Graphene-based materials for biotransistor application

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**GRAPHENE-BASED MATERIALS
FOR BIOTRANSISTOR APPLICATION**

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**Interdisciplinary Graduate School
NTU Institute for Health Technologies**

2018

GRAPHENE-BASED MATERIALS FOR BIOTRANSISTOR APPLICATION

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NTU Institute for Health Technologies**

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Abstract

A biomimetic cell membrane bioelectronic transistor consisting of a supported phospholipid bilayer atop chemical vapor deposition (CVD) graphene or solution processed reduced graphene oxide on titanium dioxide (TiO_2) has been successfully demonstrated for biosensing application. For the first time, a unique bonding interaction between titanium and carbon atom on graphene oxide has been experimentally identified by X-ray Photoelectron Spectroscopy (XPS). The covalent interaction acts as a strong surface anchorage in facilitating uniform adhesion and near to ideal surface coverage of spin coated graphene oxide on titanium bearing substrate using the in-situ solid-liquid phase exfoliation method. The liquid gated Reduced Graphene Oxide (RGO) biotransistor is sensitive to environmental changes in ionic strength of liquid environment by shift in electrical output signals at a sensitivity level of $\sim 0.030\text{mA}$ per millimolar at gate bias $V_G = -8\text{V}$ and 0.035mA per millimolar at drain bias $V_D = 1\text{V}$. Incorporation of a phospholipid membrane atop the RGO transformed the sensing transistor to a bioelectronic transistor capable of sensing attached biomolecules on functionalized lipid/RGO surface acting as pseudo gate bias which modulated the electrical signals output. Supported neutral and charged lipid membranes on RGO at active source-drain region were formed by solvent assisted lipid bilayer (SALB) formation. A modulation of transistor conductance induced by gate charges were observed by the measurement of transistor drain current. Based on the 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)/RGO biotransistor platform, a functional supported lipid bilayer (SLB) was demonstrated using biotin-streptavidin binding event. The quantification of biotin composition with respect to transistor drain current modulation was monitored which showed a linear relationship between the percentage of changes in drain current versus the percentage of biotin composition in the complex SLB. For instance, a 12%

change in drain current was measured for a 1wt % biotinylated DOPC lipid complex membrane upon streptavidin binding. The results illustrated the potential of using lipid bilayer as an efficient biofunctionalization tool on graphene oxide as a highly versatile and sensitive biosensing platform for a variety of receptor-ligand binding events.

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

19 Feb 2019

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Ng Wei Beng

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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Assoc. Prof Cho Nam-Joon

Authorship Attribution Statement

This thesis contains material from 1 paper published in the following peer-reviewed journal where I was the first and/or corresponding author.

Chapter 3 is published as Seyed R. Tabaei, Wei Beng Ng, Sang-Joon Cho and Nam-Joon Cho, “Controlling the Formation of Phospholipid Monolayer, Bilayer, and Intact Vesicles Layer on Graphene” *ACS Appl. Mater. Interfaces* 8, 1875–11880 (2016).

The contributions of the co-authors are as follows:

- Assoc Prof Cho. Nam-Joon provided the guidance on the experimental work and direction in the manuscript writing.
- I co-designed the study with Dr Seyed R. Tabaei and performed all the laboratory work at the School of Materials Science and Engineering.
- The manuscript was co-written by me and Dr. Seyed R. Tabaei.
- Dr. Sang-Joon Cho assisted in the proof-reading of the manuscript.

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Abbreviations

Acronym	Meaning
AFM	Atomic Force Microscopy
CNT	Carbon Nanotubes
CVD	Chemical Vapor Deposition
DOEPC	1,2-Dioleoyl- <i>sn</i> -glycero-3-Ethylphosphocholine
DOPA	1,2-Di-(9Z-octadecenoyl)- <i>sn</i> -glycero-3-phosphate (sodium salt)
DOPC	1,2-Dioleoyl- <i>sn</i> -glycero-3-phosphocholine
DOPS	1,2-Dioleoyl- <i>sn</i> -glycero-3-phospho-L-serine (sodium salt)
DOTAP	1,2-Di-(9Z-octadecenoyl)-3-trimethylammonium-propane (chloride salt)
DPhPC	1,2-Diphytanoyl- <i>sn</i> -glycero-3-phosphocholine
DPPC	1,2-Dihexadecanoyl- <i>sn</i> -glycero-3-phosphocholine
DPPE	1,2-Dipalmitoyl- <i>sn</i> -glycero-3-phosphocholine
DPTE	1,2-Dipalmitoyl- <i>sn</i> -glycero-3-phosphothioethanol
FET	Field Effect Transistor
FRAP	Fluorescence Recovery After Photobleaching
GFET	Graphene Field Effect Transistor
GO	Graphene Oxide
GOx	Glucose Oxidase

HRTEM	High Resolution Transmission Electron Microscopy
I_D	Source-drain current
I_G	Gate current
IPA	IsoPropyl Alcohol
IVD	In-Vitro Diagnostics
LB	Langmuir-Blodgett
PBS	Phosphate Buffer Saline
PDMS	Polydimethylsiloxane
POC	Point-Of-Care
POPG	1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol
QCM-D	Quartz Crystal Microbalance-Dissipation
RGO	Reduced Graphene Oxide
SALB	Solvent Assisted Lipid Bilayer
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
SLB	Supported Lipid Bilayer
SPR	Surface Plasmon Resonance
TEM	Transmission Electron Microscopy
V_D	Source-drain bias
V_G	Gate bias
XPS	X-ray Photoelectron Spectroscopy

Chapter 1: Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, a biosensor is defined as an analytical device that is capable of providing quantitative or semi-quantitative information using specific biochemical reactions with a physicochemical detector [1]. The Clark oxygen electrode [2] laid the basis for the first glucose biosensor (in fact the first biosensor of any type), invented by Clark and Lyons in 1962 [3] which was an enzyme-electrode biochemical-based biosensor for glucose concentration. Following this work, a variety of biosensors and sensing mechanisms were reported, such as the first immunosensor on a potentiometric transducer in 1975 [4], Surface Plasmon Resonance (SPR) [5] and Quartz Crystal Microbalance (QCM) [6] both in 1983, among many other biosensors with variations.

Among these bio-sensing mechanisms, an interesting sensing approach, i.e. Field Effect Transistor (FET)-based biosensor, has been proposed and become an emerging field because of fast development in solid-state technology. Since almost all biomolecules carry electrostatic charges and bioactivities involve electrical potential changes, the FET-based biosensor used to detect nucleotides, amino acid and cells [7-16] becomes a promising candidate for applications requiring ultra-sensitivity and fast response time. With these developments, FET-based biosensors have been addressed to be a good candidate for next generation Point-Of-Care (POC) testing. POC is a branch of In-Vitro Diagnostics (IVD). It is defined as a medical diagnostic testing at the point where patient care is needed [17, 18]. For instance, a glucometer is used at patients' house or bedside, rather than central laboratory in medical centers. To accomplish POC, its operation should be simple enough for less-trained or even un-trained person. And it dramatically decreases the time to test results, which would provide doctors instant information for correct diagnosis. Due

to the advantages of sensitivity, selectivity, miniaturization, light-weight, low-power, and seamless for fitting in electronic read-out systems, the FET-based biosensors become competitive candidates for future POC applications in contrast to bulky optical-based IVD instruments such as SPR, QCM and immunoassay which required bulky analyzers.

Graphene Oxide (GO) is analogous to graphene or unzipped Carbon Nano-Tubes (CNT) with oxygen moieties [19]. It offers several advantages over graphene or CNT, such as a tunable band-gap, facile synthesis and no use of metal catalysts hence at a much lower cost. Due to the monolayer configuration of GO which is much thinner than CNT, all of its carbon atoms are readily exposed to the atmosphere and are sensitive to surface perturbations since it has a high surface area to volume ratio, thus making GO very suitable for liquid-gated FET type sensing applications in which surface sensitivity is of paramount importance. In fact, graphene oxide has around two-fold higher effective surface area and has a greater cost-effectiveness than carbon nanotubes [20]. Additionally, it has greater homogenous surface that is responsible for highly uniform and efficient functionalization offering much more versatility in the design of various types of FET biosensors.

1.1 Molecular Structure and Properties of Graphene

The term, two-dimensional (2D) materials, first came into the limelight with the first report on the isolation of graphene in 2004 [21]. A close relative of carbon nanotubes which have a longer history, graphene is fundamentally an atomic single layer of sp^2 hybridized carbon atoms which extend in two-dimension into a sheet-like molecular structure. The 2D structure can be envisioned as the unzipping of a single-walled carbon nanotube. The graphene basal plane comprises of covalent bonding in which three of the four valence electrons of carbon (one $2s$ orbital and

two $2p$ ($2p_x$ and $2p_y$) orbital electrons) form strong σ bonds with three neighboring carbon atoms. The bound electrons in these σ bonds do not participate in electron conduction but are responsible for the chemical and mechanical stability as well as crystallinity of graphene. The fourth electron lies in the $2p_z$ orbital which is perpendicular to 2D basal plane. These electrons from the $2p_z$ orbitals collectively form the delocalized π electrons cloud above and below the basal plane of graphene, which is responsible for the electron conduction along the surface of the 2D basal plane. The three sp^2 hybridized orbitals of each carbon have a mutual 120° angle separation in the xy plane. Since each carbon atom is bonded to three neighboring carbon atoms, the extended structure constitutes an array of honeycomb lattice structure in the basal plane.

In solid state physics, the term Fermi level is used to describe the top of the collection of electron energy levels at absolute zero temperature, such that no electrons will have enough energy to rise above the Fermi level. In conventional semiconductors, the Fermi level lies within the bandgap between the valence and conduction band. Single-layer graphene has two atoms per unit cell denoted as A and B as illustrated in Figure 1.1. Due to the band crossing between the conduction and valence bands, graphene can be well represented as semimetal with vanishing Fermi surface or as a semiconductor with a diminishing bandgap. An interesting feature of intrinsic graphene is depicted in Figure 1.1 in which the Fermi level coincides with the Dirac point which is the crossing point of the linear energy dispersion curves. The implication of this intersection is that intrinsic graphene can behave like a zero-bandgap semiconductor. The position of the Fermi level could be adjusted either above or below the Dirac point by external stimulus such as voltage bias, doping of impurities and disorder in the lattice structure. When the Fermi level is in the valence band, holes are the major carriers and graphene behaves like a p-type semiconductor. When the Fermi level is in the conduction band, electrons are the major carriers and in this case the graphene will behave like a n-type semiconductor. The high tunability

of electronic properties of graphene is unique on its own in the material world. Another complementary property of graphene is its optical transparency in the visible light range. This makes graphene an attractive nanomaterial for transparent electronics and liquid crystal displays. and flexible electronics due to its atomic thickness.

With the multitude of unique properties as briefly introduced above, there has been an increasing number of reports on graphene-based application on an exponential rise in recent years. One has to be careful to assess the base materials used in this wide spectrum of application because there is not just one form of graphene. Driving by the different processing and application requirement, these graphene could be in the oxidized form which is often known as graphene oxide (GO) or reduced graphene oxide (RGO). Each of these graphene members have a slightly different molecular structure and hence materials characteristics and properties.

Figure 1.1. (a) Honeycomb atomic structure of a perfect single layer graphene showing C atoms in A and B sublattices. (b) Schematics illustrating the band dispersion of a perfect single layer graphene showing the π bands are touching one another at the K point [22] (c) 3D band structure of monolayer intrinsic grapheme and contour plot of band structure. (d) Dirac cones of intrinsic graphene, n type and p type graphene [23].

1.2 Characteristics and Synthetic Processes of the Graphene Family

Pure graphene of perfect molecular structure is challenging in terms of fabrication and process handling. So far Chemical Vapor Deposition (CVD) is the technique of choice. Fabrication cost is high and area of deposition is limited although a CVD graphene is by far the most superior in meeting high expectation of materials properties in the production of monolayer or few layer graphene with low defect density. CVD is carried out on poly and monocrystalline metallic substrates,

particularly copper and nickel surfaces. As the substrates are often not utilized in the final design, there will be a transfer step of graphene onto appropriate substrates which complicated the process such as surface contamination and structural defects. Traditionally graphene on surfaces could also be obtained by physical exfoliation of bulk graphite. The latter method is very cost effective however morphology and dimensional controls of the graphene samples are far from desirable since they are very non-uniform. In view of the above short-comings, coupled by intense interest in the last 20 years in flexible electronics has driven research effort in solution processing of graphene. Pure graphene is intrinsically hydrophobic. Many researchers in the field have hence turn to its oxidized form, graphene oxide (GO) for solution.

GO can be manufactured chemically by oxidation of crystalline graphite to produce monolayer or few layer typically in an aqueous suspension with excellent dispersion. The structure of GO is however slightly different from graphene but drastically different materials properties. The molecular structure of GO consists of single-atom-thick carbon sheets with phenol, hydroxyl and epoxide groups above and below the basal plane and carbonyl and carboxyl functional groups attached to the edges of the graphene sheets [24] (Figure 1.2). While the functional groups at the edges of GO sheets are able to form hydrogen bonds with water molecules [25], GO is essentially amphiphilic since the basal plane is hydrophobic. The maximum dispersibility of GO depends on both the solvent and the extent of surface functionalization imparted during oxidation. To date it has been found that the greater the polarity of the surface, the greater the dispersibility. Dispersibilities are typically on the order of 1-4 mg/ml in water [26].

Figure 1.2. (a) Structural model and (b) 3D view of graphene showing carboxylic groups at the edge, phenol hydroxyl and epoxide groups mainly at the basal plane. [27].

Aqueous dispersion of GO has been used as the candidate of choice for many solution coating processes such as spin-coating, drop-casting and spray-coating. Good uniformity of GO film has been demonstrated on various substrates including flexible and transparent substrates. This has opened the door to exciting opportunities for devices fabrication. However, due to the high density of structural defects in the sp^2 bonding networks and functional groups on GO, the materials properties of GO is far more inferior to CVD graphene. In fact, GO is an electrical insulator in contrast to its ultrahigh conductivity CVD graphene counterpart. In many applications and designs electrical conductivity is highly desired. Fortunately, a conductive form of solution-derived GO is achievable by a reduction of the solution coated GO to its derivatives known as Reduced Graphene Oxide (RGO). By partially restoring the π -network which is responsible for the ultrahigh conductivity in pure graphene via the removal of oxygen functionalities, solution coated RGO is able to meet most requirements.

Reduction methods of GO can be achieved through chemical, thermal or electrochemical pathways. Chemical reduction is usually achieved using strong reducing agents such as hydrazine monohydrate ($N_2H_4 \cdot H_2O$) [28], lithium aluminum

hydride (LiAlH_4) [29] or sodium borohydride (NaBH_4) [30]. Among all, hydrazine is most commonly used since it does not have high reactivity to water in contrast to the other reductants. One of the main disadvantage of chemical reduction methods is the introduction of heteroatomic or functional impurities. In the case of hydrazine, while effective at removing oxygen functionality, nitrogen tends to remain covalently bonded to the surface of the GO, likely in the form of hydrazones, amines, aziridines or other similar structures [31]. Residual C-N groups have a profound effect on the electronic properties of the resulting RGO functioning as n-type dopants [32]. NaBH_4 is most effective at reducing C=O species, while having low to moderate efficacy in removal of epoxides and carboxylic acids. Additional alcohols are the principal impurities that are generated during the reductive process. A few other reductants have been subsequently reported such as hydroquinone [33], gaseous hydrogen [34] and strongly alkaline solutions [35], however each with its own set of merits and limitations. Nevertheless, in all cases, chemically reduced RGO still present an attractive option for cost effective and large-area coating of graphene-like material that is able to meet most requirements.

Rather than using chemical reduction which often imposed secondary impurities while removing the primary functionalities, thermally mediated reduction methods have been explored [36, 37] since graphene is thermodynamically more stable than GO. Although low temperature thermal reduction could be conveniently carried out in air, an inert atmosphere has to be used for the thermal reduction above about 200°C to prevent combustion of the GO to carbon. A notable effect of thermal reduction is the structural defects created by the release of carbon dioxide [38]. It has been estimated that approximately 30% of the mass of the GO is lost, leaving behind vacancies and topological defects throughout the plane of the RGO [36]. These structural defects could adversely affect the electronic properties of the product by the introduction of scattering sites. Despite these structural defects, however, bulk

conductivities of $1000 - 2300 \text{ Sm}^{-1}$ were measured, indicating overall effective reduction and restoration of the ideal electronic structure.

