonal lattice of graphene [22]. So far, we introduced three different method of graphene fabrication. In table.1 main methods are summarized.

Consequently, the graphene has to be removed from

the copper and transferred to an insulating substrate. As depicted in Figure 4, a layer of poly(methyl-2-methylpropenoate) (PMMA) is spin-coated on the graphene/copper stack to provide mechanical stability to the graphene layer. This stack is placed on the surface of an iron (III) chloride solution, which etches the copper under the graphene. After diluting, the graphene/PMMA layer is fished on the final substrate and

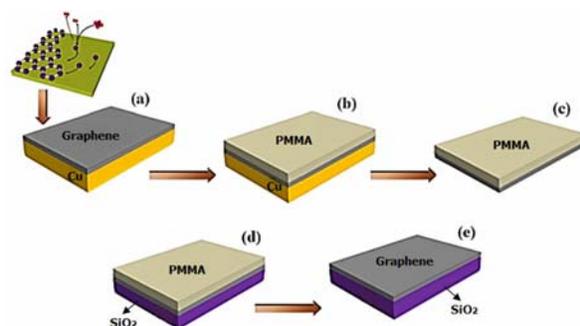


Figure 4: Graphene transfer process. (a) Graphene is grown via CVD on copper foil. (b) PMMA is spun on top of graphene. (c) Copper foil is removed with an etchant bath. (d) Graphene with PMMA is transferred to Si/SiO₂ substrate. (e) PMMA is removed [15].

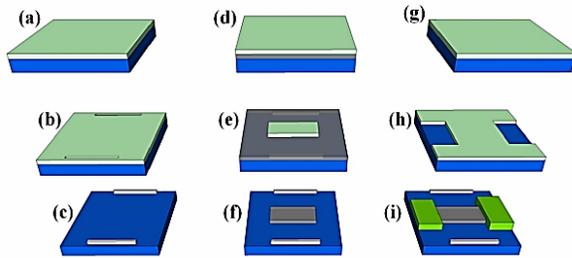


Figure 5: Graphene FET fabrication process. (a) PMMA Spin Coating on Si/SiO₂. (b) Photoresist patterning. (c) Metal deposition and photoresist removing. (d) Graphene growth on Si/SiO₂ substrate and PMMA spin coating on it. (e) Graphene channel patterning. (f) Photoresist removing. (g) PMMA spins coating. (h) Electrode patterning. (i) Metal deposition and photoresist removing [23].

the PMMA is removed by dissolving it in solvents. A final annealing step can be used to further remove residues from the PMMA [14].

To define active area and completely finish the fabrication process of GFET, multiple steps have to be done (Figure 4). First, photoresist has to be spun on Si/SiO₂ substrate then patterns for alignment marks. After that, chrome alignment marks have to be deposited via e-beam metal evaporation and remaining metal and photoresist has to strip. In next step, graphene has to transfer to Si/SiO₂ substrate. After PMMA removing, photoresist has to spin on top of graphene and patterned. Graphene has to be removed with O₂ plasma etching and remaining photoresist has to be stripped to make channel of device. For creating ohmic metal electrodes, same lithography like channel patterning and e-beam evaporation has to be used [14].

After device fabrication, a passivation layer has to spin coated and patterned on GFET. Channel is the only part of transistor that can be in contact with analyte and metal electrodes has to be insulated. The device have to wire-bonded so can be characterized. The bonding wires have to cover with silicone glue to insulate them from the analyte [23].

4. GRAPHENE FET BIO APPLICATION

a) DNA Sensor

Mohanty et al., in 2008 published first significant in-

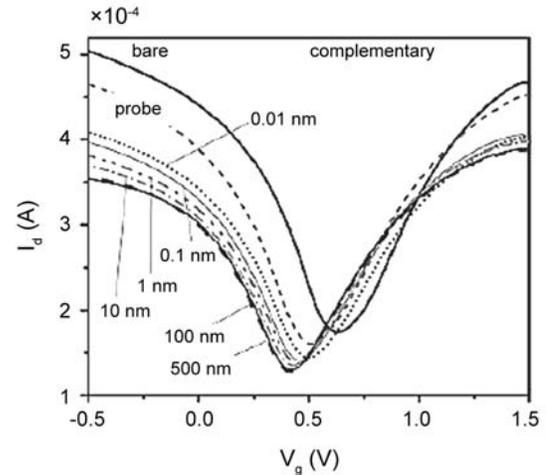


Figure 6: Transfer characteristics for the graphene transistors before adding DNA, after immobilization with probe DNA, and after reaction with complementary [24].

