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Bio sensing of Graphene-based nanocomposites: A Comprehensive Review of Current Progress and Future Prospect

Kamrun Nahar Fatema[1] & Won-Chun Oh[1*]

Department of Advanced Materials Science & Engineering, Hanseo University, Seosan-si, Chungnam, Korea

Abstract: Biosensors with high sensitivity, selectivity and a low limit of detection, reaching Nano/picomolar concentrations of biomolecules, are important to the medical sciences and healthcare industry for evaluating physiological and metabolic parameters. Over the last decade, different nanomaterials have been exploited to design highly efficient biosensors for the detection of analyte biomolecules. The discovery of graphene has spectacularly accelerated research on fabricating low-cost electrode materials because of its unique physical properties, including high specific surface area, high carrier mobility, high electrical conductivity, flexibility, and optical transparency. Graphene and its oxygenated derivatives, including graphene oxide (GO) and reduced graphene oxide (rGO), are becoming an important class of nanomaterials in the field of biosensors. The presence of oxygenated functional groups makes GO nanosheet strongly hydrophilic, facilitating chemical functionalization. Graphene, GO and rGO nanosheet can be easily combined with various types of inorganic nanoparticles, including metals, metal oxides, semiconducting nanoparticles, quantum dots, organic polymers and biomolecules, to create a diverse range of graphene-based nanocomposites with enhanced sensitivity for biosensor applications. This review summarizes the advances in two-dimensional (2D) and three-dimensional (3D) graphene-based nanocomposites as emerging electrochemical and fluorescent bio sensing platforms for the detection of a wide range of biomolecules with enhanced sensitivity, selectivity and a low limit of detection. The bio functionalization and nanocomposite formation processes of graphene-based materials and their unique properties, surface functionalization, enzyme immobilization strategies, covalent immobilization, physical adsorption, bio interactions and direct electron transfer (DET) processes are discussed in connection with the design and fabrication of biosensors. The enzymatic and nonenzymatic reactions on graphene-based nanocomposite surfaces for glucose- and cholesterol-related electrochemical biosensors are analyzed. This review covers a very broad range of graphene-based electrochemical and fluorescent biosensors for the detection of glucose, cholesterol, hydrogen peroxide (H2O2), nucleic acids (DNA/RNA), genes, enzymes, cofactors nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP), dopamine (DA), ascorbic acid (AA), uric acid (UA), cancer biomarkers, pathogenic microorganisms, food toxins, toxic heavy metal ions, mycotoxins, and pesticides. The sensitivity and selectivity of graphene-based electrochemical and fluorescent biosensors are also examined with respect to interfering analytes present in biological systems. Finally, the future outlook for the development of graphene based bio sensing technology is outlined.

* Corresponding author*: wc_oh@hanseo.ac.kr*

Keywords: graphene; graphene oxide; reduced graphene oxide; electrochemical biosensor; instrumentation; surface Plasmon resonance; field-effect transistor; electrochemical; bio imaging.