A more recent reduction method is by electrochemical means [39, 40]. The GO coated substrate served as one of the electrode pair in a two-electrode system and a liner sweep voltammetry was run in a buffer electrolyte. Reduction began at -0.60V and reached a maximum at -0.87V. Rapid reduction has been observed during the first 300s followed by a decreased rate of reduction up to 2000s. The route appears to be extremely effective at reducing the oxide functionalities and it precludes the use of hazardous reductants. The main issue with this technique is that it has not yet been demonstrated on a large sample. Scalability is a fundamental requirement of a useful protocol if graphene is to be broadly utilized.

1.3 Biochemical Functionalization of Graphene as Biosensors

As illustrated in Figure 1.3, a biosensor consists of two components, a biological recognition element (bioreceptor) and a transduction element (transducer). The role of the bioreceptor is to interact specifically with the target analyte, and the bioreceptor element can be an enzyme, antibody, nucleic acid, cell structure, organelles, microorganisms, or tissue. On the other hand, the role of the transducer is to transform the biorecognition event resulting from the interaction of the analyte with the biological element into a detectable signal that can be more easily measured and quantified. The transducer usually works in a physicochemical way, for example, the electrochemical technique, the optical technique, the piezoelectric technique, etc. The graphene family of materials can be used as the transducing platforms. The distinctive properties such as zero-bandgap semiconductor, high carrier mobility, quantum hall effect make the graphene family hot candidates for components on the surface of electrodes and electronic components of biosensors.

Biosensors are playing an increasingly important role in the modern healthcare management since they provide first-hand tool that enables the interrogation of a multitude of biomolecular processes from a macro perspective. With the advancement of peripheral technologies in the field of materials science and electronics, there had been much research in the label-free detection of biological agents including cytokine [41], antibodies [42], protozoan [43], viruses [44] and bacteria [45] using voltammetric, amperometric and potentiometric methods. The nanoelectric features of such platforms offers unmatched advantages in the development of lab-on-a-chip point-of-care (POC) diagnostics. While sensitivities of many bioelectronics platforms have been greatly improved, the construction of an efficient biomolecular interface, which is fundamental in all bioelectronics, remains a challenging issue to date. Intrinsically, biomolecules do not adhere well to non-cellular surfaces. In the case of graphene, surface functionalization is crucial to create ligand receptors for biological analytes. There are many strategies of biofunctionalization of the graphene surfaces which have been well reported. They can be broadly categorized into covalent and non-covalent interaction.

Figure 1.3. General scheme of graphene-based biosensors [46] illustrating the different level of transducer, receptor and analyte elements.

The enriched reactive oxygen functional groups of GO and RGO should render it a good solid substrate for biofunctionalization through covalent binding. This can be achieved via chemical reactions between the side groups of amino acid residues located on enzymes or proteins and oxygen functional group available on the GO and RGO surfaces. The covalent immobilization generally ensures the highest binding strength between the GO/RGO and the receptor while minimizing leakage issues and increasing operational stability towards heat, pH, organic solvents. On the other hand, covalent immobilization may result in steric modifications of the bioreceptors, leading to a decrease of functionality or enzymatic activities. In the case of GO-protein conjugates, direct immobilization could be achieved by the formation of amide bonds between the carboxylic groups on GO and the free amine groups on proteins using water-soluble coupling agent such as EHC and NHS. However, there is a possible risk of a loss or decrease in catalytic activity of glucose oxidase (GOx) due to conformational change in the case of covalent bonding

Non-covalent interaction, or physical adsorption, is by far the most simple and desirable pf physical immobilization. The interaction of the receptors with the graphene surface is via self-assembly including weak Van der Waals forces, hydrophobic, electrostatic and π - π stacking interaction. A few research groups have been exploring non-covalent π - π interaction to preserve or even enhance the activity of enzymes on GO. One of the notable findings was provided by Alwarappan et. al. [47] who reported the experimental observation of strong π - π stacking between individual hexagonal cells of the GO basal planes with GOx.

In terms of biosensing, the imperfections of graphene oxide presents conveniently themselves as anchoring points for the binding events of a variety of biological molecules. Recently, direct virus capture and destruction by GO has been reported without any receptors [48], though the experiment was not performed on clinical samples but on pure virus sample. In addition, graphene oxide being soluble in water could be easily dispensed by large-area liquid coating process on a variety

of substrates which is not easily achievable by CVD of graphene. Although intrinsically insulating, graphene oxide could be partially reduced to impart conductivity either by thermal or chemical reduction processes, while still preserving the imperfections necessary for anchoring of biomolecules. The use of RGO is particularly attractive for biosensing since RGO has been found to be more biocompatible than graphene [49] and it is much easier to fabricate and coat graphene oxide on various substrates by simple solution coating. Few groups have reported the formation of single or bilayer of phospholipid layers on reduced graphene oxide [50-54]. However, due to the flaky morphology of coated graphene oxide on the substrates, the uniform formation of lipid layers is far from ideal.

1.4 Basics of Biotransistor

Biotransistor is a term which has been coined by many biomedical engineers working on field effect transistor (FET) as a biosensing electronic platform. FET is a very well-established technology in the semiconductor industry. A very simplified overview of the working mechanism of the FET is that the electrical output is dependent on the charge density on the semiconductor surface, which could be in the form of negatively charged electrons or positively charged holes. The charge density can be modulated by the effect of applied electric field hence the name field effect transistor (FET). The same charge density modulation could be achieved by the chemical interaction of the semiconductor surface with ions or molecules. The modulation of the charge density could be manifested as electrical output signals which forms the basis of the field effect biotransistor.

In order to understand the principles of operation of a FET, we need to refer to the structure of a conventional FET making use of silicon technology as shown in Figure 1.4. The semiconducting silicon (n-type or p-type) as the substrate has

metallic electrodes at the left and right extremities which are designated as the source and drain. The semiconductor surface is insulated from a top third metallic electrode called a gate by an insulator which is often silicon dioxide in the conventional semiconductor technology. Under an applied source drain voltage V_D no current is possible between source and drain due to the depletion layers formed at the interface of semiconductor (whether n-type or p-type) and metallic electrodes at the source and drain. The situation changes when a gate voltage V_G is applied at the gate electrode. In the case of an n-type semiconductor, when a negative electric charge is loaded on the gate, under the influence of the field effect across the insulator on the underlying semiconductor, the n-type semiconductor turns into a p-type one within a very thin layer below the gate term as the inversion layer through which holes are the charge carriers between the source and drain. Charge carriers are injected from the source and collected at the drain giving rise to a source drain current I_{DS} . Since the current flow is confined within the inversion layer the zone is called a channel. In the case of a p-type semiconductor, a mirror twin situation occurs in which a positive electric charge has to be loaded on the gate to create a n-channel to inject electrons from source into the very thin layer of semiconductor below the gate and get collected at the drain giving rise to I_{DS} . Note that in both situations the V_G has to be above a threshold voltage V_{th} in order to break down the depletion layers in both cases of the n-type and p-type semiconductor. Since the current flow is controlled by the electrical field produced by the gate across the insulator on the semiconductor, such a device is termed as a field effect transistor (FET).

Figure 1.4. Cross-section of an n-channel silicon metal oxide semiconductor FET, showing that a drain current, I_D , flows between the source and drain when the applied voltage bias V_G between the source and gate terminals exceeds a threshold voltage bias, V_{th} . [55].

Figure 1.5. Schematic illustration of electrolyte-gated GFETs [56] depicting the relative position of the source, drain and gate of a three-terminal FET.

One important consideration in the electrolyte top-gate configuration is the Debye screening length which plays an important role in the performance characteristics and sensitivities of the Graphene Field Effect Transistor (GFET). The Debye screening length is the extended distance from the sensing surface into the electrolyte at which mobile charge carriers screen out the external electric field. For the electric field of the charged analyte to be sensed and hence be picked up by the GFETs, the charged analyte or the receptor-ligand interaction has to be at a distance less than the Debye screening length. As the Debye screening length is highly

dependent on the ionic strength of the electrolyte, the ionic strength of the electrolyte or buffer will have to be carefully and precisely tuned to ensure sensitivity of detection.

1.5 Overview of Objectives and Work Scope

Solution coating of GO on the substrates is still far from ideal due to the flaky morphology and scattered distribution of GO sheets which comprises of non-uniform mixture of single layer and a few layers. This has been well-reported whenever GO is coated on SiO_2 [57-62]. The micro and nano-steps and uniformity on the support surface will ultimately affect the formation of a uniform lipid layer since a lipid bilayer is only 5 nm in thickness. If the lipid layer as a biofunctionalization platform is non-uniform, biosensing performance of the bioelectronic platform will be compromised. In this preliminary study, we have shown that uniformly thin GO layer of a few layers could be formed by an in-situ solid-liquid phase exfoliation mechanism on a TiO_2 -bearing surface as compared to SiO_2 . Based on this methodology, we will extend the application of GO/ TiO_2 as a support for the lipid bilayers to be used as a variety of biosensors. Most of the reported biosensing devices are fabricated on SiO_2 . Earlier reports provided clear indication of substrate effect of different metal oxides on the formation of lipid bilayers [63, 64]. It is important to study and understand the same substrate effect on GO for the application of such hybrid lipid/GO for biosensing application. We compare the effect of SiO_2 and TiO_2 on the formation of the coated GO and to investigate the effects of different types of lipids-GO interaction on a biotransistor performance. This study constitutes the basis for building prototyped biosensors using graphene or GO. The scope of work is:

- Establishment of the formation of SLB on GO/ TiO_2 ;

- Device fabrication of SLB biotransistor by the integration of fluidics flow chamber and contact electrodes;
- Verification of biosensing capabilities of biotransistor by measurement of electrical output signals upon formation of charged SLB; and
- Demonstration of a functional SLB biotransistor by established biomolecular affinity such as streptavidin-biotin binding event.

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Chapter 2: Literature Review

There has been an increasing multitude of literature reports on using graphene FET (GFET) based on various biomolecules for biosensing application as discussed in the previous chapter [1-4]. These biomolecules range from the larger micrometer-sized microorganisms such as bacteria and living cells to nanometer-sized viruses down to small molecules such as DNA and proteins [5]. With such a wide span in lateral and areal dimensionality of the analytes, 2D graphene of comparable range of dimensionality has to be achieved. Such a fabrication challenge can nowadays be easily overcome owing to the well-established lithographic technology from the electronics industry. This is a distinct advantage of graphene over other graphitic nanomaterials such as carbon nanotubes. Over the last few decades, research communities have made great strides in the improvement of graphene synthesis and patterning and hence biosensing capabilities [6]. On an additional note of the unique advantage of using graphene for biosensing, the large surface area of graphene enhances the surface loading of desired biomolecules, either through passive adsorption, or by the covalent immobilization of biomolecules. The excellent conductivity and small band gap of graphene are beneficial for the conduction of electrons between the biomolecules and the electrode surface. In fact, graphene exhibits a large surface area of $2630 \text{ m}^2 \cdot \text{g}^{-1}$ for a single-layer, similar to that of carbon nanotubes (CNTs) [7, 8]. It also exhibits some other merits like low cost, two external surfaces, facile fabrication and modification and absence of metallic impurities, which may yield unexpected and uncontrolled electrocatalytic effects and toxicological hazards [9 -11].

In this chapter, supported lipid bilayers will be first introduced followed by literature review of hybrid graphene interfaced with lipid bilayers as biosensors. As there is a huge spectrum of electronic biosensors (such as electrochemical, optical, impedance and acoustic sensors) a full literature review covering all will not be

possible within the scope of this thesis. Therefore, the literature review provided in this chapter will only be limited to hybrid graphene or graphene oxide interfaced with lipids as a biosensing platform based on the field effect transistor (FET) to be in-line with the topic of this thesis.

2.1 Supported Lipid Bilayers (SLB)

Lipid bilayer membrane, or plasma membrane, is an important constituent of all biological cells that serve as the outer boundary between cell cytoplasm and extracellular matrix [12]. The annotation of a bilayer refers to the structure of the membrane being made up of two lipid layers with their hydrophobic tails facing inward to each other while the hydrophilic heads of each layers facing outward, one towards extracellular matrix the other towards cell cytoplasm. The biological membrane and a simplified structure of a lipid bilayer are presented in Figure 2.1 [12].

Although the biological membrane has astounding complexity associated with diverse physical-chemical properties [13] such as two-dimensional fluidity, material elasticity, thermal fluctuations, chemical diversity, and rich phase behavior [14]. Scientists have been trying to mimic such biological systems with synthetic versions. They are many direct and practical benefits of learning to devise such synthetic membranes, many of which can lead to provide new classes of biosensors, diagnostic tools, biocompatible materials, and high throughput characterization platforms for rapid and early detection of interactions between cells and their environment [15]. Within this framework, are approaches that are proving powerful involves interfacing lipid bilayer membranes to solid substrates via self-assembly and directed self-assembly methods [16-20]. These membrane-mimicking

architectures are collectively referred to as supported lipid bilayers (SLB) as shown in Figure 2.2 [21].

Figure 2.1. The watery interior of cells is surrounded by the plasma membrane, a two-layered shell of phospholipids. The phospholipid molecules are oriented with their fatty acyl chains (black squiggly lines) facing inward and their water-seeking head groups (white spheres) facing outward. Both sides of the membrane are lined by head groups, mainly charged phosphates, adjacent to the watery spaces inside and outside the cell. All biological membranes have the same basic phospholipid bilayer structure. In actuality, the interior space is much larger relative to the volume of the plasma membrane depicted here [12].

Supported membranes are typically formed at the solid-liquid interface when vesicular microphases of lipids spontaneously rupture and spread on hydrophilic surfaces. Alternative strategies employ Langmuir-Blodgett troughs to successively

transfer two lipidic monolayers from the air-water interface onto planar surfaces [22]. The precursor phases in these approaches can consist of purified and synthetically tailored lipids and their complex mixtures in predetermined compositions, providing molecular-level control over their structure, chemical compositions, phase state and lateral fluidity. Moreover, because these constructs interface membrane architecture with solid substrates, they are amenable to a brand suite of surface analytical tools for molecular-scale characterization of their structure and dynamics and enable chip-based bioanalytical assays, sensors and platforms [23-33].

Figure 2.2. Schematic illustration of basic supported-membrane formation processes. (a) Vesicles adsorb, fuse, and rupture at solid surfaces; (b) lipid stacks spontaneously spread upon hydration; and (c) two successive transfers of Langmuir–Blodgett monolayers deposit pre-equilibrated lipid mixtures to form (d) single, supported bilayers on hydrophilic substrates. The dark-blue area is the intervening layer of hydration water between the substrate and the lipid [21].

2.2 Graphene Field-Effect Transistor (GFET) based on Supported Lipid Bilayer (SLB)

The investigation of lipid membranes on graphene-based materials is not as widely studied as compared to other nanobiointerfacial systems such as the interaction of nucleic acid with graphene [34] and lipid bilayers formation on organic materials [35, 36] and carbon nanotubes [37]. The study of the interaction between graphene oxide and lipid membranes was started very recently in 2011 as reported by Frost et al. [38]. Alternating nanocomposite structures of graphene oxide and lipid membranes formed by liposome rupture were fabricated and adsorption kinetics were studied real-time by quartz crystal microbalance with dissipation monitoring (QCM-D), complemented by post-adsorption surface analytical techniques such as dual polarization interferometry (DPI) and atomic force microscopy (AFM). Supported lipid bilayers were first formed on SiO₂ [38] followed by introduction of graphene oxide solution. It was reported that graphene oxide, being negatively charged, only adsorb on positively charged POPC:POEPC (3:1) lipid membranes but not on negatively charged POPC:POPS (3:1) membranes due to electrostatic repulsion. Upon the second layer of liposomes adsorption, the interaction with graphene oxide was not strong enough to induce liposomes rupture, similar to the case of TiO₂ and Au. Adsorbed liposomes on graphene oxide underwent rupture only upon subsequent graphene oxide adsorption on the liposomes. The observation indicated the requirement for both sides of the liposomes to be deformed by graphene oxide for rupturing event.

