

# Advances in Polymer/Graphene Nanocomposite for Biosensor Application

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## Abstract

In this article, polymer/graphene nanocomposite has been focused with reference to their application in biosensor. In this regard, various type of biosensors-based on polymer/graphene nanocomposite has been explored. Major categories of biosensors studied include electrochemical biosensor, flexible biosensor, field effect biosensor, and glucose biosensor. Owing to excellent electrical, mechanical, thermal, and biomedical features, polymer/graphene nanocomposite has been found to improve the performance of different type of biosensors.

## Keywords

Polymer/Graphene, Nanocomposite, Glucose biosensor

## Introduction

Theory of crystals pointed out that diverse contribution of thermal fluctuation in crystal lattice of low dimensionality may lead to variation of atoms relative to interatomic distance at any finite temperature [1]. The argument was greatly supported by experimental observation. Thus, with decrease in melting temperature of film decreases the film stability. The films becomes unstable (decompose or segregate into islands) at thickness of typically dozens of atomic layers. Thus, atomic monolayers have so far been recognized only as an integral part of larger 3D structures generally grown epitaxially on the monocrystals with matching crystals lattices [2, 3]. Until 2004, the common wisdom was flaunted by experimental discovery of graphene and other free standing 2D atomic crystals. At that time, 2D materials were presumed not to exist without a 3D base. These crystals were achieved on top of non-crystalline substrates as suspended membranes and non-crystalline substrates. Significantly, the 2D crystals were found not only to be continuous but to comprise high crystal quality. The later was clearer in the case of graphene in which the charged carriers may cover thousands of interatomic without scattering [4]. The name given to flat monolayers of carbon atom is graphene. The carbon atoms are tightly packed into two-dimensional honey comb lattice which is the basic building block of graphitic material of all the other dimensionalities as shown in figure 1. By wrapping it can be changed to 0D fullerenes, rolled into 1D nanotube, or 3D graphite. For 60 years, theoretical graphene or 2D graphite has been studied and is extensively employed for describing the features of several carbon-based graphene. It also provided outstanding condensed matter analogue of (2+1) dimensional quantum electrodynamics which propelled graphene into thriving theoretical toy model. On the other hand, however known as an integral part of 3D materials, graphene was not assumed to exist in free state. It was described as academic constituent and was supposed to be unstable with respect to the formation of unstable structures

such as fullerene, nanotube, and soot [5, 6]. In biosensors, polymer/graphene nanocomposites have range of important applications.

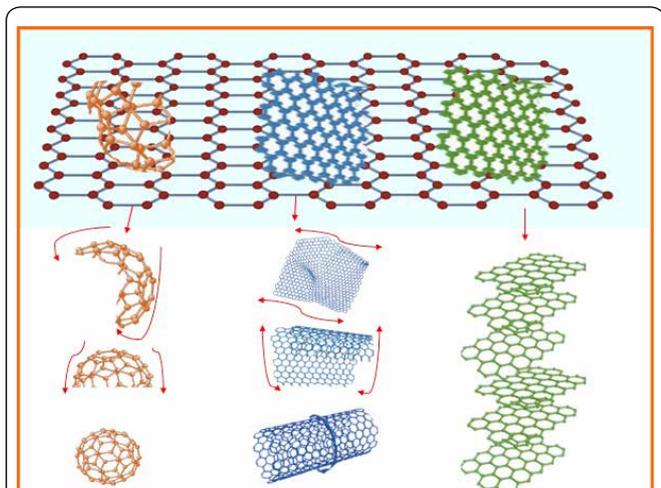


Figure 1: Graphene (2D building material for carbon material) wrapped up into 0D buckyball, rolled into 1D nanotubes, and stacked into 3D graphite.