Introduction

Biosensors with high sensitivity, able to detect femto- or picomolar concentrations of analyte molecules, are of paramount importance not only in biomedical applications such as glucose monitoring and clinical diagnostics1 but also in the agriculture. [2] and food industries [3] and environmental monitoring. [4,5] The development of highly sensitive devices and new approaches that can provide efficient point-of-care testing with high accuracy and low cost is an urgent need in the healthcare industry. [6–8] In addition, in vivo biosensors have received attention since they enable the long-term monitoring of target analytes within live cells with high sensitivity, selectivity and biocompatibility. [9,10] Notably, biosensor research is considered to be an important field since it covers a wide range of sensing capabilities, including pulse, heart rate, blood pressure, body motions, blood oxygen level, glucose, cholesterol, antibodies, nucleic acids, proteins, cancer cells, toxins in food products, and heavy metals in drinking water. [11–13] Numerous approaches have been explored, including colorimetric biosensors, [14] potentiometric biosensors, [15] electrochemical biosensors, [16] fluorescent biosensors, [17] and Raman spectroscopy-based platforms. [18] Compared with other detection methods, an electrochemistry- [19,20] and fluorescence-based [21] approach offers a much less expensive, more facile and highly sensitive detection method, which enables the monitoring of different analytes, fast response–recovery times and very low detection limits. [22] After the discovery of buckminsterfullerene (C60) molecules in 1985, the field of nanotechnology focused intently on developing new materials and devices within the 1–100 nm scale because nanoscale materials show unique chemical and physical properties compared to their counterpart bulk materials. [23] A wide variety of nanoscale materials have been developed, including zero-dimensional (0D) nanoparticles (such as metallic and semiconducting nanoparticles), [24,25] one dimensional (1D) nanostructures (nanowires, Nano rods, nanotubes), [26] and two-dimensional (2D) nanostructures including graphene nanosheet (GNs), transition metal di chalcogenides (TMDs), etc., [27] with substantial progress on their synthesis, processing, characterization and potential applications. Over the past two decades, these nanoscale materials have been used in many applications, including light-emitting diodes, memory devices, communication devices, magnetic disks, solar cells, batteries, fuel cells, super capacitors, and catalysts. [28,29] Interfacing with various probe biomolecules has been studied in order to develop highly sensitive biosensors with significantly enhanced sensitivity.30 Due to the size and unprecedented physical properties of these nanomaterials, the development of biosensors with extremely small dimensions and substantially improved performance is possible, introducing new opportunities in the development and commercialization of next generation biosensors for biomedicine and healthcare fields. [31] Since the initial isolation of graphene from bulk graphiteand characterization in 2004 by Geim and Novoselov, [32] intensive research efforts have been directed toward 2D graphene nanomaterials and their potential applications. [33–36] Graphene (GR) is a 2D sheet of carbons with atomic thickness that exhibits unique electrical, optical, mechanical and thermal properties. [37] Graphene nanosheet can be easily exfoliated from earth abundant graphite and are considered allotropically similar to fullerenes and carbon

nanotubes. Graphene can be easily processed into single-layer, few-layer or multi-layer nanosheet, [38] stretchable ultrathin films, [39] papers, [40] nanoribbons, [41] and foams. [42,43] Single-layer graphene nanosheet exhibit a high mechanical strength with a Young's modulus of 1.1 TPa, [40] thermal conductivity of _5000 W m K^{-1}, [44] high carrier mobility (200 000 cm^2 V^{-1} s^{-}1), [45] high optical transparency toward visible light (_2.3% absorption) [46] and a large specific surface area (2630 m^2 g^{-1}). [47] As a result, graphene-based materials (GBMs) have been explored for a wide range of applications, including bulk-heterojunction [48] and dye-sensitized solar cells, [49] energy storage devices, [50] electronic skin and touchscreen-panel devices, [51] field effect transistors, [52] light-emitting diodes (LEDs), [53] gas and chemical sensors, [54] Nano medicine, [37] drug delivery, [55] and many other applications.56 Graphene and its oxidized derivatives, such as graphene oxide (GO), which contain various oxygen functional groups (hydroxyl, carboxyl and epoxy functional groups), have emerged for potential use in biosensors. [57] The presence of these functional groups makes GO sheets strongly hydrophilic and allows the integration of various types of inorganic nanoparticles, including noble metals, metal oxides, semiconducting nanoparticles, quantum dots (QDs), and nanoclusters (NCs), to enhance the performance of sensors based on them. [58,59] Moreover, the reduction of GO into reduced GO (rGO) results in a high density of defects that leads to high electrochemical activity compared with that of CVD-grown graphene, which is particularly useful for developing electrochemical biosensors. Graphene-based nanocomposites also inherit unique morphological structures and properties useful for sensing. [60] The 3D interconnected hierarchical structures of graphene nanocomposites facilitate the diffusion of different types of biomolecules and preserve their bio catalytic functions to optimize bio sensing functionality. [61–63] Graphene-based hybrids with polymers [64,65] and surface-decorated metal nanoparticles58 have been explored for bio sensing due to their excellent biocompatibility, high surface area, and site-selective conjugation with biomolecules. Various nanostructures have been explored for biosensors, including the detection of glucose and hydrogen peroxide, [66] cancer biomarkers, [67–69] nucleic acids, [11] antibodies, [70] heavy metals, [71,72] pathogenic bacteria, [73] and many other targets [19,74–77] However, no comprehensive review focused on a wide range of electrochemical and fluorescent biosensors utilizing graphene based nanocomposites is yet available in the literature. There are no reviews on graphene-based fluorescent biosensors. Therefore, this review is intended to summarize the recent advances in both electrochemical and fluorescent biosensors based on graphene nanocomposites, including graphene, GO, rGO/polymer nanocomposites, graphene/inorganic NP nanocomposites, 3D graphene integrated with various metal/metal oxide nanoparticles, and polymer hydrogel networks. Biosensor systems to detect glucose, hydrogen peroxide (H_2O_2), cholesterol, dopamine (DA), ascorbic acid (AA), uric acid (UA), nucleic acids (NAs), cofactors nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP), cancer biomarkers, pathogens, food toxins, metal ions and pesticides are discussed. The discussion focuses primarily on advances in enzymatic and nonenzymatic electrochemical platforms as well as conceptual advances in fluorescent bio sensing and amplified detection techniques. The selectivity of graphene-based electrochemical and fluorescent biosensors are also examined in biological systems with respect to interfering analytes. This review will provide a single reference source for researchers in biosensors, graphene, materials science, nanotechnology,