In place of insulating graphene oxide, conductive RGO was used as a support for DOPC bilayers by Tsuzuki et al. [39]. Hydrazine-reduced RGO was fabricated on SiO₂/Si substrates followed by self-assembly of electrically neutral DOPC atop RGO using vesicle fusion. The DOPC vesicles in buffer solution (100mM KCl, 25mM HEPES/pH 7.4 NaOH) was prepared by freeze-thaw treatment and extrusion

through a 100nm polycarbonate filter and sonicated to obtain unilamellar vesicles. The RGO/SiO₂/Si substrate was incubated in the vesicles solution with 10mM of CaCl₂ at 45°C for 60min. Excess vesicles in the liquid was subsequently washed out by exchanging the vesicles solution with fresh buffer solution not containing any vesicles. It was concluded by AFM imaging and height measurement complemented by FRAP observation that while exposed SiO₂ were coated by DOPC lipid bilayers, RGO-coated SiO₂ surfaces were coated by a lipid bilayer atop a single lipid layer.

Okamoto et al. [40] performed a fluidity evaluation of supported lipid bilayer using pegylated and unpegylated DOPC, DOPE and DPPTE on GO by vesicles fusion following the same protocol as Tsuzuki [39]. Quantum dot was conjugated on the lipid bilayers to qualitatively evaluate the fluidity of the single particle tracking method. It was noted that the diffusion coefficients of lipids in the membranes on GO is lower than that on SiO₂ due to the nanoscale hydrophilic and hydrophobic domains on the GO that affected the lipid assembly of the membranes hence affecting the lateral diffusion of lipids.

The effect of substrates on mechanically exfoliated graphene has been shown to have a significant impact on the formation of lipid membranes by vesicles fusion and rupture [41]. DOPC single layer was formed on graphene attached on hydrophilic regions while no lipid layers were formed on graphene attached on hydrophobic regions on sapphire substrate. The permeability of the hydrophilicity of the support substrate through the graphene films have been demonstrated to extend over a few graphene monolayers in liquid environment.

Other than the widely used vesicle fusion method, there have been reports using self-spreading method [42], dip-pen nanolithography (DPN) [43] and Langmuir-Blodgett (LB) [44] technique to form lipid membranes on graphene-based surfaces. In the self-spreading technique [42], a small amount of the dried lipid in chloroform was transferred onto the surface of the substrate followed by gentle immersion in a

buffer solution (100mM NaCl, 10mM Tris-HCl of pH 7.6). In contrary to the results in [40], there was no formation of L- α -Phosphatidylcholine lipid membrane observed on the GO although single lipid bilayer could be well formed on SiO₂. It was believed that the hydrophilicity of the GO surface was insufficient to satisfy the energy balance requirement for the self-spreading process which depends on the hydrophilic-hydrophilic interaction between the solid surface and the spreading lipid bilayer. A direct patterning of DOPC and DOPC:DOPA on graphene which was prepared by mechanical cleavage from graphite was achieved by DPN using phospholipids (L-DPN) [43]. Submicron arrayed patterns of inverted phospholipid bilayers are formed on a base lipid monolayer atop graphene by directing writing of phospholipids using cantilever arrays in air. These inverted bilayers in air reorganized into single lipid monolayers on graphene upon immersion into buffer solution. Electronic doping of pristine graphene by charged phospholipids such as DOPC:DOPA was observed based on peak shifting in the Raman spectroscopy. In the LB technique [44], DOPC with defective holes and defect-free DPPC lipid monolayers are formed on RGO. The uncoated defective regions on RGO by DOPC could be due to the hydrophobic nature of the aromatic rings of the scaffold plane of graphene since the hydrophilic tails of the lipid molecules prefer to associate with the RGO sheets. In the case of DPPC, the gel-like state of the lipids at room temperature with the tails densely packed and oriented parallel to the film normal seemed to facilitate the formation of a uniformly covered lipid monolayer on the RGO, both on the hydrophobic aromatic rings regions as well as the hydrophilic defect regions.

There have not been many reports on using lipid membranes/graphene-based electronic platforms for biosensing applications. Ang et al. [45] reported a transistor based on a lipid bilayer membrane chemical vapor deposition (CVD) monolayer graphene platform for electrical detection of membrane disruption via an electrolyte-gated configuration using silver paste as source and drain electrodes. Poly Dimethyl

Siloxane (PDMS) well was fabricated and used to hold the liquid samples and to isolate the source and drain electrodes from the electrolyte. Lipid bilayers were formed by vesicles fusion (incubation of 2-3h at 60°C) on the CVD graphene after soaking the graphene/substrates in distilled water for one day. They demonstrated the effect of neutral and charged lipid membranes on the electrical outputs of the electrical signals via modulation of the impurity potential on the underlying graphene monolayer on SiO₂/Si substrates. Corresponding voltage shifts in the charge neutrality point of graphene were observed upon the formation of charged lipid membranes using negatively charged POPC/POPG (ratio = 2/1) and positively charged POPC/DOTAP (ratio = 2/1). As a proof of concept, the authors demonstrated the recovery of the original electrical output upon the thinning and disruption of POPC/POPG membrane on graphene respectively by the addition of membrane-disrupting agent, magainin 2 peptides, as shown in Figure 2.3, from 5nm to 3nm. The disruption of the membrane reduced the field effect from the negatively charged lower membrane leaflet because of the charge screening by the ionic solution within the Debye distance. Following the report of charged lipid bilayers on graphene field effect transistor [45], Kiani et al. [46, 47] proposed an analytical model for the elucidation of total conductance of graphene as a support for atop lipid bilayers in an electrolyte-gated configuration as a function of two parameters, namely the type of electric charges of the phospholipids, and the thickness of the lipid bilayer. The proposed model presented mathematical results that were in good agreement with the experimental data reported by Ang et al. [45].

Figure 2.3. Membrane thinning effect of Magainin 2 peptides on gram-positive bacteria biomimetic membrane. AFM and epi-fluorescence (inset) images of membrane (a) before and (b) after addition of 1 μM Magainin 2 peptides. Peptide-induced membrane thinning was evident especially at the edge of the lipid membrane patches. (c) Conductivity curves of biomimetic membrane-GFET in 10mM NaF with increasing Magainin 2 concentration and (d) the corresponding shifts in Dirac points. (e) Schematic diagram showing sensing concept of membrane thinning effect. Brown ovals represent peptides [45].

A more recent publication [48] reported on a nanosensing device using a supported lipid bilayers on CVD graphene for the measurement of ion channel currents as shown in Figure 2.4. The authors demonstrated the formation of a uniform lipid bilayer acting as a biomimetic barrier that insulated the graphene both from the electrolyte environment in terms of solution pH and ionic strength. The formation of the lipid bilayers followed the same procedure as Ang et al. [45] of soaking the graphene on substrates in water and incubation at 60°C. The authors demonstrated the voltage shifts of the Dirac point of graphene with ionic strength changed from 10mM to 1000mM and in solution pH of 4, 7 and 10. However after the formation

of DPhPC, the membrane-coated graphene was impervious to the change in the ionic strength and pH of electrolyte displaying no shift in Dirac point of underlying graphene. Upon the introduction of proteins such as alamethicin and gramicidin A, their nanosensing device detected current pulses when the proteins formed pores in the lipid bilayers leading to an opening of the underlying graphene to the electrolyte.

Figure 2.4. (a) Photograph of a GFET device. (b) Top view of GFET. (c) Schematic illustration of grapheme SLBs platform. (d) Fluorescence images of SLBs on grapheme surface (scale bar is 100 μm). (e) Illustration of SLBs on grapheme surface. (f) Raman spectrum of monolayer grapheme. (g) The drain-source current vs liquid gate voltage characteristics of bare GFET and covered by SLBs at 100mM KCl and $V_{th} = 100$ mV. (h) The liquid gate current vs liquid gate voltage characteristics of GFET with bare grapheme and covered by SLBs at 100 mM KCl [48].

2.3 Advantages of using Supported Lipid Bilayer on Graphene for biosensing application

An important consideration in the electrolyte gated immuno GFET is the Debye length which is a physical distance from the sensing surface within which the binding of the analyte has to occur in order for the field effect signals to be picked up. The Debye length is caused by the screening effect arising from an electron double-layer formed by counter-ions in the electrolyte [49]. The Debye length is highly dependent on the ionic strength of the electrolyte or specimen under test such that higher ionic strength translates to a shorter Debye length as illustrated in Figure 2.5. For clinical samples such as blood, serum and urine which contain rich level of mineral salts, or undiluted phosphate-buffered saline solution (PBS buffer, ion concentration of 0.15 M), the dimensions of some antibodies (10–12 nm) are large enough to surpass the thickness of the Debye length (1 nm) at the electrolyte-immunosensor interface. In this case, the analyte charges will be at a farther distance from the surface than the Debye length and will be shielded from the sensing channel, in this case, the graphene layer, by the counter-ions in the buffer. Although, there are conditioning steps which could be performed on the specimen such as centrifugation, filtration and dilution to increase the Debye length, alternative solution for immuno GFET to achieve a meaningful LOD and high sensitivity has been in progress to handle high ionic strength specimen.

Figure 2.5. A schematic illustrating the effect of the strength of ionic buffer solution on the screening length (λ_D). Green: sensor platform, Purple: bioreceptor, Pink: Antigen [49].

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Chapter 3: Controlling the Formation of Phospholipid Monolayer, Bilayer, and Intact Vesicle Layer on CVD Graphene

ABSTRACT

Exciting progress has been made in the use of graphene for bio- and chemical sensing applications. In this regard, interfacing lipid membranes with graphene provides a high-sealing interface that is resistant to nonspecific protein adsorption and suitable for measuring biomembrane-associated interactions. However, a controllable method to form well-defined lipid bilayer coatings remains elusive, and there are varying results in the literature. Herein, we demonstrate how design strategies based on molecular self-assembly and surface chemistry can be employed to coat graphene surface with different classes of lipid membrane architectures. We characterize the self-assembly of lipid membranes on CVD-graphene using quartz crystal microbalance with dissipation, field-effect transistor, and Raman spectroscopy. By employing the solvent-assisted lipid bilayer (SALB) method, a lipid monolayer and bilayer were formed on pristine and oxygen-plasma-treated CVD-graphene, respectively. On these surfaces, vesicle fusion method resulted in formation of a lipid monolayer and intact vesicle layer, respectively. Collectively, these findings provide the basis for improved surface functionalization strategies on graphene toward bioelectronic applications.

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3.1 Introduction

Graphene-based materials attract wide interest across the chemical sciences because of the unique electrical and mechanical properties of graphene [1]. An ongoing area of interest involves interfacing graphene with biological systems, especially for sensor applications [2-5]. For such purposes, there is great demand for fabricating lipid bilayer coatings on graphene. Such coatings can serve as biofouling-resistant layers in bioelectronics devices and can enable surface functionalization and the possibility to host membrane-associated biomolecules. Figure 3.1 presents three different model membrane platforms, including the supported lipid monolayer [6-8], bilayer (SLB) [9], and adsorbed vesicle layer [10], which can form on solid supports depending on the corresponding surface chemistry.

Figure 3.1. Schematic illustration of three model membrane systems. **(a)** Supported monolayer and **(b)** bilayer provide a natural environment for surface immobilization of membrane-associated molecules at the immediate vicinity ($\sim 5\text{--}10$ nm) of the surface and impart biocompatibility and biofunctionality to the sensor surface. **(c)** Intact vesicles adsorbed on a substrate can be used to study the effects of membrane curvature.

Although there has been some promise in fabricating lipid nanostructures, a controllable method to form well-defined lipid bilayer coatings remains elusive, and there are varying results in the literature. Early work by Ang et al., [11] reported lipid bilayer formation on graphene by using the vesicle fusion method conducted at high temperature (60°C), and the conclusion was based on interrogating the fluidic

properties of the lipid adlayer. By contrast, Hirtz et al., [12] reported formation of a lipid monolayer on graphene by dip-pen lithography, as confirmed by direct morphological measurements with atomic force microscopy. The discrepancies suggest that a clear understanding of why different kinds of lipid membrane architectures can form on graphene is lacking especially from the perspective of how surface chemistry and the chosen fabrication method influence the fate of the molecular self-assembly process.

In the conventional vesicle fusion method, when small (<100nm) unilamellar vesicles are added to a solid surface, they adsorb to the surface, and provided the adhesion energy between the membrane and the support is high enough, they rupture and subsequently fuse to form a continuous bilayer or monolayer on hydrophilic or hydrophobic surfaces, respectively [13]. However, if the energetics are insufficient, then the surface-adhered vesicles remain intact [14]. The energetics of the system are controlled by the lipid composition, the material properties of the support [15], and the environmental conditions such as ionic strength [16], solution pH [17], and osmotic pressure [18]. The outcome is determined by a delicate balance between intermolecular interactions, i.e., van der Waals [19], electrostatic [20], and hydration forces [21]. Under physiological conditions, successful bilayer formation using vesicle fusion is mainly applicable to silicon-based materials such as silicon dioxide, glass, and mica. The solvent-assisted lipid bilayer (SALB) [22, 23] method is an alternative membrane formation technique that does not require lipid vesicles. The method involves lipid deposition onto a solid surface in an alcohol, e.g., isopropanol, followed by gradual replacement of the alcohol by an aqueous buffer to drive self-assembly of a lipid bilayer or monolayer on hydrophilic or hydrophobic surfaces, respectively. This self-assembly pathway sidesteps vesicle rupture, which is usually the preventive stage in the transformation of vesicles to a bilayer. The SALB method has been applied successfully on a wide variety of surfaces including SiO₂, Au, Al₂O₃, and hydrophobic alkanethiol-coated gold [22, 24].

In this work, we have investigated how the surface properties of graphene affect the self-assembly of phospholipid molecules on CVD-graphene, which is known to be typically hydrophobic [25], and compared the results with those obtained from oxygen-plasma treated graphene (denoted as O₂-graphene), which increases the surface hydrophilicity. Two fabrication strategies were chosen, the vesicle fusion and the SALB methods, and the corresponding membrane formation processes were tracked by quartz crystal microbalance with dissipation (QCM-D) monitoring. In addition, we probed the modulation of the electronic state of graphene by utilizing charged lipids, as determined by electrical measurements and Raman spectroscopy.

3.2 Materials and Methods

Lipid Preparation.

Zwitterionic lipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cationic lipid 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (chloride salt) (DOEPC), and anionic lipid 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DOPS) were purchased from Avanti Polar Lipids (Alabaster, AL). To prepare the lipid samples for vesicle preparation, chloroform solutions of lipids with desired composition were first dried using a gentle stream of nitrogen gas and subsequently stored under vacuum overnight to remove traces of chloroform. Vesicles were prepared in tris buffer (10 mM Tris, 150 mM NaCl, pH 7.5) via extrusion through 50 nm diameter, track-etched polycarbonate membranes as previously described [26]. For SALB experiments, DOPC lipid powder was first dissolved in isopropanol at 10 mg/mL lipid concentration. The stock was diluted to a 0.5 mg/mL lipid concentration before experiments.

Quartz Crystal Microbalance with Dissipation (QCM-D) Monitoring.

A Q-Sense E4 (Q-Sense AB, Gothenburg, Sweden) instrument was used to monitor the adsorption kinetics of lipids onto graphene-coated 5 MHz, AT-cut piezoelectric quartz crystals. The custom-made graphene-coated QCM-D crystal was obtained from Cheap Tubes Inc., Grafton, USA. Graphene was grown on copper using chemical vapor deposition (CVD) and transferred onto the SiO₂ coated QCM-D crystal (QSense AB). Changes in frequency (ΔF) and energy dissipation (ΔD) were recorded as functions of time, as previously described [27]. The measurement data was collected at the $n = 3-11$ overtones, and the reported values were recorded at the third overtone ($\Delta F_n = 3/3$). All samples were introduced at a flow rate of 50 $\mu\text{L}/\text{min}$ using a peristaltic pump (Ismatec Reglo Digital) under continuous flow conditions. Based on the volume of the QCM chamber, amount of required sample is about 200 μL . The temperature of the flow cell was fixed at 24.00 ± 0.5 °C.

Oxygen Plasma Treatment.

Before experiment, sensor surfaces were treated with oxygen plasma at 5 W for 30 s (March Plasmod Plasma Etcher, March Instruments, California) immediately before use.

Raman Spectroscopy.