## Graphene

Graphene is an outstanding material. It has large theoretical surface area (2630 m<sup>2</sup>/g), high Young’s modulus (~1.0 TPa), thermal conductivity (~5000 Wm<sup>-1</sup> K<sup>-1</sup>), optical transmittance (~97.7%), and excellent electrical conductivity (10<sup>2</sup> S/m) at high pressure. Graphene merits attention for applications such as transparent conducting films among other perspective applications [7]. Over 40 years, graphene has been studied experimentally and for measurement of transport properties in micromechanically exfoliated layers of graphene (grown on SiC). Large surface area graphene have been grown on copper substrate. Variety of studies involving the employment of chemically modified graphene have been found in literature [8, 9]. A dynamic yet flexible graphene provides essentially infinite perspectives for its functionalization and modification of the carbon backbone. Comparison of relevant electronic and thermal properties of graphene with other related carbon material (Si, Cu, and single walled CNT) is shown in table 1.

Table 1: Comparison of physical properties of graphene with other materials.

Physical Properties	Graphene	Carbon nanotube	Si	Cu
Melting point (K)	3800	3800	1687	1357
Thermal conductivity (10 <sup>3</sup> W/mK)	3-5	1.75-5.8	0.15	0.385
Current density (A/cm <sup>2</sup> )	> 10 <sup>8</sup>	> 10 <sup>9</sup>	-	10 <sup>7</sup>
Electron mobility (cm <sup>2</sup> /(V.s))	> 10,000	> 10,000	1400	-
Mean free path (nm)	1 × 10 <sup>3</sup>	> 10 <sup>3</sup>	20-30	40

The graphene honeycomb lattice is comprised of two equivalent sub lattices of carbon atoms bonded together with  $\sigma$  bonds. Each carbon atom in lattice has  $\pi$  orbital that links to a demoralized electron network. Freely suspended graphene

has intrinsic ripples as addressed by Monte Carlo stimulation and transmission electron microscopy (TEM) studies [10]. The microscopic suggestions were estimated to have lateral dimension of about 8 to 10 nm height displacement of about 0.7 to 1 nm. Thus, due to outstanding thermal, mechanical, and electrical properties of graphene filler, they have been employed in several of applications in the form of polymer nanocomposite due to interaction of nanofiller with the polymer. Various current reviews have been appeared about graphing and relevant constituent [11-17]. The appraisals on the chemistry of graphene or exfoliated platelets have been originated from graphene oxide (GO). A brief summary of graphene production techniques are shown in table 2.

Table 2: Various techniques used for fabrication of graphene.

Technique used	Graphene synthesis
Chemical vapor deposition (CVD)	Production of single layer graphene
Liquid exfoliation method	Large scale production of graphene from graphite powder
Mechanical technique	Formation of atomic layer of graphene on SiO <sub>2</sub> (~300 nm)
Epitaxial growth	Graphene synthesis at high temperature (~1300 °C) on SiC substrate

## Biosensor

The molecular sensors link biological mechanism for recognition using physical transduction route. They offer a novel class of portable and inexpensive instruments that allow sophisticated physical measurement to be undertaken rapidly at decentralized location [18]. Moreover, adoption of biosensor for practical applications rather than the measurement of blood glucose is recently restricted by insensitivity, expense, and inflexibility of available transduction routes. Thus, the progress of biosensing route is through population conductance of molecular ion channels by recognition events. The approach mimics biological sensory functions and can be employed with various types of receptors comprising nucleotides and antibodies [19]. Schematic of ion channel biosensor is shown in figure 2. The route is very flexible and even flexible form is sensitive to picomolar protein concentration. It is essentially an element of impedance whose dimensions can readily be decreased to become an integral constituent of microelectronic circuit. It is utilized in extensive range of applications and in complex media comprising blood. These sensors might comprise cell typing, detection of viruses, large proteins, antibodies, DNA, electrolyte, pesticides, drugs, and other low molecular weight constituents [20].

The reactive elements of ion channel switch include gold electrode to which a membrane containing gramicidin ion channels was combined to antibodies. The molecular structure of the tied membranes results in an ionic reservoir being formed among the gold electrode and membrane. The ionic reservoir can be electrically processed by connection through gold electrode. In the presence of an applied potential, when the channels are conductive, ions flow occurs between the external

solution and reservoir [21, 22]. When the mobile channels diffusing with in outer half of membrane become cross-linked to antibodies immobilized at the surface of membrane, the ionic current is switched off. This inhibits the dimer formation with channels immobilized with the membrane inner half. The measurement of number of dimers has been carried out from the electrical conduction of membrane. The switch has high gain, single channels flourishing the flux of up to million ions per second. Thus, verification on the quantitative model of biosensors has also been carried out. The analyses recognition comprising multiple detection sites performed by employing the structure is displayed in figure 3.