vestigation on graphene and DNA interaction. They showed that chemically modified graphene is excellent device for biocellular and bimolecular scale. They have investigated single bacterium and DNA interaction with graphene sheet [23]. First, they had immobilized single strand DNA on graphene sheet. After that, complementary DNA tagged with fluorescent and at the end hybridized with target DNA. They saw this process affects current and electrical field that makes method feasible for DNA sequencing detection.

In other work, Dong et al. [24] have used transfer CVD graphene from Ni substrate to glass to fabricate graphene transistor. Their device detects hybridization of target DNAs to the probe DNAs pre-immobilized on graphene with 0.01 nM sensitivity. They decorated the Au nanoparticles on graphene sheet and could increase upper limit of detection from 10 to 500 nM [22]. According to Dong et al. research, device conductance shows amipolar behavior subjecting to applied gate voltage. They have showed that V_{g-min} is sensitive to probe DNAs immobilization and hybridization. As shown in Figure 6, increasing in analyte concentration shifts minimum gate voltage (V_{g-min}) to the left and decreases GFET current for the same applied gate voltage.

Chen et al. [23] fabricated low noise GFET on large area graphene. Their GFET sensitivity achieves the concentration as low as to 1 pM. They investigated graphene surface cleanness effect on interaction be-

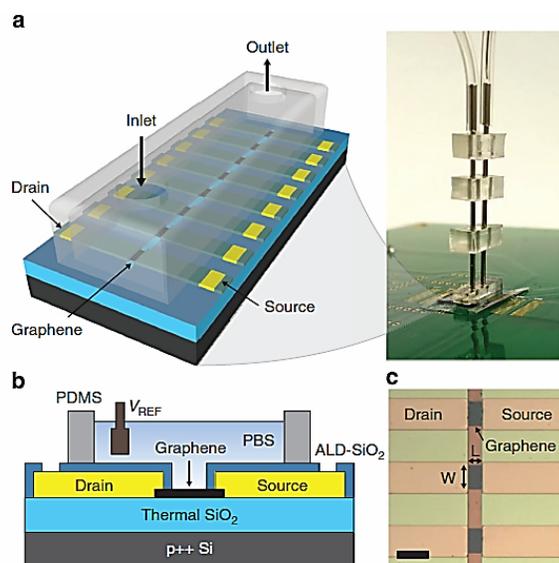


Figure 7: Graphene FET array and experimental set up [26].

tween DNA and graphene. They have used gold transformation instead of PMMA to improve GFET electrical properties (125%) [25].

Most of methods are using single graphene ISFET on chip for detecting DNA strands. Recently, Xu et al. [26] have introduced new multiplexed DNA array Graphene ISFET. They have created 8-FET DNA sensor array based on CVD-graphene with maximum sensitivity of 100 pM. Its sensitivity was 10 times better than other state-of-the-art CVD graphene FET [27]. It also better than commercial optical DNA sensing that has sensitivity in order of 1 pM [28]. They have used graphene in two different functions as a site specific detection of target DNA and as an electrophoretic electrode for specific-site probe DNA immobilization (Figure 7).

b) PH Sensor

One of the first applications of solution gated GFET was to sense pH of a solution. First pH sensor was in-

troduced with multilayer graphene sheet as a channel that was grown on SiC substrate [11]. According to that research sensitivity was 99 mV/pH and difference in graphene layer number had almost no effect on sensitivity. Ag/AgCl was used as gate electrode.

Donkerl et al. have used epitaxial graphene on 6H-SiC substrate to fabricate ion solute gate FET array for pH sensing. They have investigated charge carrier mobility and concentration as a function of electrolyte gate potential. They have used UV-lithography to fabricate 40 μm *20 μm gate length transistor [29].