A 488 nm excitation laser and a 100 $\times$ objective lens of a confocal spectrometer (alpha 300R, WiTEC, Germany) were used for Raman spectrum collection with a laser power of <1 mW to avoid sample damage.

Contact angle.

Water contact angle measurement was done at ambient laboratory temperatures using a Dataphysics OCA 15Pro instrument. The static water contact

angle was measured as the angle from the tangent to the horizontal of 6 μL sessile drops of deionized water.

Field-Effect Transistor Fabrication and Measurement.

Using a stainless-steel shadow mask of channel length 4000 μm and width of 2000 μm , chromium electrodes were first sputtered on the CVD graphene/ SiO_2 /Si substrate as an adhesion layer before the subsequent sputtering of gold on top of the chromium electrodes. The thickness of the resultant Cr/Au was about 10/100 nm serving as the source and drain. Polydimethylsiloxane (PDMS) (with 10 wt % curing agent) (Sylgard 184) was fabricated as the encapsulating fluidic chamber and assembled on top of the source and drain electrodes using precured PDMS (with 50 wt % curing agent) as an adhesion layer. After overnight drying in air at room temperature, the edges were reinforced to the substrates by air-cured epoxy glue. The PDMS fluid chamber (Figure 3.2) is designed with an inlet and outlet to allow the continuous injection of liquid sample via tubing connection and a peristaltic pump (ISM 833). The electrical performance of the FETs was determined by measuring the standard two-probe configuration of the top-gated device's static characteristics (drain-source current I_D versus gate voltage V_G at constant drain-source voltage V_D of 0.1 V). These characteristics were measured using the Keithley 4200 Semiconductor Characterization System with an Ag/AgCl gate electrode.

Figure 3.2. Photographs of polydimethylsiloxane (PDMS) microfluidic device with integrated electrodes (left) and the chamber in its assembled form (right).

3.3 Results and Discussion

3.3.1 Lipid monolayer formation on CVD graphene

We first investigated the lipid self-assembly on pristine CVD graphene. Figure 3.3a shows the Raman spectrum of a CVD graphene sheet that was first grown on Cu and then transferred onto the QCM-D crystal. The negligible defect-related D band at around 1350 cm^{-1} and the sharp symmetric 2D peak at 2678 cm^{-1} indicated the high crystallinity of the CVD-graphene film. In general, the Raman spectrum of a monolayer sheet of graphene will show a 2D band approximately 2–2.5 times more intense than the G band at around 1586 cm^{-1} [28]. The intensity ratio, I_{2D}/I_G , of <2 in our spectrum suggested that the CVD graphene on a QCM-D crystal was at least a two-layer graphene sheet, rather than a monolayer.

Figure 3.3. QCM-D analysis of lipid membrane formation on pristine CVD-graphene. **(a)** Representative Raman spectrum from CVD-graphene on a QCM-D crystal. Inset shows a photograph of a water droplet and the contact angle on CVD-graphene. **(b)** QCM-D frequency shift (ΔF , top panel) and dissipation shift (ΔD , bottom panel) for the third overtone were measured as a function of time during the formation of a lipid monolayer on a QCM-D crystal coated with CVD graphene. The arrows indicate the injection of (i) isopropanol, (ii) lipid mixture (0.5 mg/ml of DOPC in isopropanol), and (iii) Tris buffer (10mM Tris, 150mM NaCl, pH 7.5), producing a final ΔF and ΔD of $-12.5 \pm 1\text{ Hz}$ and $0.7 \pm 0.2 \times 10^{-6}$, respectively). **(c)** QCM-D responses during the adsorption of vesicles onto CVD-graphene. DOPC vesicles were added at $t = 5\text{ min}$ (i) after establishing a baseline for the frequency and dissipation shifts.

We next examined the hydrophobicity of the CVD-graphene by conducting water contact angle (WCA) measurements. As shown in the inset of Figure 3.3a, a water droplet placed on top of the pristine graphene gave a static contact angle of 72.2°. Our pristine graphene was therefore slightly less hydrophobic than other graphene samples previously studied, which were reported to have WCA in the range of 87–127° [29]. In our case, the lower value was most likely due to the adsorption of air moisture and oxygen. Figure 3.3b represents the time course of QCM-D frequency (ΔF , top panels) and dissipation (ΔD , bottom panels) shifts during SALB formation on CVD graphene. A typical SALB formation experiment involved baseline recording in the aqueous buffer solution, injection of the isopropanol (i), addition of lipid mixture in isopropanol (ii), and solvent exchange back into the initial buffer solution (iii). For CVD-graphene, the final frequency value after complete solvent replacement was -12.5 ± 1 Hz. It has previously been established that planar bilayer formation leads to changes in frequency of about -26 Hz [13]. Thus, the value obtained in our experiments corresponded to $\sim 50\%$ of the value expected for bilayer formation, indicative of a lipid monolayer deposition [13], as would be expected from the hydrophobic nature of CVD-graphene [25]. Similarly, for vesicle adsorption on CVD-graphene (Figure 3.3c), we observed a one-step monotonic kinetic behavior that resembled a monolayer lipid assembly with a final frequency shift of 13 ± 3 Hz. However, the relatively high standard deviation (3 Hz) suggested that the adlayer was not homogeneous. In addition, the dissipation value obtained upon vesicle fusion on CVD graphene ($1.3 \pm 0.5 \times 10^{-6}$) was relatively high.

3.3.2 Lipid bilayer formation on oxidized CVD graphene

Next, to investigate further the self-assembly of phospholipids, we treated the CVD-graphene surface with oxygen plasma (5 W for 30 s) and compared the results of two self-assembly processes: the SALB formation method and the VF method.

Upon treating the CVD graphene with oxygen plasma (denoted as O₂-graphene), there was a sharp decrease in the 2D peak intensity along with extensive peak broadening (Figure 3.4a). The appearance of the prominent D peak at approximately 1350 cm⁻¹ confirmed the oxidation of graphene to mainly graphene oxide, which typically has a much higher proportion of structural defects. When the sample was mildly treated with oxygen plasma, the contact angle of the water droplet decreased to 31° (see the inset of Figure 3.4a) on O₂-graphene, which corresponded well to the range of 30–60° reported in the literature [30]. The oxidation of graphene induces the formation of epoxides and hydroxyl groups on the basal plane and of carboxyl groups at the edges of the graphene sheets [30]. In particular, defective sites containing hydroxyl groups are mainly responsible for the hydrophilic nature of graphene oxide.

To follow the assembly of lipid bilayer, a typical QCM-D response was measured for the SALB formation on O₂-graphene as shown in Figure 3.4b. Once the SALB procedure was completed as previously described, the ΔF values were consistent with previous QCM-D responses for planar lipid bilayer formation (-27.5 ± 0.5 Hz), and the energy dissipation ($0.9 \pm 0.3 \times 10^{-6}$) was acceptable for a homogeneous and defect-free bilayer ($<0.5 \times 10^{-6}$). These results suggest that the SALB formation method is necessary and sufficient to form a planar lipid bilayer on an O₂-graphene surface. In marked contrast, the addition of vesicles onto the O₂-graphene caused the frequency to decrease and dissipation to increase monotonically until they reached the final values of -52 ± 8 and $3.8 \pm 1.5 \times 10^{-6}$ Hz respectively. When adsorbed vesicles remain intact on the surface forming a vesicular layer, a significant quantity of water is trapped inside as well as between the adsorbed vesicles, which is detected as a mass gain by the QCM-D frequency signal, and this additional hydrodynamically coupled solvent mass dissipates a large amount of energy. Therefore, the QCM-D measurement results indicate that adsorbed vesicles did not rupture and remained intact on O₂-graphene. Our results agreed well with a

related study by Okamoto et al. [31], which demonstrated that, vesicles remain intact on graphene oxide in the absence of Ca^{2+} ion. These data therefore indicate that O_2 -graphene can support a layer of intact vesicles.

Figure 3.4. QCM-D analysis of lipid membrane formation on oxygen plasma treated graphene. (a) Raman spectrum and illustration of the static water contact angle for O_2 -graphene. QCM-D curves for (b) SALB formation (arrows indicate injection of (i) isopropanol, (ii) lipid mixture, and (iii) buffer) and (c) vesicle fusion on O_2 -graphene producing a final ΔF values of $\sim 27.5 \pm 0.5$ and $\sim 52 \pm 8 \times 10^{-6}$ Hz, which correspond to a plane bilayer and an absorbed vesicle layer, respectively.

3.3.3 Electrolyte-gated supported lipid membrane FET

The electrical response of atomically thin CVD-graphene to charged molecules at the interface provides a sensitive modality for the detection of biomolecular binding. To characterize the electronic effect of charged lipids, we measured the electrical response of the lipid monolayer on a CVD-graphene sheet, fabricated as a three-terminal field-effect transistor (FET) (Figure 3.5a,b). Monolayers of different charges were prepared by the vesicle fusion method, and the drain–source current (I_{DS}) was measured as a function of gate bias (V_{G}). As shown in Figure 3.5c, the characteristic V-shaped curve, indicating the Dirac point of graphene, was retained when neutral and charged lipids were coated on the graphene. A noticeable difference was the shifting of the Dirac point upon lipid adsorption. The Dirac point shifted toward negative gate voltage when positively charged DOPC:DOEPC (4:1) monolayer was formed; conversely, it shifted toward positive

gate voltage when a positively charged DOPC:DOPS (4:1) was coated. The observed voltage shift due to the charged lipids corresponded well with the trend observed by Ang et al [11] and was a consequence of the charge-impurity potential generated by the charged membranes atop the CVD-graphene. The shift in the electrically neutral Dirac point was a direct manifestation of the modulations of the hole and electron concentrations in the graphene; these modulations were induced by the charged membranes via a field effect across the ultrathin water interlayer between the lipid membranes and the graphene. It should be noted that the Dirac point upon the formation of a neutral lipid membrane was slightly positive at +0.4 V because of the presence of negatively charged impurities trapped on the surface of the SiO_2/Si substrate. In addition, the magnitude of the Dirac point shift in the presence of negatively charged lipids was larger than that in the presence of positively charged lipids for the same concentration of net lipid charge. It is possible that the positively charged lipids induced a partial nullification of the impurity potential generated by the underlying negatively charged surface impurities on the SiO_2/Si substrate.

Figure 3.5. Electrical measurement of lipid-coated graphene. **(a)** Schematics of a lipid-coated graphene in liquid-gated FET device. **(b)** Photograph of polydimethylsiloxane (PDMS) microfluidic device with integrated electrodes. **(c)** IV curves for three-terminal field effect transistor (FET) graphene coated with zwitterionic DOPC (rectangles), positively charged DOPC:DOEPC (20%) (triangles), and negatively charged DOPC:DOPS (20%) (circles) monolayers formed by vesicle fusion.

3.3.4 Raman spectroscopy of lipid membrane supported graphene

We further measured the influence of lipid membrane formation on graphene using Raman spectroscopy. Figure 3.6a shows a representative Raman spectrum for DOPC bilayer on silicon dioxide. Consistent with previous reports [32], an intense C–H stretching band appeared at 2871 cm^{-1} even though the symmetric and asymmetric components of C–H stretching were not resolved. On CVD-graphene, prominent peaks corresponding to the different molecular vibration modes of C–C and C–H bonds in the neutral DOPC monolayer were clearly identified in comparison to the characteristic G and 2D peaks of graphene, as shown in Figure 3.6b.

Interestingly, the symmetric and asymmetric components of C–H stretching were resolved on graphene. Raman spectroscopy was repeated on graphene coated with positively charged DOPC:DOEPC (4:1) and negatively charged DOPC:DOPS (4:1) monolayers. Upon closer examination of the 2D peaks in Figure 3.6c, a shift in the wavenumber was detected for the charged lipids. Although positively charged lipids induced an upward shift, i.e., a higher wavenumber for the 2D peaks, negatively charged lipids displayed a downward shift.

Figure 3.6. Raman spectroscopy analysis of lipid membrane formation on graphene. Raman spectra with band assignments for a DOPC bilayer on (a) SiO₂ and (b) CVD-graphene. (c) Raman spectra of CVD graphene coated with lipid monolayer of different charge, showing the shift of the 2D peak.

The Raman shift could be explained as the n and p doping effects of the negatively and positively charged lipids, respectively, on graphene [12]. The Raman results supported the charge-impurity potential effect of the charged lipids, which was demonstrated in the Dirac point shift in the $I_{DS}-V_G$ measurement shown in Figure 3.5c. Interestingly, the magnitude of the Raman peak shift was smaller for the positively charged lipids than it was for the negatively charged lipids. The unbalanced shift magnitude again reflected the same observation from the $I_{DS}-V_G$ curves, which can be attributed to the presence of surface charges from impurities on the SiO_2/Si substrate. Das et al [33] performed in situ Raman measurements on a graphene transistor, and on the basis of density function theory (DFT) and experimental measurements, they correlated the position of the 2D peak to the doping level. Using their calibration (see Figure 3 of ref [33] for the position of the 2D peak as a function of doping), the Raman shift in our measurement (2688 cm^{-1} for DOPC:DOPS) corresponded to a change in the carrier density of $\sim 3 \times 10^{13}\text{ cm}^{-2}$. Considering that each phospholipid occupies a 0.68 nm^2 surface area [34] and noting that 20% of the surface groups are charged, the estimated value of this impurity charge density was $\sim 2.86 \times 10^{13}\text{ cm}^{-2}$, which was in good agreement with the calculated charge density. These results also further supported the formation of a lipid monolayer, rather than a bilayer, on the hydrophobic graphene. Taken together, both vesicle fusion and SALB result in formation of a lipid monolayer on the hydrophobic pristine CVD-graphene. On the hydrophilic oxygen-plasma-treated graphene (O_2 -graphene), SALB and vesicle fusion resulted in formation of a bilayer and intact vesicle layer, respectively.

3.4 Conclusions

QCM-D was for the first time used to monitor lipid self-assembly on a graphene surface. The characterization of lipid attachment on graphene demonstrates that depending on the preparation condition CVD-graphene can support the formation of a lipid monolayer, a lipid bilayer, or intact vesicles. Such capabilities provide a sensitive bioelectronic interface that is also biocompatible and antifouling. In addition, the significant modulation of the electronic properties of graphene by charged lipid membranes indicates the huge potential of using a lipid-graphene interface as an ultrasensitive platform for sensing membrane-related processes, using Raman spectroscopy and electronic measurements.

3.5 References

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Chapter 4: Towards large area ultrathin graphene oxide coating by solid-liquid phase interfacial exfoliation

ABSTRACT

Surface corrugations and coating inhomogeneities of monolayered and few layered graphene oxide have long been the challenging obstacles for many researchers who capitalized on the solution processibility of graphene oxide. Such structural defects which occurred in the form of wrinkles, ripples and foldings on various functional substrates have an atomic-level impact on the electronic properties of the 2D material and hence are detrimental to the physical and electrical properties of the 2D material. The maximum performance of graphene oxide based devices is often compromised. There is a myriad of contributing factors such as thermal contraction, solvent entrapment, uneven surface anchorage which are difficult under the constraints of wet processing method. Herein, we have identified experimentally a unique bonding interaction between titanium atom on the substrates and carbon atom on graphene oxide which acts as a strong surface anchorage in achieving uniform adhesion and hence near to ideal surface coverage on the titanium bearing substrate. The same silicon-carbon interaction was not detected under the same experimental condition on silicon oxide substrate. As a consequence, while titanium oxide surface was fully coated with graphene oxide, the surface coverage on silicon surface was far from ideal. Following the strong adhesion of the first layer of graphene oxide on the titanium bearing substrates, subsequent intercalation of appropriate solvent molecules by repeated rinsing and washing sequences via our postulated solid-liquid phase exfoliation of upper layers of graphene oxide which are weakly bonded by van der Waals forces to the first layer of anchored graphene oxide could be achieved with

1 to 2 monolayers. The exfoliation efficacy and kinetics have been experimentally by Raman surface imaging and quartz crystal mass monitoring. The technique of applying a titanium adhesion layer followed by in-situ solid-liquid exfoliation is applicable to a wide variety of functional substrates and serves to solve the long-standing issue of surface corrugations and coating inhomogeneities of wet-processed graphene oxide coating.