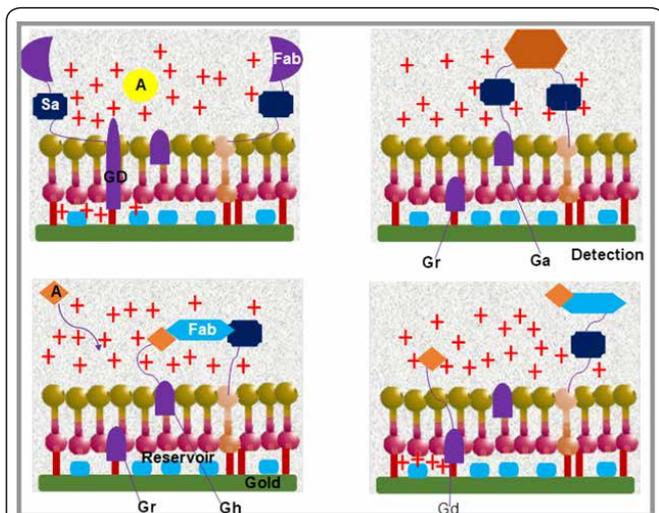


Figure 2: Schematic ion-channel switch (ICS) biosensor.

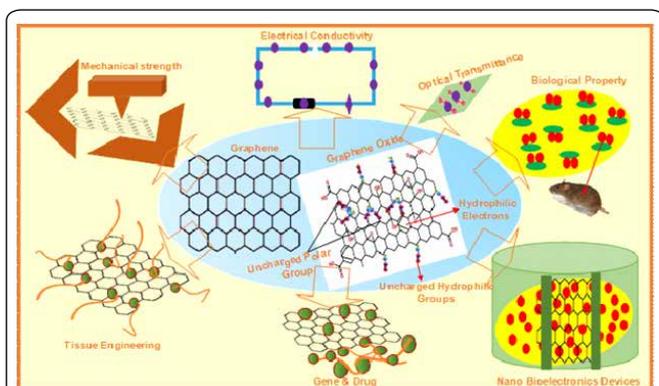


Figure 3: Representation of graphene-based electrochemical sensor in DNA, protein, and cancer cell detection.

The structure is collected employing a combination of sulphur gold chemistry and physisorption [23]. The membrane comprises of lipids and channels which remains immobilized on gold surface, while some of these diffuse literally with in the membrane plane. The antibodies of mobile channel cause scanning of an area of the order of  $1 \text{ mm}^2$  less than 5 min. Thus, with the channels of low density and immobilized antibodies of high density, each channel may access up to 103 more capture antibodies in comparison to that of gating mechanisms triggered by direct analyte binding to channels. The sensitivity and speed of biosensor response may be adjusted in direct proportion to the number of binding sites accessible to each channel of mobile.

This permits for quantitative recognition of analyte from sub-picomolar concentration of less than 10 min [24].

## Polymer/Graphene in Biosensor

The discovery of the capability of carbon to form two-dimensional (2D) modification in the form of graphene led to extensive growth in material features and its perspective application in electronics. It is recognized that graphene comprises excellent set of electrical features including high charge mobility at low concentration and maximum perspective ratio of surface area to volume and low noise level [25]. Because of this combination, the adsorption of an extremely low amount of impurity on graphene surface can remarkably alter the total conductivity. Graphene is an outstanding material for creation of numerous sensors. It is displayed that graphene is capable of detecting absorption of even single molecule [26, 27]. The attached molecules of gas, depending on their charge and graphene conductivity, act as donor or acceptors that cause modification in concentration of mobile charge carrier. Consequently, the resistance of graphene film is reduced or enhanced depending on the absorbed type of molecule. It should be noted that considerable disadvantage of graphene-based gas sensors is the lack of selectivity [28]. In general, an alteration in conductivity does not recognize that which of the particular molecule is adsorbed on graphene surface. However, some molecules contribute to conductivity with opposite signs, so that the total change in the resistance is nearly zero [29]. The problem is the selection of graphene based sensors. It can be solved by employment of antigen-antibody reaction. The constituent of such pair can only be interfaces with each other and not with any other type of protein. It is well recognized that certain stages of many diseases involve emergence of antigen markers that are specific of one disorder or group of numerous disorders. The antigens may cause binding to specific antibodies preliminary immobilized on graphene sensor surface [30]. Thus, the antigen-antibodies reaction may lead by similarity with gas sensors to alteration in resistivity of graphing film. By using antigen-antibodies pair, it is perspective to solve the sensitivity problem in biosensors and broad possibilities for application of graphene-based sensors in medicine and biology. This approach may direct to creation of miniature biosensor capable of the detection of diagnostically remarkable markers in biological fluids in the regime of rapid analysis for diseases which are presently only detected by laborious and time consuming procedure of enzyme immunoassays [31, 32].