Wang et al. have fabricated oxide on graphene bio-FET for pH sensing. They passivated graphene channel with 25/20 nm SiO_2/HfO respectively as a sensing/immobilizing and protection layer. In this research surface of SiO_2 functionalized with APTMS ((3-Aminopropyl) trimethoxysilane) as a pathway for DNA and protein immobilization. They have investigated sensitivity of device on PBS (Phosphate-buffered saline) solution pH. Best sensitivity was 34 mV/pH [30].

Kwak et al. [31] have used flexible GFET on PET as a glucose sensor. Fabricated sensors sensitivity was in the range of 3.3-10.9 mM. This accuracy is enough for reference examination or screen test for diabetes diagnostic.

The sensor uses detection of H_2O_2 as a function of glucose. They saw that, as the H_2O_2 concentration increases, i.e. from 0 mM to 4.4 mM, the Dirac point where the channel conductance is minimized was shifted towards lower value of V_g [31].

In Table 2, voltage sensitivity of different GFET pH sensors report in literature is compared. Main reason for huge difference in sensitivity is not been well understood until present; but defects, contaminations and unintentional chemical functionalizations have an important role in GFET quality and so sensitivity.

Table 2: Comparison of graphene PH Sensor sensitivity according to different graphene sheet fabrication process.

	Ang et al. [11]	Ohno et al. [12]	Fu et al. [32]	Cheng et al. [33]
Sensitivity (mV/pH)	99	27	6	18
Graphene source	Epitaxial	Exfoliation	CVD	Exfoliation

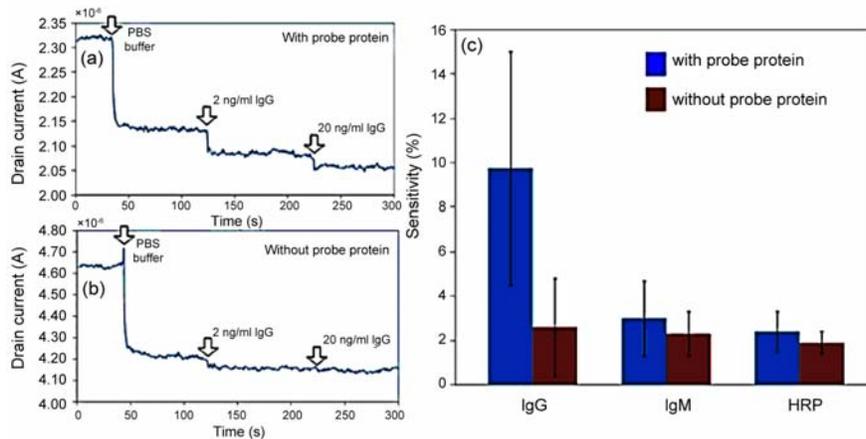


Figure 8: Dynamic response of the VG sensor exposed to different concentrations of IgG (a) with and (b) without probe proteins. For all measurements, the drain-source voltage V_d was fixed at 0.01 or 0.1 V. (c) Comparison of the sensor sensitivity in response to complementary IgG (2 ng/mL), mismatched IgM (0.2 mg/mL), and mismatched HRP (0.2 mg/mL). Error bars were obtained from five samples tested for each analyte [35].

c) Protein Sensor

GFETs, because of their large area and high mobility are really excellent device for protein, anti body and antigen detection. In recent years, there has been lots of research for improving sensitivity of GFET biosensors. In a recent research, Mao and his coworkers [34] investigated vertically aligned graphene FET grown on gold electrode with PECVD method. The sensor sensitivity was 12 pM and showed really good selectivity for Immunoglobulin G (IgG) protein. They showed that vertically-oriented graphene (VG) facilitates the deposition of gold nanoparticle-antibody conjugates on transistor. In other work they showed that attaching

gold nanoparticle-antibody will decrease transistor current. They have used thermally reduced graphene FET to investigate immune system of human body. Binding of antibody and antigen can be detected with I-V characteristics of vertically aligned GFET (Figure 8) [35]. Okamoto et al. used graphene flake as a channel for GFET for detection of heat shock protein (HSP). They could reach good sensitivity of 100 pM.