4.1 Introduction

Atomically thin two-dimensional materials such as graphene offer a wide spectrum of unique material properties that will revolutionize many applications. The presence of epoxy, carboxyl and hydroxyl groups on graphene oxide (GO) [1, 2] distinguishes hydrophilic GO from hydrophobic graphene in two ways. Firstly, solution coating of graphene-based thin film became feasible using GO dispersion for economical large area coating. Secondly, functional groups on GO can conveniently act as effective sites for immobilization of molecules via covalent conjugation [3] for improved sensitivity of biosensor[4]. There are a variety of solution coating methods reported to date, such as spray-coating [4-6], drop-casting [7] and Langmuir-Blodgett (LB) [8, 9] assembly. However, due to the atomic thickness of GO and poor adhesion with the substrates, wrinkles and folds [10] were easily formed which are detrimental for device performance. Since GO dispersion is composed of nano- or micron sized GO flakes suspended in solution, very often a flaky morphology with a high degree of overlapping flakes with intermittent uncoated regions are often reported [10].

Figure 4.1. AFM images (5 x 5 μm) of chemically RGO (a) without and (b) with SDBS. Insets show digital photographs of the corresponding samples in an aqueous 1 mg/mL solution.

Figure 4.2. (a) AFM image of exfoliated GO sheets spin-coated on silicon, z-range 10 nm. (b) Zoom-in of the sheets, z-range 8 nm. Labels show the number of layers in different areas. (c) Height profile taken along the white line in a. (d) Height histogram performed on b. The peak numbers correspond to the number of layers present in the area.

Self-assembled monolayers such as (3-aminopropyl) triethoxysilane (APTES) [11, 12], 2-aminoethanethiol (AET) [13], hexanethyldisilazane (HMDS) [14] and quaterthiophene molecules (T4) [15] functionalization on silicon dioxide (SiO_2) and other functional substrates have been reported to improve surface adhesion of spin-coated GO film by promoting electrostatic attraction. However there is still a major portion of uncoated surface as characterized by flaky surface morphology (Figure 4.2). Thermal vibrations, edge instabilities, thermodynamically unstable (interatomic) interactions, strain in 2D crystals, thermal contraction, dislocations, solvent trapping, pre-strained substrate-relaxation, surface anchorage and high solvent surface tension during wet processing have been discussed to be the contributing factors for the formation of wrinkles or ripples of graphene or graphene oxide on various substrates [16]. Theoretical calculation using the classical Kirchhoff-Love plate theory has also been actively performed to study the buckling of monolayer graphene on substrates [17]. All the above studies are indicative that a stronger nature of interaction between the substrate and GO film is necessary for uniform and wrinkle-free coating. It has been recently reported residual oxygen atoms on chemical vapor deposited (CVD) monolayer graphene is able to form covalent Ti-O bonds with titanium adhesion layer [18] on various functional substrates. Consequently, the titanium adhesion layer promoted wrinkle-free graphene monolayer. Overcoming the issue of non-homogeneity in coated GO film by enhancing the adhesion energy, in our opinion, should be the choice of approach to reduce wrinkling and promote atomically smooth surface coverage. Given the abundance of oxygen atoms in GO, we envision strong covalent interaction between GO flakes and titanium (Ti) -bearing substrates.

4.2 Materials and Methods

Materials

Highly doped p-type (boron) 4-inch silicon substrates (500 ± 25 μm thickness) with a low resistivity of 0.001-0.005 $\Omega\cdot\text{cm}$ and a thermally oxidized silicon dioxide (SiO_2) (300nm thickness) were purchased from Silicon Prime Wafers. Titanium oxide (TiO_2) with a thickness of 100nm on glass slides were purchased from Deposition Research Lab Inc.

Chemicals

Acetone, isopropyl alcohol (IPA), chloroform were purchased from VWR chemicals. Hydrazine monohydrate were purchased from Sigma-Aldrich. All reagents were of analytical grade and used without further purification. Ultrapure deionized water with an electric resistance of 18.3 $\text{M}\Omega$ was used for all solution preparations.

Graphene Oxide Coating

All substrates were ultrasonically cleaned by acetone, isopropyl alcohol (IPA) and Milli-Q water in sequence for 1min each before subjecting to 1min of oxygen plasma treatment (Harrick Plasma, PDC-002, Ithaca, NY) to generate a hydrophilic surface. Aqueous suspension of graphene oxide of 1.0 mg/ml diluted from a raw concentration of 4.0 mg/ml was sonicated for about 10min before spin-coating at a speed of 6000 rpm x 1 min on the substrates using a spin-coater (WS-650MZ-23NPP, Clarion Safety System). Chemical reduction was subsequently carried out to partially convert graphene oxide to graphene generally termed as reduced graphene oxide. This was achieved by enclosing the samples within a saturated hydrazine monohydrate vapor environment at a temperature of 90°C in an oven over 12 hrs.

Atomic Force Microscopy (AFM)

An atomic force microscopy (MultiMode 8-HR, Bruker, USA), combined with an optical microscope was used for tapping mode AFM imaging on graphene oxide coated substrates. Model SCANASYST-AI (Bruker, USA) was used as the AFM tip in all measurement at room temperature of 25°C. Each sample was scanned at five different locations to produce five images each of 5 μm by 5 μm and 10 μm by 10 μm imaged area. Area quantification on these AFM images was subsequently carried out statistically using ImageJ to quantify the percentage of surface coverage.

Raman Spectroscopy

A 488nm excitation laser and a 50x and 100x objective lens of a confocal spectrometer (alpha 300R, WiTEC, Germany) were used for Raman spectrum collection and 2D mapping respectively. Over a 40 μm by 40 μm area, 2D mapping was scanned at 100 points per line horizontally and vertically (spectral resolution of 3cm^{-1}) with a laser power well $<1\text{mW}$ to avoid laser heating of the sample. Unpolarized Raman spectra were recorded at room temperature on reduced GO thin films deposited on 300-nm-thick SiO_2 and 100-nm-thick TiO_2 . The laser spot size was estimated to be less than 3 μm . According to recent studies, the peak position of the second-order Raman D-peak, $f(2D)$, considerably blueshifts with increasing number of layers in graphene. Therefore, we were able to reconstruct the number of layers in our samples by mapping $f(2D)$ in our films.

Quartz-Crystal Microbalance-Dissipation (QCM-D)

A Q-Sense E4 instrument (Q-Sense AB, Gothenburg, Sweden) was used to monitor the lipid deposition process in real time. Changes in the resonance frequency

and energy dissipation of a 5 MHz AT-cut piezoelectric quartz crystal were recorded at the 3rd, 5th, 7th, 9th and 11th overtones. The QCM-D SiO₂ coated substrates are composed of quartz crystals coated with the following layers and corresponding thicknesses in sequential order: 10nm Cr, 100nm Au, 10nm Ti, and last 50nm SiO₂. Layers were coated by using the physical vapor deposition (PVD) method. The QCM-D Au substrates are composed of quartz crystals coated with the following layers and corresponding thicknesses in sequential order: 10nm Cr and then 100nm Au. The layers were also coated by PVD method. All measurements were done under flow-through conditions at a flow rate of 50 μ L/min by using a Reglo Digital peristaltic pump (Ismatec, Glattbrugg, Switzerland) (which could be seen in Fig. 1). All surfaces were treated with oxygen plasma at 180 W or 1 min (March Plasmod Plasma Etcher, March Instruments, Concord, CA) immediately before use.

X-Ray Photon Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) analysis was performed using PHI Quantera II spectrometer (Physical Electronics Inc., Minneapolis, USA) using mono Al-K α radiation (1486.6 eV, 15 kV) with reference to C 1s line at 284.5 eV. An Ar⁺ ion beam sputtering at 2 keV was used to investigate the depth profile of the sample. The sputtering rate was calibrated based on SiO₂. The total pressure in the main vacuum chamber during analysis was typically 10⁻⁹ torr. To obtain detailed information about chemical structure, oxidation states, etc., high resolution spectra were recorded from individual peaks at 20 eV pass energy. Each specimen was analyzed at an emission angle of 0° as measured from the surface normal. Assuming typical values for the electron attenuation length of relevant photoelectrons, the XPS analysis depth (from which 95% of the detected signal originates) ranges between 5 and 10 nm for a flat surface. Since the actual emission angle is ill defined in the case of rough surface (ranging from 0° to 90°), the sampling depth may range from 0 to

≈ 10 nm. Binding energies were referenced to the C 1s peak at 284.7 eV (aromatic hydrocarbon) or 284.4 eV (graphitic carbon).

High Resolution Transmission Electron Microscopy (HRTEM)

Analysis was carried out using a FEI Tecnai F20 field emission TEM operated between 80 kV and 200 kV as required, with a tip extraction voltage of 4.5 kV and with an attached Gatan Orius SC600A CCD camera. Gatan DigitalMicrograph software was used for image capture and analysis. Electron fluence rates were calculated using conversion formulae provided by FEI and were dependent on magnification, beam spread, camera emulsion setting and fluorescent plate exposure time. Magnification (2,500 times) and beam spread (0.08 m diameter at the viewing screen) were kept constant, with changes to spot size being used to alter the electron fluence rate. This was kept in a typical range of ~ 10 to $\sim 20 \text{ e}^- \text{nm}^{-2} \text{s}^{-1}$.

Standard conditions used were: 200 kV accelerating voltage at an ambient temperature with the sample on a special TEM copper grid pre-coated with a thin layer of TiO_2 was commercially purchased (Substratek™ Ted Pella Inc) and the same fabrication process was repeated on the TEM copper grid. Variations on these standard conditions were investigated which included using 80 kV accelerating voltage, cooling the sample to -190°C .

Electrical Measurement

Using a stainless steel shadow mask of channel length 100 μm and width of 2000 μm , chromium electrodes were first sputtered on the RGO as an adhesion layer before the subsequent sputtering of gold on top of the chromium electrodes. The thickness of the resulted Cr/Au was about 10/100 nm serving as the source and drain.

PDMS (with 10wt% curing agent) was fabricated as the encapsulating fluidic chamber and assembled on top of the source and drain electrodes using pre-cured PDMS (with 50 wt% curing agent) as an adhesion layer. After overnight drying in air at room temperature, the edges were reinforced to the substrates by air-cured epoxy glue. The PDMS fluid chamber is designed with an inlet and outlet to allow the continuous injection of liquid sample via tubing connection and a peristaltic pump (ISM 833). The electrical performance of the FETs was determined by measuring the standard two-probe configuration of top-gated device's static characteristics (drain-source current I_D versus gate voltage V_G at constant drain-source voltage V_D of 1 V, or drain-source current I_D versus drain-source voltage V_D at constant gate voltage V_G of 0V). These characteristics were measured using the Keithley 4200 Semiconductor Characterization System with an Ag/AgCl gate electrode.

4.3 Results and Discussion

4.3.1 In-situ solid-liquid phase exfoliation of GO on TiO₂

We describe herein a simple and scalable solution coating of uniform GO film and reduced graphene oxide (RGO) on titanium oxide (TiO₂) substrates and compared with SiO₂. Aqueous GO solution of 1.0 mg/ml concentration containing GO multilayered flakes was spin-coated on cleaned substrates schematically represented in Figure 4.3. During spin-coating (see Methods for details), GO flakes were lodged onto the surface of the substrates while the suspending solvent were spin-casted away or evaporated from the surface. A chemical reduction using hydrazine in the vapor phase subsequently removed most of the oxygen-containing functional groups yielding a conductive reduced GO film. For applications in which the conductive form of GO is not required, the reduction step could be omitted hence

the versatility of this process. Spin-coating typically produced multiple layers of GO coating [19, 20] in which each graphite planes are held together by Van der Waals forces. These weaker form of interaction between each layer could be disrupted by external stimulus such as shear forces in the liquid phase [21, 22]. If the first monolayer of GO could be covalently bonded to the Ti-bearing substrate, the upper layers could be exfoliated at the solid-liquid interface. Hence, cycles of rinsing were carried out using various solvents to achieve exfoliation of the multi-layered GO flakes to achieve an ultrathin covalently bonded GO on the substrate.

Figure 4.3. Formation of exfoliated graphene oxide (GO) on metal oxide substrates by spin-coating, hydrazine-induced chemical reduction of GO to reduced graphene oxide (RGO), followed by liquid-solid phase in-situ exfoliation on the substrate surfaces.

4.3.2 Surface morphology and exfoliation kinetics of GO on TiO₂

Atomic force microscopy (AFM) of the reduced GO films reviewed a contrasting surface morphology between SiO₂ (Figure 4.4c) and TiO₂ (Figure 4.4d). There are large regions on the SiO₂ substrates where there was no coverage of GO sheets. The interaction between SiO₂ and GO sheets is probably not sufficiently

strong to induce full coverage of the substrate surface during the spin-coating. A very different morphology was observed on the TiO_2 surface on which there were no observable flakes and no step profile was detected as shown in Fig. 4.4. Wrinkle-free surface morphology was observed over a large area of $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$ (Figure 4.4d).

Figure 4.4. (a) Schematics of AFM imaging using the tapping mode. (b) Schematics of Raman spectroscopy using laser wavelength of 488nm. (c) AFM images of RGO on SiO_2 (scanning area is $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$). (d) AFM images of RGO on TiO_2 (scanning area is $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$). (e) Raman mapping of RGO on SiO_2 (scale bar is $4\text{ }\mu\text{m}$). A flaky and unevenly RGO coated SiO_2 is contrasted against the uniformly coated TiO_2 . (f) Raman mapping of RGO on TiO_2 (scale bar is $4\text{ }\mu\text{m}$). The dark region corresponds to the uncoated SiO_2 and TiO_2 substrate since there are no D and G Raman peaks arising from RGO. Regions of high raman intensity indicate a thicker RGO film. The unevenly coated RGO on SiO_2 hence show up as a region of uneven high and low intensity distribution. (g) Raman spectra of RGO on SiO_2 and TiO_2 . (h) Raman spectra of thick (before exfoliation) and thin RGO (after exfoliation) on TiO_2 . Defect-related D peak at $\sim 1350\text{ cm}^{-1}$ and graphitic G peak at $\sim 1600\text{ cm}^{-1}$ were distinctly observed for both RGO samples. The broad and diffused 2D peak at ~ 2740

cm^{-1} of lower intensity is indicative of a multi-layered film and it superimposed with another peak at $\sim 2930\text{cm}^{-1}$ which represents C-H bonds in the structure because of the defect bands in the GO layers. Defects in the context of RGO refer to the presence of epoxides and hydroxyl groups on the basal plane and carboxyl groups at the edges. The defective sites especially the hydroxyl groups are responsible for the hydrophilic nature of graphene oxide in contrast to the hydrophobic properties of perfect graphene.

Single Raman spectrum of reduced GO on SiO_2 and TiO_2 was shown in Figure 4.4g. Defect-related D peak at $\sim 1350\text{cm}^{-1}$ and graphitic G peak at $\sim 1600\text{cm}^{-1}$ were distinctly observed for both reduced GO samples on both substrates. The broad and diffused 2D peak at $\sim 2740\text{cm}^{-1}$ of lower intensity is indicative of a multi-layered film [23] and it superimposed with another peak at $\sim 2930\text{cm}^{-1}$ which represents C-H bonds in the structure because of the defect bands in the GO layers. Defects in the context of reduced GO refer to the presence of epoxides and hydroxyl groups on the basal plane and carboxyl groups at the edges. The defective sites especially the hydroxyl groups are responsible for the hydrophilic nature of graphene oxide in contrast to the hydrophobic properties of perfect graphene.

Overlapping flakes of reduced GO were observed on SiO_2 surface with a step height profile of $\sim 2\text{-}3\text{ nm}$ as shown in Figure 4.5a. While pure graphene flake has a theoretical thickness of 0.34 nm , GO and reduced GO sheets are thicker with a reported thickness of about $1\text{-}1.4\text{ nm}$ [3] due to the presence of functional groups, structural defects and absorbed water molecules [24, 25]. Hence the AFM step profile on SiO_2 corresponds to 2-3 layers of random stacking of GO sheets. While root-mean-square (rms) roughness measurement on SiO_2 in Figure 4.5b indicated 0.571 nm over a $10 \times 10\text{ }\mu\text{m}$ area and 0.534 nm for a $3 \times 3\text{ }\mu\text{m}$ region, comparatively rms roughness on TiO_2 was down to 0.258 nm over a $10 \times 10\text{ }\mu\text{m}$ region and 0.289 nm for $3 \times 3\text{ }\mu\text{m}$ region.