## Types of Polymer/Graphene-based Biosensor

Since graphene and its relevant derivatives have fascinated the interest of numerous scientific fields mainly energy technology, nanoelectronics, sensors, bioscience, and biotechnologies because of their electric-electronic and physiochemical features. Generally, these exceptional features comprise excellent electrical conductivity, high surface area, strong electrical conductivity, good thermal conductivity, high charge mobility, good optical transparency, and ease

of biological as well as chemical functionalization [33, 34]. Because of outstanding physiochemical features, graphene-based nanomaterial presents great opportunities into wide variety of biomedical applications. Accordingly, they comprise various types of biosensors mainly graphene-based DNA biosensors, graphene-based glucose biosensors, graphene-based Hb biosensors, graphene-based cholesterol biosensor, and graphene-based electrochemical biosensors, etc. [35].

### Electrochemical biosensor

The electrochemical detection is highly sensitive to electrically active molecules which is the feature of electrical detection. It also presents detection selectivity due to the oxidation and reduction of different molecules at different potential. Graphene nanofiller is excellent conductor of electrical charge. The heterogeneous transfer of electrons occurs at the defects in basal planes or at the edge of graphene [36, 37]. Thus, the excellent surface area of graphene facilitates greater amount of defects and thus electroactive sites. Graphene has been used in numerous schemes for glucose sensing. This is reflected by the fact that electrochemistry is crucial for the glucose sensing for diabetic patients. Graphing has also used for electrochemical immunosensing. In immunosensing the direct electrochemical detection of antibody antigen detection is generally not possible and so electrochemically active labels must commonly be employed [38]. First graphene can be utilized as electrode surface for sensitive detection of label. This case was utilized for graphene enhanced detection of  $\alpha$ -fetoprotein, which is biomarker for cancer. The modification of graphene sheets was carried out by antibodies, then the  $\alpha$ -fetoprotein was added and consequently secondary antibodies loaded with microspheres bearing horseradish peroxidase enzyme were sensitive labels [39, 40]. The second approach utilizes graphene as label bearing nanocarriers. More generally a gold nanoparticles was modified with probe antibody to phosphorylated protein p53. Thus, the conjugation of secondary antibodies was carried out with graphene oxide for the generation of large amount of electrically active molecules and thus large signals were produced [41]. The sensing depends on conjugation of graphene oxide with antibody to offer assessable binding sites.

### Electrode in glucose biosensor

One of the most clinically critical diseases in the world is diabetes and it is significant to make a quantitative determination of the glucose level in blood for diagnosis of this disorder. This metabolic disorder results in insulin deficiency and hyperglycemia and it is reflected by blood glucose concentration lower or higher in comparison to that of normal range of 80-120 mg dL<sup>-1</sup>. The disease causing severe conditions comprising death and disability [42]. Accordingly, the treatment and diagnosis of this disease needs close monitoring of blood glucose level. Graphene supplies highly sensitive and cost efficient material for the production of glucose biosensors. The graphene-based glucose biosensors with polyethylenimine/graphene-functionalized ionic liquids nanocomposite modified electrode have been synthesized [43]. It comprises extensive linear glucose response, efficient