chemistry, and electrochemistry. Therefore, this review will attract a wide range of audiences from diverse research areas and stimulate further interest in graphene-based biosensors for future sensor industries. The biosensor system illustrated in **Figure 1** shows a typical platform, consisting of a bio receptor interfaced with a transducer. The bio receptor has to be capable of recognizing the biomolecule element, for instance, enzymes, antibodies, DNA, RNA, and cells. In an incorporation, the biological signal that can be detected in various quantities by the receptor is transduced by physical, chemical, optical, thermal or electrochemical actions into observable information and analyzed quantitatively.

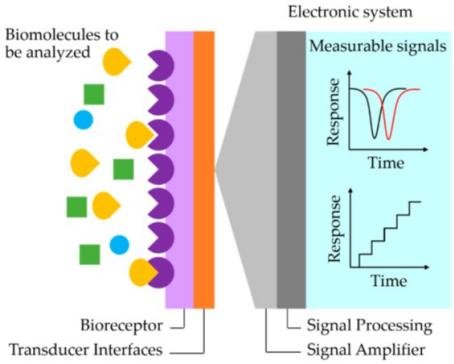

Figure 1. Schematic illustration of a typical biosensor system.

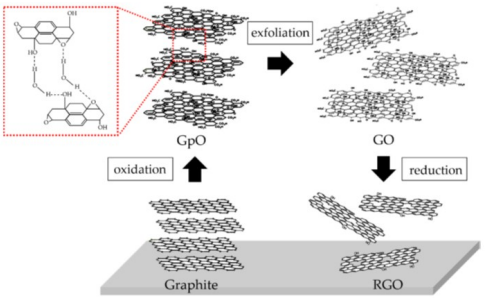

Figure 2. Figure 4. Schematic structure of chemical synthesis of graphite oxide (GPO), graphene oxide (GO); reduced graphene oxide (RGO). [78]

Experimental

Chemical Synthesis of Graphite Oxide and Graphene Oxide

(1) Brodie method (1859), the GpO was prepared using Ceylon as a raw material resulting in a purification to give 99.96% carbon. A boiled mixture of concentrated nitric and sulfuric acids called carbonic acid was used as an oxidizing agent. As observed from elemental analysis, the oxidized Ceylon graphite included C: O:H contents as 67.79:30.37:1.84 and the C-to-O ratio was 2.23. Therefore, the material was termed graphic acid, and was the very first sample of graphite oxide prepared experimentally.

(2) Staudenmaier method (1898), this method is very similar to Brodie's. The graphite oxide is prepared in a mixture of concentrated sulfuric acid and fuming nitric acid. In addition, potassium chlorate oxidizing agent is also added and reacted over 4 days. By rinsing in water and dispersing in diluted hydrochloric acid, sulfonate ions were removed. Finally, the graphite oxide was dried at 60 _C for 2 days. The graphite oxide prepared by this method was found to have an elemental composition of C: O:H of 58.73:23.28:17.99. The C-to-O ratio was 2.52, which indicated the lowest degree of oxidation; (3) The Hummers and Offeman method (1958) was developed when it was realized that the usage of nitric acid requires a lot of time for oxidizing graphite, has the potential for explosion and the release of highly corrosive vapor. The Hummers and Offeman method is a less hazardous way to oxidize graphite. The oxidizing agent is a mixture of concentrated sulfuric acid, sodium nitrate and potassium permanganate. The entire process needs 1–2 h to complete the reaction. As a result, the graphitic oxide had a C-to-O ratio between 2.1–2.9. The color of the product in aqueous solution is referred to the degree of oxidation. The product gives a bright yellow color for the most oxidized graphite while the green to black color refers to poor graphitic oxidation having too high C-to-O ratios. Currently, the Hummers and Offeman method is the most commonly used and is commonly known as the Hummers method. [78]