Figure 4.5. AFM images of exfoliated RGO on (a) SiO₂ and (b) TiO₂ and multiple line scans for each sample. Based on AFM line profile, the remaining RGO on TiO₂ corresponded to 1 or 2 monolayers.

Figure 4.6. 2D raman mapping of RGO on (a) SiO_2 and (b) TiO_2 are monitored stepwise after sequential liquid-solid phase in-situ exfoliation of RGO on the coated surfaces. The raman mapping images are converted into contour plots using Matlab to capture the shift in the Raman D and G peaks which correlated to the thickness ie. number of layers of RGO. The shade of color corresponds to the range of RGO layers as indicated in the legend. The line bar represents 10 μm for the images.

A 2D Raman mapping was carried out on both surfaces as shown in Figure 4.6a(i) and 4.6b(i) showing the evolution of the original surface intensity upon

rinsing with buffer, ethanol, IPA and milliQ water followed by 2 cycles of ethanol and milliQ water, in each case. High Raman intensity of the original reduced GO/TiO₂ was contrasted against the low intensity after rinsing the samples as shown in Figure 4.4h. Since the characteristics D and G peaks are preserved, the reduced signals indicated a much thinner film.

Raman mapping of original surfaces on SiO₂ in Figure. 4.6a(i) indicated non-uniform coating with scattered dark regions while TiO₂ in Figure 4.6b(i) showed a much more uniform surface coverage over a 40 x 40 μm area. While SiO₂ presented a typical flaky coated morphology in correspondence with AFM images, surface uniformity on TiO₂ was much smoother. In general, the reduced GO intensity is higher on TiO₂ suggesting a much thicker film even though both surfaces were coated under the same process parameters. This effect could have been related to the extensive substrate effect of the TiO₂ arising from the higher polarity of Ti-O bonds. Ti has a lower electronegativity than Si and the high electronegativity of oxygen could have induced a more much more polarized covalent bond state in the Ti-O as compared to Si-O. An analysis based on the dielectric constant of the different metal oxides concluded in the same outcome that substrates with a higher dielectric constant such as TiO₂ over SiO₂ have a greater adhesion force for graphene-based materials as reported by other authors [26]. The higher affinity for GO on TiO₂ facilitated the uniform coating during the spin coating by ensuring all GO flakes were lodged on all uncovered TiO₂ surfaces. As for SiO₂, it is obvious that some GO flakes slide pass the surface during spin coating without getting lodged resulting in uncoated patches. Since the starting raw material consists of mostly single layer, the upper layers of GO flakes physisorbed on top of each other under the effect of the substrate which extends beyond the first layer. Under extensive hydrodynamic flow upon rinsing, the top few layers of GO were “exfoliated” leaving behind the strongly attached first or two layers. As a matter of fact, even if the top layers were attached by Van der Waals forces, shear-induced interlayer sliding in a solvent has been

shown to cause exfoliation above a minimum shear rate [21]. The end result of the shear induced exfoliation at the solid-liquid interface is shown in Figure 4.6a(i) and Figure 4.6b(i) in which the Raman mapping showed a much thinner coating with much reduced intensity. For SiO_2 with decreased affinity for GO, high shear rate might actually dislodge the originally coated GO from the surface of SiO_2 . As evidenced in Figure 4.6a(i), we observed an increased portion of uncoated surfaces corresponding to dark patches without Raman signals on SiO_2 .

In contrast to pristine graphene, GO and reduced GO do not have a well-defined 2D peak upon which layer determination could be carried out easily based on peak shift [27]. Using a combined intensity of D and G peaks from the 2D Raman mapping we map the number of reduced GO layers in the film over a $40 \times 40 \text{ }\mu\text{m}$ spatial region. Figure 4.6a(ii) and 4.6b(ii) show the corresponding contour map in terms of the number of layers generated from 2D Raman mapping in Figure 4.6a(i) and 4.6b(i). At the end of the final cycle of rinsing, a thinner surface coating of 1 – 2 layers was revealed indicating that most of the upper layers of the stacked GO sheets were being exfoliated since they were not as strongly bounded to the substrate as the first layer of GO sheet which was bounded covalently to TiO_2 . The liquid phase exfoliation of the top-layered GO sheets allows for reasonably good nanoscale control over the film thickness and surface coverage.

In order to quantify surface coverage of the GO coating on the substrates, freeware ImageJ [28] was used to calculate the percentage coverage of surface area based on five samples for each of the SiO_2 and TiO_2 substrates and over three different AFM image size of $5\mu\text{m}$ by $5\mu\text{m}$, $10\mu\text{m}$ by $10\mu\text{m}$ and $20\mu\text{m}$ by $20\mu\text{m}$. The average results were shown and plotted in Figure 4.7. For all three image sizes, surface coverage on TiO_2 was almost 80% was consistently higher than that on SiO_2 which could be as low as only 40%. It was noted that the 80% of surface coverage of exfoliated GO on TiO_2 seems lower as compared to observation made via Raman

mapping as shown in Figure 4.6 probably due to the higher resolution of AFM as compared to Raman spectroscopy of which the laser spot size was $\sim 3\mu\text{m}$ in diameter.

(c) 5um x 5um AFM image

(d) 10um x 10um AFM image

(e) 20um x 20um AFM image

Figure 4.7. Examples of 5um by 5um of AFM images for RGO on (a) SiO₂ before and after exfoliation; and on (b) TiO₂ before and after exfoliation. Statistical analysis of surface coverage of RGO on SiO₂ and TiO₂ each before and after exfoliation for an area of (c) 5um by 5um; (d) 10um by 10um; e. 20um by 20um. Area quantification is performed using Image J [28].

To support the hypothesis of solid-liquid phase exfoliation of graphene oxide on TiO_2 by multiple solvents, we monitored the real-time kinetics of the rinsing process using quartz-crystal microbalance-dissipation (QCM-D) as shown in Figure 4.8. A few hundred hertz of frequency increase was recorded during the sequential rinsing of GO on TiO_2 as monitor in Figure 4.8(a) corresponding to the exfoliation and dislodging of the top GO layers especially in ethanol while no changes was observed on SiO_2 in Figure 4.8(c) since the coverage was much lower on SiO_2 and the coated GO was much thinner.

Figure 4.8. Solid-liquid phase exfoliation of RGO in terms of (a) frequency shift and (b) dissipation on TiO_2 . Solid-liquid phase exfoliation of RGO in terms of (c) frequency shift and (d) dissipation on SiO_2 . RGO coated on TiO_2/QCM and SiO_2/QCM sensors are exfoliated sequentially in milliQ water, buffer, ethanol, IPA and finally milliQ water.

4.3.3 Atomic interaction between GO and TiO₂ substrate

We performed XPS to further elucidate the interfacial science between reduced GO and the substrates. The high-resolution C 1s XPS spectrum of reduced GO on TiO₂ in Figure 4.9b reveals that the interfacial interaction between reduced GO and TiO₂ is more than Van der Waals interaction. The C 1s XPS spectrum indicates the presence of three types of carbon bonds at 284.9, 286.4, and 288.8 eV that can be assigned to a C 1s orbital of C-C, C-O, and O-C=O, respectively [29]. The C-C peak is the most prominent arising the main frame carbon in the benzene rings while C-O and O-C=O are due to the remaining unreduced hydroxyl and epoxide groups on GO. Notably, an additional peak at 284.1 eV is observed from the spectrum of reduced GO on TiO₂ corresponding to the presence of Ti-C bond which has been reported similarly in the case of TiO₂-graphene nanocomposites [29]. Fitting of the Ti 2p XPS indicated the Ti-C bonding state to be specifically related to Ti-C 2p₁ and Ti-C 2p₃ (Figure 4.9c). In comparison with the XPS spectrum of reduced GO on SiO₂, no carbide interaction was detected except peaks corresponding to C-C, C-O and O-C=O as shown in Figure 4.9a. The C-Ti bond formation probably involves a charge transfer due to the highly polarized Ti-O via the hydroxyl and epoxide groups on reduced GO. Simulated calculation studies based on density function theory (DFT) clearly indicated the feasibility of Ti-C bond formation by examining the electron density differences at the interface of the thin film [29]. Prevalent peaks corresponding to TiO₂ 2p₁ and 2p₃ bonds are present in the Ti XPS fitting for reduced GO on TiO₂. Hydroxyl and epoxides in RGO serve as strong anchoring sites for the binding between and TiO₂ [30] since Ti atoms on the surface are under-coordinated and preferentially bonded to the epoxide O atoms from reduced GO. Based on calculation of electron density changes before and after the formation of the interface, there is very little change for the case of pristine graphene with TiO₂ indicating no true chemical bonding is taking place. This is in direct

contrast to significant electron density shift in the presence of epoxides on reduced GO. This presents an important consideration for the reduced GO-TiO₂ heterostructures and correspond very well with our experimental observation.

Figure 4.9. X-ray Photon Spectroscopy (XPS) spectra of RGO on (a) TiO₂ and (b) SiO₂, each in terms of carbon 1s orbital and Ti 2p orbital. (c) Fitting of the Ti 2p on TiO₂ substrate.

Scanning electron microscopy (SEM) on exfoliated GO on TiO₂ was used to investigate the microstructure and morphology as shown in Figure 4.10. A bilayer GO was clearly observed at the edge of the fractured sample (Figure 4.10b) while an atomically smooth morphological feature (Figure 4.10a) was observed on the basal

plane of the substrate. In order to investigate the lattice structure of GO on TiO_2 substrate by transmission electron microscopy (TEM), a special TEM copper grid pre-coated with a thin layer of TiO_2 was commercially purchased and the same fabrication process was repeated on the TEM copper grid. HR-TEM images in Figure 4.10e – g indicated the presence of lattice fringes of graphene of which interplanar distance d_{002} for RGO material has been reported to be $\sim 3.34 \text{ \AA}$ [31].

Figure 4.10. (a) SEM of RGO on TiO_2 . Line bar represents 1 μm . The middle boundary represents the fractured edge of the TiO_2 substrate. (b) SEM of single and double monolayered RGO at the edge of fractured TiO_2 substrate. The line bar represents 1 μm . (c) Optical images of RGO coated on TiO_2 thin film on TEM grids. Line bar represents 50 μm in the top image and 10 μm in the middle and bottom images (d). Schematics of side-view of the TiO_2 of 10-20 nm thickness deposited on copper TEM grids which is commercially purchased. RGO is spin-coated on the TiO_2 thin film on the TEM grid and exfoliated as per normal procedure. (e) HRTEM of RGO on TiO_2 . Line bar represents 50 nm. (f) and (g) HRTEM of RGO on TiO_2 . Line bar represents 10 nm.

4.4 Conclusion

For the first time we have identified experimentally a unique bonding interaction between titanium and carbon atom on graphene oxide which acts as a strong surface anchorage in facilitating uniform adhesion and near to ideal surface coverage of spin coated graphene oxide on titanium bearing substrate. The same silicon-carbon interaction was not detected under the same experimental condition on silicon oxide substrate. Following the strong adhesion of the first layer of graphene oxide on the titanium bearing substrates, subsequent intercalation of appropriate solvent molecules by repeated rinsing and washing sequences via solid-liquid phase exfoliation of upper layers of graphene oxide which are weakly bonded by van der waals forces to the first layer of anchored graphene oxide, large area uniform coating could be achieved within 1 to 2 monolayers. The exfoliation efficacy and kinetics have been experimentally by Raman surface imaging and quartz crystal mass monitoring. The technique of applying a titanium adhesion layer followed by in-situ solid-liquid exfoliation is applicable to a wide variety of functional substrates and serves to solve the long-standing issue of surface corrugations and coating inhomogeneities of wet-processed graphene oxide coating.

4.5 References

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Chapter 5: Supported lipid membranes on solution-processed reduced graphene oxide for biosensor application

Abstract

Planar lipid bilayers on solid supports mimic the fundamental structure of biological membranes and can be investigated using a variety of surface sensitive techniques. A biomimetic membrane bioelectronic transistor of a supported phospholipid bilayer atop reduced graphene oxide on titanium dioxide (TiO_2) has been successfully demonstrated for biosensing application. The liquid gated reduced graphene oxide (RGO) transistor is sensitive to environmental changes in ionic strength of liquid environment by shift in electrical output signals at a sensitivity level of $\sim 0.030 \text{ mA}$ per millimolar at gate bias $V_G = -8 \text{ V}$ and 0.035 mA per millimolar at drain bias $V_D = 1 \text{ V}$. Incorporation of a phospholipid membrane atop the RGO transformed the sensing transistor to a bioelectronic transistor capable of sensing attached biomolecules on functionalized lipid/RGO surface acting as pseudo gate bias which modulated the electrical signals output. Supported neutral and charged lipid membranes on RGO at active source-drain region were formed by solvent assisted lipid bilayer (SALB) formation. A modulation of transistor conductance induced by gate charges were observed by the measurement of transistor drain current. Based on the 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)/RGO biotransistor platform, a functional supported lipid bilayer (SLB) was demonstrated using biotin-streptavidin binding event. The quantification of biotin composition with respect to transistor drain current modulation was monitored which showed a linear relationship between the percentage of changes in drain current versus the percentage of biotin composition

in the complex SLB. For instance, a 12% change in drain current was measured for a 1wt % biotinylated DOPC lipid complex membrane upon streptavidin binding. The results illustrated the potential of using lipid bilayer as an efficient biofunctionalization tool on GO as a highly versatile and sensitive biosensing platform for a variety of receptor-ligand binding events.

5.1 Introduction

Tapping onto the development of the electronics industry, there is a huge surge in bioelectronics-based diagnostics in recent years based on the electrical modulation of biological events. With the advancement of peripheral technologies in the field of materials science and electronics, there had been much research in the label-free detection of biological agents including cytokine [1], antibodies [2], protozoan [3], viruses [4] and bacteria [5] using voltammetric, amperometric and potentiometric methods. The nanoelectric features of such platforms offers unmatched advantages in the development of lab-on-a-chip point-of-care (POC) diagnostics. Generally, transistor biosensors employ a semiconductor material as the active sensing element which often requires surface immobilization or functionalization due to specific binding events between receptor-ligand pairs. The binding process modulates the electrical parameters such as charge density on the sensing surface. The modulation in the electrical signals could be easily captured by the integration of electrodes on the surface. In the case of transistors source to drain current or gate current which are very often in the micron regime could be easily measured by integration of microfluidic channel design to enclose the liquid environment.

While sensitivities of many bioelectronics platforms have been greatly improved, the construction of an efficient biomolecular interface, which is

fundamental in all bioelectronics, remains a challenging issue to date. Intrinsically, biomolecules do not adhere well to non-cellular surfaces. To this end, supported lipid bilayers (SLB) serves as an attractive model of study for biosensing [6, 7] since it enables biofunctionalization of many surfaces including metal and inorganic solids by covalent coupling or it can be separated from the solid support by an ultrathin layer of water or soft polymer cushions [8] as illustrated in Figure 5.1.

Figure 5.1: Schematics of different examples of tethered lipid bilayers separated from substrates by an ultrathin soft hydrated polymer cushions of various forms [7] .

SLB separated from the solid surface by nanometer-thick waters or soft polymer cushions maintain the thermodynamic and structural properties of free bilayers. In addition, the SLB composition could be easily tailored to incorporate receptors for

the investigation of receptor-ligand binding interactions and appropriately modified to study many other biologically important events. Therefore, the driving force to use SLB for the design of biosensors are three-fold: (i) as a very thin electrical insulator, (ii) as a matrix for the incorporation of receptors (such as lipid-coupled antigens or antibodies), and (iii) for the suppression of nonspecific ligand binding.