In other work, Kim et al. reported self aligned reduced graphene oxide FET for label free detection of

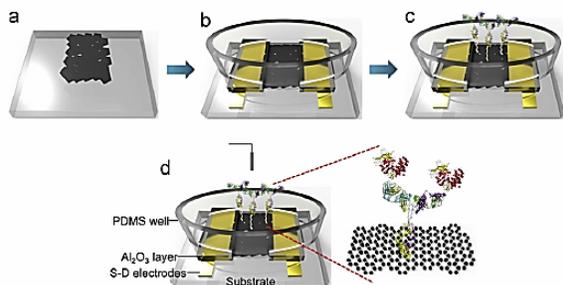


Figure 9: Schematic of R-GO FET fabrication and detection of PSA-ACT complex. (a) Self-assembly of GO nanosheet. (b) Formation of Ti/Au source and drain electrodes. (c) Functionalization of R-GO channel by linker molecules. (d) Illustration of R-GO FET immunosensor with Pt reference electrode in the analyte solution [36].

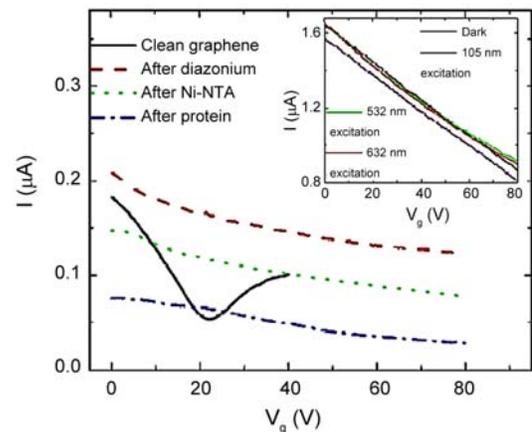


Figure 10: Current-gate voltage characteristic (I-VG) of graphene FET after each step required for functionalization with fusion protein GST-BT5: as prepared (black), after diazonium treatment (red dashed), after Ni-NTA attachment (green dotted), and after incubation in protein (GSTBT5) solution (blue dot-dash) [36].

prostate cancer [36]. They showed that analyte concentration has linear effect on gate minimum voltage and conductivity. They used PSA-ACT on to different pH environment. For pH= 7.4 increasing in analyte concentration makes V_{g-min} shift to left and for pH= 6.2, increasing in analyte concentration results in V_{g-min} shift to right. In addition to gate voltage, conductivity increases for pH= 7.4 and decreases for pH= 6.4 with analyte concentration increase (Figure 9).

Lu et al. [37] reported robust method to bind poly-histidine-tagged protein to graphene FET and used fluorescent protein to investigate photoelectric effect in device. They have applied three different light beams with 405, 502 and 632 nm wave length. They saw that only 405 nm light has impact on I-V characteristics of device. They saw significant $I-V_G$ shift is observed only for violet illumination, with negligible change for green or red light. They conclude that current decrease may reflect a net dipole associated with charge redistribution in GFP upon photo excitation or GFP-GFET charge transfer since GFP is reported to be a light induced electron donor (Figure 10) [37]. It can be concluded that GFETs device may be superior to electrode device in certain aspect.

5. CONCLUSIONS

In this review, fabrication, characterization and application of GFET has been discussed in biosensor device application. Our focus was in DNA and protein detection for new methods of cancer detection. Due to excellent electrical and mechanical properties like high carrier mobility and capacity graphene has shown really amazing exclusivity in bio area. Along with its great results, GFET has shown numerous challenges for mass production. First one is large area production of high quality graphene. Nowadays most promising method to achieve high surface area graphene is CVD method. Another challenge is graphene tendency to absorb hydrocarbons that contaminates the surface of device and degrades its performance. One of the fabrication steps that introduce contamination in graphene sheet is lithography. Photoresist is the main reason of contamination in this step. With these advantages consideration, it is obvious that graphene will brings

amazing future for electronic and biosensor future application.

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