reproductivity, and strong stability. The exploration on the efficiency of chitosan in dispersing graphene and constructed glucose biosensors with desired sensitivity was also studied [44]. Thus, it was shown that chitosan helped to form well-integrated graphene suspension and immobilized the biomolecules. Graphene-based biosensors displayed high sensitivity (37.93  $\mu\text{A mM}^{-1}\text{cm}^{-2}$ ) and long term stability of measuring glucose. Graphene/metal nanoparticle-based biosensors have also been developed to detect glucose. Qui et al. [45] synthesized Pt/PANI/GN-based sensor comprised a detection limit of 50 mM for H<sub>2</sub>O<sub>2</sub>. After immobilization of GO, Pt/PANI/GN modified electrode indicated a detection limit of 0.18  $\mu\text{M}$  for glucose.

### Graphene paper as flexible biosensor

Flexible biosensor comprising dramatic shape alteration are instrumental for the progress of portable point of care medical products, minimally invade implantable devices, and complex diagnostic platform. Biosensors based on electrochemical read out offers a major class of analytical tools in clinical applications [46]. A capable building block of intriguing actual technological and scientific interest is graphene. It has two-dimensional single atom thick sheet of sp<sup>2</sup> hybridized carbon atom arranged in a honeycomb lattice with outstanding mechanical, electrical, and thermal features. Individual water soluble graphene sheets are heavily oxygenated comprising hydroxyl and epoxy functional groups on their basal planes (in addition to carboxyl and carboxyl groups located at the sheet edges). It is collected by flow directed vacuum filtration process into interlocking tile arrangement yielding free standing and highly flexible GO paper [47]. Thus, the presence of oxygen moieties provides GO sheets with the hydrophilic character and imparts versatile high volume processing possibilities from stable aqueous suspension into GO paper and other microscopic assemblies. They also heavily disrupt the conjugated sp<sup>2</sup> network of basal plane of single graphene sheet and drastically degrade their electronic features [48].

### Field-effect transistor biosensor

Graphene is a single layer of graphite that has the ability to solve the problem with CNT-based Field-effect transistor (FET). They are ideal two dimensional crystals that displays an extremely high mobility of  $\sim 10^4 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$  and large carrier capability of  $\sim 10^{12} \text{ cm}^{-2}$  even at room temperature without doping. As their electrical features are sensitively effected by surface conditions, gas molecule sensors by employing graphene field effect transistor (GFET) have been reported [49]. However, many electrical features of GFET have been investigated for the electrical detection of biological and chemical sensing using GFET. The mechanism of action for GFET sensors is their biological and chemical species absorbed on the graphene surface. It acts as electron acceptors or donor resulting in alteration in conductance. To realize GFET biological sensors, it is significant to investigate the electrical features under low electrical field because of avoidance of biomolecules oxidization [50]. The general size of graphene obtained by this route was 10  $\mu\text{m}$ . The formation

of Ti/Au (5 nm/30 nm) was carried out by conventional e-beam lithography, metal deposition, and lift-off route. The degenerately doped silicon substrate was also employed for black gate. Numerous pH solutions were made by mixing of 10 mM phthalate buffer solution at pH of 4, a 10 mM phosphate buffer solution at pH of 6.8, and 10 mM borate buffer solution at pH of 9.3. The leakage current between the Au electrode and solution was found to be negligible. Initially, the investigation was carried out on the transport behavior of GEFT in electrolyte. In an electrolyte, the electrical double layer acts as the top-gate insulator with thickness generally defined by Debye-Huckel equation. The thickness was dependent upon the ionic strength and was as small as 1–5 nm in an electrolyte with the concentration of several millimolar. The GFET was immersed in the electrolyte and silicon rubber was placed on GFET to allow the graphene surface channels to be filled with numerous buffer solution and analyses for sensing and electrical measurements [51].

## Summary

Graphene is an outstanding nanofiller with excellent electrical conductivity and durability under atmospheric conditions. Because of these properties, they have been used in almost all fields. Thus, the applications of graphene in biosensors have been summarized. The types of polymer/graphene have been used as glucose biosensor, electrical field transistor, electrochemical biosensor, and graphene paper. Accordingly, polymer/graphene nanocomposite has outstanding properties and has been employed on large scale in biosensors.

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