We have previously reported well-established protocols for the formation of uniform SLB on various substrates such as Au, SiO₂, TiO₂ and Al₂O₃ [9-11]. In the context of an electrical biosensor, any charge modulation on the membranes have to be transduced into electrical signals by a semiconducting underlayer materials. Surface smoothness has been shown to be an important parameter in the successful formation of a uniform lipid bilayer [11]. Two-dimensional materials such as graphene and graphene oxide which are basically monolayer of sp² hybridized carbon sheets or derivatives of the former for the case of graphene oxide. In the case of graphene, surface functionalization is crucial to create ligand receptors for biological analytes. In terms of biosensing, the imperfections of graphene oxide present conveniently themselves as anchoring points for the binding events of a variety of biological molecules. Recently, direct virus capture and destruction by graphene oxide has been reported without any receptors [12]. In addition, graphene oxide being soluble in water could be easily dispensed by large-area liquid coating process on a variety of substrates which is not easily achievable by chemical vapor deposition (CVD) of graphene. Although intrinsically insulating, graphene oxide could be partially reduced to impart conductivity either by thermal or chemical reduction processes, while still preserving the imperfections necessary for anchoring of biomolecules. The use of RGO is particularly attractive for biosensing since RGO has been found to be more biocompatible than graphene [13] and it is much easier to fabricate and coat graphene oxide on various substrates by simple solution coating. Few groups have reported the formation of single or bilayer of phospholipid layers on reduced graphene oxide [14-18]. However, due to the flaky morphology of coated

graphene oxide on the substrates, the uniform formation of lipid layers is far from ideal. In the previous chapter, we have shown that uniform RGO could be potentially formed by solution coating as a much more uniform layer on TiO₂ as compared to SiO₂. Based on this methodology, we will extend the application of RGO/TiO₂ as a support for the lipid bilayers to be used in a variety of biosensors.

5.2 Materials and Methods

Materials

Highly doped p-type (boron) 4-inch silicon substrates (500±25 μm thickness) with a low resistivity of 0.001-0.005 Ω.cm and a thermally oxidized silicon dioxide (SiO₂) (300nm thickness) were purchased from Silicon Prime Wafers. Titanium oxide (TiO₂) with a thickness of 100nm on glass slides were purchased from Deposition Research Lab Inc.

Chemicals

Acetone, isopropyl alcohol (IPA), chloroform were purchased from VWR chemicals. Hydrazine monohydrate were purchased from Sigma-Aldrich. Phosphate buffer solution (PBS) was purchased from Lonza. Polydimethylsiloxane (PDMS) was made by sylgard 184 silicone elastomer kit purchased from Dow Corning. Zwitterionic lipid, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), anionic lipid, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (DOPS) and cationic lipid, 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (DOEPC) were purchased in powder form from Avanti Polar Lipids Inc. Aqueous suspension of graphene oxide of 4.0 mg/ml concentration was purchased from Graphenea Inc. The suspension contains >95% of monolayer graphene oxide flakes. All reagents were of analytical grade and used without further purification. Ultrapure deionized water with an electric resistance of 18.3 MΩ was used for all solution preparations.

Graphene Oxide Coating

All substrates were ultrasonically cleaned by acetone, isopropyl alcohol (IPA) and Milli-Q water in sequence for 1min each before subjecting to 1min of oxygen plasma treatment (Harrick Plasma, PDC-002, Ithaca, NY) to generate a hydrophilic surface. Aqueous suspension of graphene oxide of 1.0 mg/ml diluted from a raw concentration of 4.0 mg/ml was sonicated for about 10min before spin-coating at a speed of 6000 rpm x 1 min on the substrates using a spin-coater (WS-650MZ-23NPP, Clarion Safety System). Chemical reduction was subsequently carried out to partially convert graphene oxide to graphene generally termed as reduced graphene oxide. This was achieved by enclosing the samples within a saturated hydrazine monohydrate vapor environment at a temperature of 90°C in an oven over 12 hrs.

Raman Spectroscopy

A 488nm excitation laser and a 50x and 100x objective lens of a confocal spectrometer (alpha 300R, WiTEC, Germany) were used for Raman spectrum collection and 2D mapping respectively. Over a 40um by 40um area, 2D mapping was scanned at 100 points per line horizontally and vertically (spectral resolution of 3cm^{-1}) with a laser power well $<1\text{mW}$ to avoid laser heating of the sample. Unpolarized Raman spectra were recorded at room temperature on reduced GO thin films deposited on 300-nm-thick SiO_2 and 100-nm-thick TiO_2 . The laser spot size was estimated to be less than 3 um. According to recent studies, the peak position of the second-order Raman D-peak, $f(2D)$, considerably blueshifts with increasing number of layers in graphene. Therefore, we were able to reconstruct the number of layers in our samples by mapping $f(2D)$ in our films.

Quartz-Crystal Microbalance-Dissipation (QCM-D)

A Q-Sense E4 instrument (Q-Sense AB, Gothenburg, Sweden) was used to monitor the lipid deposition process in real time. Changes in the resonance frequency and energy dissipation of a 5 MHz AT-cut piezoelectric quartz crystal were recorded at the 3rd, 5th, 7th, 9th and 11th overtones. The QCM-D SiO₂ coated substrates are composed of quartz crystals coated with the following layers and corresponding thicknesses in sequential order: 10nm Cr, 100nm Au, 10nm Ti, and last 50nm SiO₂. Layers were coated by using the physical vapor deposition (PVD) method. The QCM-D Au substrates are composed of quartz crystals coated with the following layers and corresponding thicknesses in sequential order: 10nm Cr and then 100nm Au. The layers were also coated by PVD method. All measurements were done under flow-through conditions at a flow rate of 50 uL/min by using a Reglo Digital peristaltic pump (Ismatec, Glattbrugg, Switzerland) (which could be seen in Fig. 1). All surfaces were treated with oxygen plasma at 180 W or 1 min (March Plasmod Plasma Etcher, March Instruments, Concord, CA) immediately before use.

Electrical Measurement

Using a stainless steel shadow mask of channel length 100um and width of 2000um, chromium electrodes was first sputtered on the RGO as an adhesion layer before the subsequent sputtering of gold on top of the chromium electrodes. The thickness of the resulted Cr/Au was about 10/100nm serving as the source and drain. PDMS (with 10wt% curing agent) was fabricated as the encapsulating fluidic chamber and assembled on top of the source and drain electrodes using pre-cured PDMS (with 50 wt% curing agent) as an adhesion layer. After overnight drying in air at room temperature, the edges were reinforced to the substrates by air-cured

epoxy glue. The PDMS fluid chamber is designed with an inlet and outlet to allow the continuous injection of liquid sample via tubing connection and a peristaltic pump (ISM 833). The electrical performance of the FETs was determined by measuring the standard two-probe configuration of top-gated device's static characteristics (drain-source current I_D versus gate voltage V_G at constant drain-source voltage V_D of 1V, or drain-source current I_D versus drain-source voltage V_D at constant gate voltage V_G of 0V). These characteristics were measured using the Keithley 4200 Semiconductor Characterization System with an Ag/AgCl gate electrode.

5.3 Results and Discussion

5.3.1 QCM-D of SLB on RGO

Surface sensitive technique of QCM-D was used to study the formation kinetics of DOPC lipid bilayers on RGO. By measuring the change in resonance frequency and dissipation of the oscillation of the quartz crystal we can gain much information on the absorption and viscoelastic properties of the absorbed film. For instance, a negative frequency shift indicates the addition of mass onto the crystal surface which decreases the crystal's resonance frequency of oscillation. On the other hand, high energy dissipation indicates a non-rigid film which has been adsorbed on the surface of the crystal. Furthermore, the frequency and dissipation response kinetics can be modeled to better understand the property changes of the film. For example, bilayer thickness (~5nm) can be determined from the change in frequency using the Sauerbrey relationship [19]:

$$\Delta m = -C_{QCM} \frac{\Delta f_n}{n}$$

where Δm is the adsorbed mass, C_{QCM} is the mass sensitivity constant ($17.7 \text{ ng cm}^{-2} \text{ Hz}^{-1}$ at $f = 5 \text{ MHz}$) and Δf_n is the change in the resonance frequency at the n th harmonic.

First of all, SALB was adopted for bilayer formation on RGO/SiO₂ and RGO/TiO₂ surfaces as shown in Figure. 5.2a to 5.2d. A measurement baseline was first established by flowing aqueous buffer (10mM Tris buffer of pH 7.5 with 150mM NaCl), followed by isopropyl alcohol solution at $t = 10 \text{ min}$. Afterward, 0.25 mg/mL DOPC lipid in isopropyl alcohol was deposited onto the RGO-coated substrates at $t = 25 \text{ min}$, and lipid attachment reached equilibrium. There was a slight downward frequency shift which corresponded to the attachment of lipids onto both RGO-coated SiO₂ (Figure 5.2a) and TiO₂ (Figure 5.2b) surfaces.

After lipid attachment in isopropyl alcohol, a solvent-exchange step was performed at $t = 40 \text{ min}$ and in order to reintroduce aqueous solution (without lipid) and complete the SALB procedure. The final frequency and dissipation shifts are reported in Table 1. On RGO-coated SiO₂ [20], the final frequency and energy dissipation shifts were -26.4 Hz and 0.9×10^{-6} , respectively. By contrast, on RGO-coated TiO₂, the final frequency and energy dissipation shifts were -35.7 Hz and 1.2×10^{-6} , respectively. The frequency shift of -26.4 Hz for the RGO-coated SiO₂ is in agreement with past measurements on SiO₂ while a higher frequency shift of -35.7 Hz for the RGO-coated TiO₂ is very similar to the case of SLB on Al₂O₃ [21] without RGO coating. The higher frequency shift could be attributed to a thicker water hydration layer between the lipid bilayer and the substrate.

The SALB was compared against lipid bilayer formation on RGO/TiO₂-coated QCM sensors by the method of amphipathic, Alpha helical (AH) peptide-induced vesicles rupture [19]. In this latter method, vesicles freeze-thawed for 7 cycles [20] were first prepared and diluted with 10 mM Tris, 150 mM NaCl pH 7.5. Fig. 5.2e shows the frequency shift with dissipation as Tris buffer containing DOPC

lipid vesicles was injected after establishing a baseline for 20min. The negative frequency shift upon the injection of DOPC vesicles at time = 20min indicated the irreversible adsorption of the vesicles onto the RGO-coated surface since added mass would give rise to a decrease in the resonance frequency of oscillation of the QCM crystals [19]. The corresponding increase in dissipation signified the viscoelastic nature of the adsorbed DOPC vesicles on the RGO surface, in contrast to a rigid layer. The adsorbed DOPC vesicles remained unruptured on the RGO surface until a hepatitis C virus protein, AH peptide, is added. The DOPC vesicles expanded and eventually ruptured forming a DOPC membrane on RGO with a final frequency and dissipation response values of $\sim 25\text{Hz}$ and $\sim 1 \times 10^{-6}$ respectively.

Table 1. QCM-D Measurement Responses Obtained for Supported Lipid Bilayers Formed via the SALB and AH-induced Vesicles Rupture Procedure.

Substrate	Procedure	Final Frequency (Hz)	Final Dissipation (10^{-6})
RGO-SiO ₂	SALB	-26.4	0.9
RGO-TiO ₂	SALB	-35.7	1.2
RGO-TiO ₂	Vesicles rupture	-35.1	1.0

Figure. 5.2. QCM-D of DOPC lipid bilayers by SALB on (a, b) RGO/SiO₂; (c, d) RGO/TiO₂ and by (e) AH peptide-mediated ruptures on RGO/TiO₂.

5.3.2 Electrolyte-Gated RGO Transistor

We checked the electrical properties of the RGO materials by probing the IV characteristics of RGO formed on SiO₂/Si substrate. For simple verification, we have used silver (Ag) ink as the electrodes on a RGO/SiO₂(300nm) on highly doped p-type silicon substrate (p⁺⁺Si) sample as shown in Figure 5.3a. The highly doped Si substrate served as the bottom gate in this three terminal configuration with Ag as the top contacts as source and drain. Increasing drain current I_D was observed with increasing negatively biased gate V_G (Figure 5.3b) indicating positively charged carriers as the major carriers in the RGO. In other words, the RGO is a p-type material.

Since AFM and Raman spectroscopy demonstrated a more uniform coating of RGO on TiO₂ in comparison to Al₂O₃ and SiO₂, we used only TiO₂ substrates for the subsequent sets of experiments. A liquid-gated three-terminal configuration was used for the dependence study of RGO transistor on electrolyte ionic strength or concentration. As shown in the schematics of Figure 5.3c and 5.3d, evaporated Ti/Au (~10/100nm) was used as the electrodes on RGO/TiO₂/glass with an insulating resin isolating the liquids from the source/drain electrodes and acting as a mini-trough to hold the liquid. $I_D V_G$ measurement in Figure 5.3e indicated an increasing drain current I_D with increasing negatively biased gate bias V_G , analogous to the $I_D V_G$ trend on SiO₂/Si. The mechanism of the liquid gating effect on the RGO transistor is based on the ionic screening effect of the NaCl electrolyte in which the ions are in low concentration. An increasing ionic strength in the electrolyte by increasing NaCl concentration from 2.5mM to 10mM was monitored by the RGO transistor as increasing I_D at negative gate bias. The mechanism for the current modulation could be clarified by the schematics shown by the schematics shown in Figure 5.3f. At negative gate bias V_G under the ionic screening effect, chloride anions will be accumulated at the electrolyte/RGO interface leading to an accumulation of positive charge carriers in the RGO. With more chloride ions accumulation at the interface at

increased ionic strength, the increased positive charge accumulation gave rise to an increased drain current I_D under negative gate bias. It is noted that when $V_G = 0V$, there is still a I_D corresponding to $\sim 180 \mu A$ under $V_D = 1V$ since the RGO is rather conductive by itself under zero gate bias. A slight deviation in the $I_D V_G$ curve was observed at high ionic strength of 10mM when the ionic screening effect of the ions in the buffer was probably at the breakdown threshold leading almost to the connection between gate and drain terminal. It has been demonstrated in another report double electric layer caused by the ionic screening effect would have been completely broken down at an ionic strength of 100mM KCl [22].

Figure 5.3. (a) Dimensional layout and design of RGO/SiO₂ non-electrolyte transistor. (b) $I_D V_G$ curves of RGO/SiO₂ indicating a p-type RGO channel. (c) Top-view and (d) side-view schematics of a RGO/TiO₂ transistor with a PDMS well for electrolyte. (e) Sensitivity dependence of I_D on ionic strength of RGO/TiO₂ in an electrolyte-gated configuration; (f) Schematics diagram to explain the charge distribution and field effect on the conductance in RGO channel.

5.3.3 Supported Lipid Bilayer (SLB) on RGO Biotransistor

The SALB method established in our group was used for the formation of the lipid bilayer on the RGO using the same device platform we used earlier for the ionic strength study. More detailed description of the SALB formation mechanism can be found in other reports [23]. DOPC in chloroform was first evaporated using nitrogen gas. The dried DOPC were dissolved in IPA to achieve a lipid concentration of 0.1mg/ml and dropped onto the channel region between the electrodes, contained by the boundaries of the resin. After some time of incubation, the DOPC-containing IPA was exchanged with PBS buffer by gentle flushing to induce the formation of DOPC lipid membrane on the RGO. Subsequently the channel region was gently flushed

with deionized water and a three terminal liquid gated configuration was established for $I_D V_G$ measurement using NaCl electrolyte of different concentration. As shown in Figure 5.4a, there was no drastic shift in $I_D V_G$ curves under different ionic strength indicating the passivation of the RGO by the DOPC membrane since lipid membrane is known to be impermeable to large ions like sodium and chloride ions. The screening effect due to the ionic strength of our NaCl solution can be approximated from the Debye length using the Graham equation [17] for a 1:1 electrolyte as:

$$\lambda_D = \frac{0.304}{\sqrt{[NaCl]}} nm$$

At the range of NaCl concentration from 2.5mM to 10mM used in our experiment, the Debye length works out to be extremely small from ~3nm to ~6nm. As manifested by the $I_D V_G$ curve deviation at 10mM in Figure 5.3e, a Debye length of 3nm for 10mM might be near to the breakdown threshold of the electric double layer. Typical lipid bilayer is ~5nm thick which could have well insulated the RGO from the electrolyte at a high ionic strength of 10mM.

In order to achieve a more uniform lipid membrane coverage on RGO we have used a modified design of a smaller channel region of length/width ratio of 100um/2000um for subsequent experiments to investigate the effect of charged lipid membranes on RGO transistor. DOPC:DOPS (70:30) of 70 weight % DOPC and 30 weight % DOPS was used as the negatively charged lipids, while DOPC:DOEPC (70:30) of 70 weight % DOPC and 30 weight % DOEPC was used as the positively charged lipids. SALB was used for the formation of the respective lipid membranes on RGO by flowing liquid samples through a PDMS transparent chamber attached onto RGO/TiO₂/glass using a flow system with the aid of a peristaltic pump.