Result & Discussion

Graphene-based electrochemical biosensors

Enzymatic Biosensor

Detection and determination of biomolecules are clinically highly significant for diagnosis and treatment of various diseases. Enzymatic biosensor is a very well accepted system for sensing biomolecules based on their electrochemical reaction (oxidation or reduction) with the enzyme, which is immobilized on the electrode surface. The electrochemical output signal corresponds to the concentration of the analyte molecule. The analytical performance of such a biosensor mainly depends on the electron transfer between the metal active site of the enzyme and the electrode surface. **Figure 3** shows a schematic of enzymatic biosensor. Enzyme can be directly immobilized on the electrode surface to achieve direct electron transfer between the electrode and the enzyme. However, it might result in the denaturation of the enzyme and hence affect the biosensor response. To improve enzyme adsorption, improve stability and enhance the direct electron transfer, nanomaterials have been widely used as immobilization matrix, a mediator between the enzyme and the electrode. As explained in the Introduction, graphene and its oxidized derivative-based nanomaterials show excellent electrochemical properties, viz. high electrical conductivity, access to defect sites, large surface area, better electrocatalytic activity and excellent electron transfer rates, which are promising for fabricating enzymatic biosensors [80].

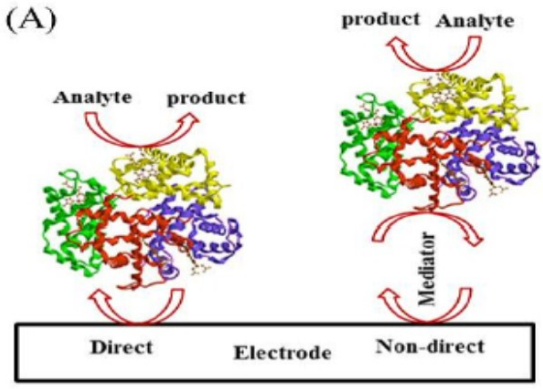

Figure 3. Schematic of the direct and mediated electrochemical biosensor. [80]

Non-Enzymatic Biosensor

Electrochemical detection of clinically important biomarkers using non-enzymatic electrodes fabricated using graphene-based nanomaterials is an another important application. non-enzymatic sensor for bilirubin detection, an important biomarker of jaundice, using ErGO. In a screen-printed carbon electrode. They found that the ErGO electrode performed much better than the MWCNT electrode in terms of detection limit, sensitivity, and range of detection. It is attributed to the faster electron transfer rate and higher electrical conductivity of ErGO. Further, the selectivity was ensured by using Nafion membrane coating. **Figure 4** shows a schematic of the non-enzymatic bilirubin sensor developed using ErGO and MWCNT. It offers a low-cost, reliable, and miniaturized point-of-care electrochemical sensor for bilirubin. Similarly, graphene nanomaterials based non-enzymatic electrodes were reported for the detection of dopamine, ascorbic acid (AA) and uric acid (UA). Detection of dopamine in the presence of AA is challenging due to the overlapping of electrochemical oxidation potential. [80]

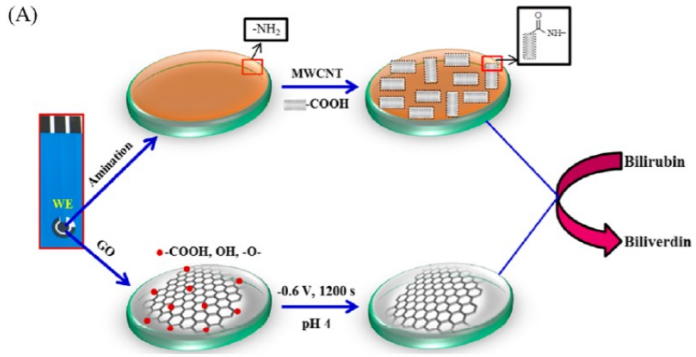

Figure 4. (**A**) Schematic showing the preparation of the MWCNT based (top row) or electrochemically reduced graphene oxide (ErGO) based (bottom row) bilirubin sensors.