IPA was first flowed through the PDMS chamber followed by lipid-containing IPA of 0.1mg/ml concentration. There was no lipid layer formation

during the incubation of lipid-containing IPA on the RGO. Lipid layer was only formed on the substrate upon exchanging the IPA with the PBS buffer by flowing it through the chamber. In order to check the dielectric robustness and uniformity of the SLB of DOPC thus formed on the RGO, $I_G V_G$ was monitored as shown in Figure 5.4a, which indicated a zero I_G over the range of ionic strength up to 10mM KCl of buffer with V_G sweep. The zero gate current I_G confirmed the electrical isolation between the gate and drain due to the ~5nm of SLB uniformly spread across the RGO.

The linear $I_D V_D$ characteristics of DOPC/RGO was measured before the same device was flushed with sodium dodecyl sulfate (SDS) for cleaning off the DOPC membrane followed by PBS buffer, pure isopropyl alcohol (IPA) and IPA containing DOPC:DOPS. After some time of incubation DOPC:DOPS membrane was formed after exchange using PBS buffer. Upon the formation of DOPC:DOPS lipid membrane, the drain current increases from 0.200mA to 0.225mA noticeably at a drain voltage V_D of 1V as compared to the case of a neutral DOPC membrane as shown in Figure 5.4b. The increased drain current is due to the increased positive charge carriers in RGO attracted by the negative charges on the head groups of DOPS at the membrane/RGO interface. On the other hand, the positive charges on the head groups of DOPC at the membrane/RGO interface induced repulsion to the positive charges in RGO leading to a drop in the positive charge carriers as illustrated by the schematics in Figure 5.4b. We have thus herein successfully demonstrated that the molecular charges on the atop SLB can effectively act as an pseudo gate bias to modulate the conductance in the RGO channel by controlling the major charge carriers subjected to electrostatic influence.

(a)

(b)

Figure 5.4. Schematics and I_D - V_D curves under different V_G of liquid gated biosensor: (a) RGO/TiO₂ in PBS; (b) DOPC/RGO/TiO₂ in PBS; DOPC:DOPS(70:30)/RGO/TiO₂ in PBS; and DOPC:DOEPC(70:30)/RGO/TiO₂ in PBS.

5.3.4 Functionalized Supported Lipid Bilayer (SLB) on RGO Biotransistor

A functionalized lipid/RGO film was demonstrated as a sensing platform using the well-studied binding events between biotin and streptavidin. In order to clearly illustrate the binding events, we have modified the probing measurement set-up appropriate for kinetics study by monitoring the drain current versus time. In a similar fashion we prepared neutral biotinylated DOPC complex membrane composition on a RGO/TiO₂ surface. As shown in Figure 5.5a, IPA containing the biotinylated DOPC lipids was injected via the PDMS chamber on the sensing platform at zero gate bias but with a constant drain bias of $V_D = -1V$. The drain bias was chosen where there was most observable modulation in drain current. As expected there was a drop in drain current I_D upon exchanging the chamber with Tris buffer indicating the formation of the biotinylated DOPC on the RGO surface. The same drop in I_D was also observed in previous comparison experiments using pure DOPC. Subsequently 0.01 mg/ml of streptavidin in Tris buffer was injected to monitor binding interactions. A further drop in I_D was administered which could be explained by considering the negatively charged nature of streptavidin. When the streptavidin binds to the biotinylated sites on the upper leaflet of DOPC membrane, the bottom leaflet of DOPC which has no access to the streptavidin, became pseudo polarized with positive charges. The positively polarized bottom leaflet of DOPC exerts the same effect as a positively charge membrane such as in the earlier case of DOPC:DOEPC in causing a drop in positive charge carriers in the RGO surface below the membrane. The effect reduces the amount of charge carriers in the active RGO region between the source and drain leading to a drop in I_D . This is reflected as a drop in I_D from $\sim 1.15\mu A$ to $1.05\mu A$ as shown in Figure 5.5a which corresponded to a drop in I_D of $\sim 8.7\%$. To illustrate the quantification and confirm the modulation of drain current caused by the binding events, we prepared different composition percentage of the biotin content in the DOPC ranging from 0.05 to 1.00 wt % and

repeated the measurements. A plotted graph in Figure 5.5b clearly indicated a linear dependence of the concentration of biotin composition percentage versus the percentage drop in the drain current such that when biotin composition was increased to 1.0 wt %, there was a larger percentage drop in I_D of 12 % since more streptavidin would have been bound to the biotinylated lipid bilayer. It is noted that the percentage of biotin incorporated in the lipid bilayers has a direct proportion to the concentration of streptavidin bound onto the biotinylated lipid bilayer. Hence, the experiment presented a proof of concept for a highly versatile and adaptable biosensing platform for quantification of targeted biological analytes. The effect of the streptavidin on the RGO transistor revealed that it is the bottom leaflet of the membrane that dictates the charge transfer in the biotransistor rather than the top leaflet of the membrane.

(a)

Figure 5.5. Biotinylated supported lipid bilayer on graphene FET biosensor for detection of streptavidin-biotin interaction. (a) Kinetics study of source-drain current vs time on biotin-streptavidin binding. (b) Plot of percentage drain current shift vs biotin concentration upon streptavidin binding.

Figure 5.6. Biotinylated supported lipid bilayer on graphene FET biosensor for detection of streptavidin-biotin interaction. Left: Plot of drain current shift $\Delta I/I$ percentage versus Streptavidin concentration in linear scale. Right: Plot of drain current shift $\Delta I/I$ percentage versus Streptavidin in log scale. Streptavidin concentration is in mg/ml.

The limit of detection (LOD) of a biosensor is expressed in units of concentration and, following IUPAC definition, indicates the smallest solute concentration than a given analytical system is able to distinguish with a reasonable reliability of a sample without analyte [24]. The guidance for limit of detection can also be found in the international Conference on Harmonization's (ICH) Q2 Validation of Analytical Procedures: Text and Methodology [25]. Generally, the limit of detection is defined as the concentration that gives rise to an instrument signal of three times the standard deviation of the background signal [25]. In order to address the LOD of the biotinylated lipid bilayer FET biosensor developed in this thesis, another two plots have been added in Figure 5.6 so that the LOD can be directly extracted based on the concentration of analyte ie. streptavidin. Based on the dynamic linear range in Figure 5.6, the lowest limit of the linearity range is 0.667 ng/ml of streptavidin. In other words, the LOD of the biosensor will be at least 0.667 ng/ml of streptavidin if not lower. The LOD demonstrated herein is comparable to many of other reported FET-based biosensor in the literature. Cheng et al. [26] reported an LOD of 1 ng/ml and 10 ng/ml for cytokeratin fragment 21-1 (CYFRA 21-1) and neuron-specific enolase (NSE) respectively which are important tumor markers for lung cancer differentiation, while Hideshima et al. [27] reported an LOD of 10 ng/ml of AFP liver cancer biomarker. With reference to Figure 5.7 [28], the LOD reported here is better than many of reported organic FET biosensor and on par with other reported graphene FET biosensor. Going beyond the regime of FET-based biosensor, the LOD of biotinylated lipid bilayer FET biosensor reported herein also compares favorably with other standard biosensors such as surface plasmon resonance (SPR). Li. et al. [29] reported using gold nanoparticles (GNPs) modified with streptavidin (SA) to enhance signal specifically via biotin-streptavidin interaction. The developed GNPs enhanced sandwich SPR biosensor successfully fulfilled the sensitive detection of biotin-functionalized carcinoembryonic antigen (CEA) in the range of 1-60 ng/ml with a detection limit of 1.0 ng/ml.

Figure 5.7. A summary of FET-based biosensor performance, in the form of demonstrated LOD and demonstrated dynamic range. Blue color: Silicon nanowire biosensors (SiNW); Green color: Organic FET biosensors (Organic FET); Red Color: Graphene FET biosensors (Graphene FET) [29].

It is noted that within the constraint of shadow mask design, the channel width of the graphene FET in this thesis is relatively large at 1500um. It has been reported that level of background noise signal is directly proportional to the sensing area of the transistor channel [30]. There will be much room for improvement if the channel width can be reduced further perhaps for future work using semiconductor processes such that the signal to noise ratio can be enhanced to push to a lower LOD since lower background noise will lead to a lower LOD. Although, commercially available biosensor such as SPR has claimed a high sensitivity of up to 1 nM for a 20 kDa protein [31], it suffers a few shortcomings. SPR cannot easily discriminate between specific and non-specific interactions with the sensor surface. Elaborate washing does not completely remove the non-specifically bound material; thus, reference material or control samples are needed to correct for the non-specific binding [32]. Although the sensitivity for high molecular weight molecules is good since SPR is mass sensitive but binding of low molecular weight compounds is more

difficult to detect [31]. The supported lipid bilayer FET biosensor does not have the above issues since the working mechanism of FET biotransistor is based on charge accumulation and depletion in the semiconductor channel hence independent of mass. With further improvement of the LOD for the FET biotransistor, it might be a competitive alternative to SPR in the near future.

5.4 Conclusion

A hybrid biomimetic membrane platform comprising a phospholipid bilayer atop reduced graphene oxide on TiO_2 have been successfully demonstrated for biosensing application. For the first time in the literature we have presented device-level biosensing capabilities of a functional lipid membrane on RGO fabricated by economical and scalable solution coating processes. The low temperature characteristics of the lipid/RGO processing method is applicable for a variety of biomedical analytical tools such as Quartz Crystal Microbalance (QCM) and Surface Plasmonic Resonance (SPR). A morphologically smooth amorphous-like RGO film on TiO_2 was achieved which facilitated the formation of various types of lipid layers including charged membranes. It was demonstrated that the RGO transistor device is sensitive to a liquid environment exemplified by conductance modulation caused by different ionic strength. An increase in salt ionic concentration from 0mM to 10mM induces charge carriers accumulation in RGO leading to increased drain current output. Such ionic strength dependence was drastically shunted when the sensing RGO platform was passivated by the formation of a lipid biomembrane. In addition, it was shown that positively charged membrane of DOPC:DOEPC has a drastic modulation impact on the conductance of RGO in which the majority positively charged carriers were annihilated by the accumulation of negative charge carriers in the RGO under the biomembrane. Finally, a model system based on the

hybrid device platform has been demonstrated using biotin-streptavidin binding event to exhibit the quantification of biotin composition with respect to transistor drain current modulation at 12 % for a 1 wt % biotinylated DOPC lipid complex membrane formed on RGO/TiO₂.

5.5 References

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Chapter 6: Conclusion

6.1 Summary of Study

We have successfully demonstrated by the study performed in this thesis a viable field effect transistor (FET) as a biosensor using a hybrid platform of lipid bilayer supported on reduced graphene oxide (RGO) as the biosensing platform. In this FET architecture, the supported lipid bilayer acts the ultrathin dielectrics while reduced graphene oxide acts as the semiconductor analogous to the conventional structure of a semiconductor transistor. The unique advantages of using FET as biosensor has been discussed in chapter 1 which includes sensitivity, suitability for miniaturization, light weight and low-power which are all prime prerequisite for point-of-care (POC) diagnostics. The lipid membrane is well suited as dielectrics due to its inherent and ultrathin thickness which is an important consideration for biosensing in a FET in consideration of the Debye length which is an important performance metrics for a FET working in an electrolyte. As for the semiconducting channel, graphene appears as the best choice due to its ability to detect both positively and negatively charged biomolecules due to its bipolar nature, low charge transfer resistance and fast electron transfer kinetics as discussed in chapter 1. More specifically for the formation of lipid membrane atop, graphene offers the versatility of supporting a monolayer or bilayer lipid by simple modification of surface properties to be either hydrophobic or hydrophilic. As demonstrated in chapter 3, hydrophobic surface of a chemical vapor deposition (CVD) graphene renders the formation of a lipid monolayer of $\sim 2.5\text{nm}$ while a hydrophilic surface induces the formation of lipid bilayer of $\sim 5\text{nm}$. The formation of lipid monolayer and bilayer is achieved via lipid vesicles fusion and solvent assisted solvent exchange. The study in this thesis extends the application of CVD graphene in biosensing application to solution-processable graphene oxide in view of the scalability and cost effectiveness

of graphene fabrication. To this end, a first of its kind solid-liquid phase exfoliation technique was developed by capitalizing on the unique bonding interaction between carbon atoms of graphene and titanium atoms from a titanium-bearing substrate as reported in chapter 4. The unique bonding interaction acts as a strong surface anchorage in facilitating uniform adhesion and near to ideal surface coverage of spin coated graphene oxide on titanium bearing substrate. The technique of applying a titanium adhesion layer followed by in-situ solid-liquid exfoliation is applicable to a wide variety of functional substrates and serves to solve the long-standing issue of surface corrugations and coating inhomogeneities of wet-processed graphene oxide coating.

One of the noteworthy advantages of using lipid membrane as a biosensing platform, other than its inherent biocompatibility that resembled a natural cell membrane, is the versatility and ease in the control of lipid composition and incorporation of appropriate receptors for a wide spectrum of analytes. The feasibility to achieve conductance modulation in the semiconducting channel of reduced graphene oxide (RGO) by attachment of charged biomolecules on the lipid membranes was performed in chapter 5 demonstrating the effect of negatively and positively charged lipids in the lipid composition, although the same has been demonstrated for CVD graphene in chapter 3. Finally, a biotinylated lipid bilayer for streptavidin recognition was demonstrated in chapter 5 with a limit of detection (LOD) of 0.667 ng/ml streptavidin. The demonstrated LOD herein is comparable to many other reported biosensors based on FET and in fact also on par with standard biosensor such as surface plasmon resonance (SPR) as discussed in chapter 5.

6.2 Challenges and Opportunities for Future Work

This dissertation demonstrated the great potential of graphene as a highly versatile materials for biomedical diagnostics application. Despite the encouraging results, the development of such devices for real-life clinical applications presents certain challenges that still need to be addressed. A critical issue derives from the sample itself. Clinical samples are complexes containing numerous biological components and the biomedical engineer and scientist can only work with rough estimates of anticipated matrix effects. In the biosensor operation should also consider variations such as air stability in terms of design, suitability of samples such as in the form of whole blood, plasma or serum. It is well known that natural plasma membrane which is essentially lipid bilayers contain a large family of transmembrane proteins and peripheral proteins, hence it will not be surprising to expect the synthetic lipid bilayers in the demonstrated devices herein to exhibit high affinity to various physiological components such as serum albumin, intralipids in the sample. Hence a complete interference study will have to be performed in order to quantify the specificity and selectivity of the supported lipid membranes as a practical biosensor towards a target analyte.

Being an organic entity, graphene establishes good biointerface between biotic and abiotic system because of its stable interaction with bio-entities, high adsorption capacity for biorecognition proteins and target molecules and high electrical sensitivity since every carbon atom in graphene responds to charges induced by bio-entities, negating the necessity for an oxide passivation layer. Though the dissertation has focused on in-vitro application, there are huge future potential of building in-vivo and implantable graphene-based medical devices, by capitalizing on the intrinsic properties of graphene as a highly flexible and conformable materials coupled with excellent chemical inertness. In the context of in-vivo application of graphene-bearing devices, it is crucial to highlight that graphene-based

nanomaterials have been reported to be toxic dependent on various factors such as lateral size, surface structure, functionalization, charge, impurities, aggregations, and corona effect [1-3]. In this aspect, long-term and short-term biocompatibility of graphene will have to be assessed such as cytotoxicity, irritation, sensitization, genotoxicity, carcinogenicity, hemocompatibility and pyrogenicity as per ISO 10993 standard: Biological evaluation of medical devices. Ultimately some form of modification might be necessary to render the graphene oxide support to be safe for in-vivo application. On a similar note, titanium dioxide (TiO₂) is considered as an inert and safe material and has been used in many applications for decades. However, as a long-term material incorporated in an implantable device, potential leaching effect of TiO₂ nanoparticles from the support will have to be clinically assessed in terms of toxicology. Mechanistic toxicological studies show that TiO₂ nanoparticles predominantly cause adverse effects via induction of oxidative stress resulting in cell damage, genotoxicity, inflammation, immune response etc. [4-6]. Hence the biocompatibility of TiO₂, other than graphene oxide will have to be scrutinized. It will require the concerted effort of clinicians, materials scientists and biomedical engineers to bring smart graphene-based medical devices onto the center stage in responding to a huge variety of unmet clinical needs in future biomedical challenges.

6.3 References

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