Immunosensor

Graphene nanomaterial-based electrodes have also been used to develop electrochemical immunosensor. The specific interaction between the antigen and antibody confirms the high selectivity and sensitivity of the immunosensor. Electrochemical immunoassays are well known for their simplicity, high sensitivity and selectivity, large-scale manufacturability, volume miniaturization, and rapid analysis. It can be a sandwich type sensor or a label-free type immunosensor. Several electrochemical immunosensor were reported using graphene nanomaterials for the detection of well-known biomarkers such as carcinoembryonic antigen (CEA), Interleukin-6 (IL-6), human chorionic gonadotropin (hCG), and prostate specific antigen (PSA). **Figure 5A** shows a schematic of the sandwich immunosensor electrode in which four different antibodies were immobilized that are specific to their antigens. Mao et al. used label-free electrochemical immunosensor to detect PSA using graphene sheet–methylene blue–chitosan nanocomposite. Figure 5B shows a schematic of the label-free electrochemical immunosensor fabrication. [80].

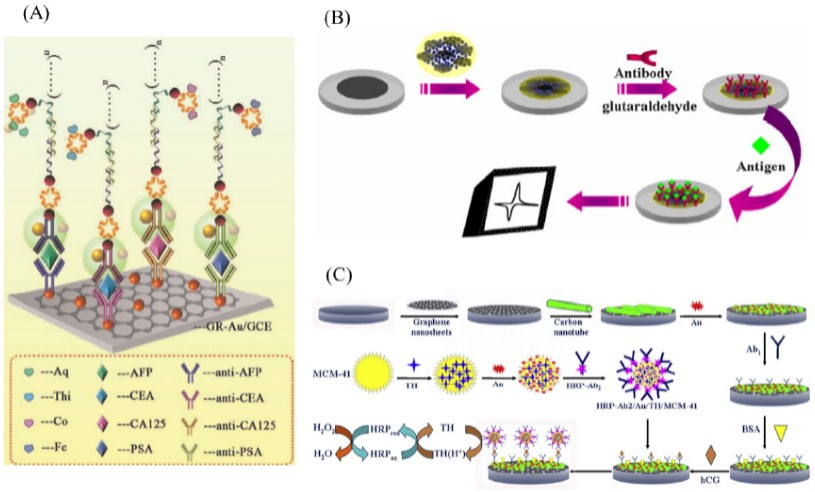

Figure 5. (**A**) Schematic illustration of the principle of sandwich-type simultaneous detection of four antigens. (**B**) Fabrication steps of the label-free electrochemical immunosensor. (**C**) Fabrication process of Au/TH/MCM-41 nanomaterials and the measurement protocol of the electrochemical immunosensor. [80]

Glucose biosensors

The development of electrochemical glucose sensors to date can be categorized into three generations. In the first generation of glucose enzyme electrodes, the measurements relied on the oxygen consumed by the enzyme-catalyzed reaction. Specifically, an enzymatic reaction occurred between glucose and the GOx enzyme electrode in the presence of oxygen, producing hydrogen peroxide (H_2O_2), and the glucose level was monitored through the amount of enzymatically generated H_2O_2. However, Enzyme-based electrochemical glucose sensors have attracted significant attention over the past 40 years because of their high selectivity, simplicity, and sensitivity. Enhanced electrical contact of redox enzymes with the electrode surface is of fundamental interest for the development of mediator-free third-generation electrochemical glucose biosensors with high sensitivity and selectivity. Depending on the graphene derivative,

the conductivity and electrocatalytic activity can differ significantly. For example, the electrical properties of graphene can change upon reduction to GO or rGO as well as upon chemical functionalization. Graphene-based materials provide a large specific surface area, excellent electrochemical properties, biocompatibility and plentiful oxygenated functional groups such as hydroxyl, carbonyl, carboxyl and epoxy groups, all of which facilitate the effective immobilization of redox enzymes through either physical adsorption or covalent conjugation for subsequent glucose sensing. Graphene-based nanocomposites have been widely used to fabricate enzymatic glucose biosensors the **Figure 6** showing schematic fabrication of glucose biosensor [79]

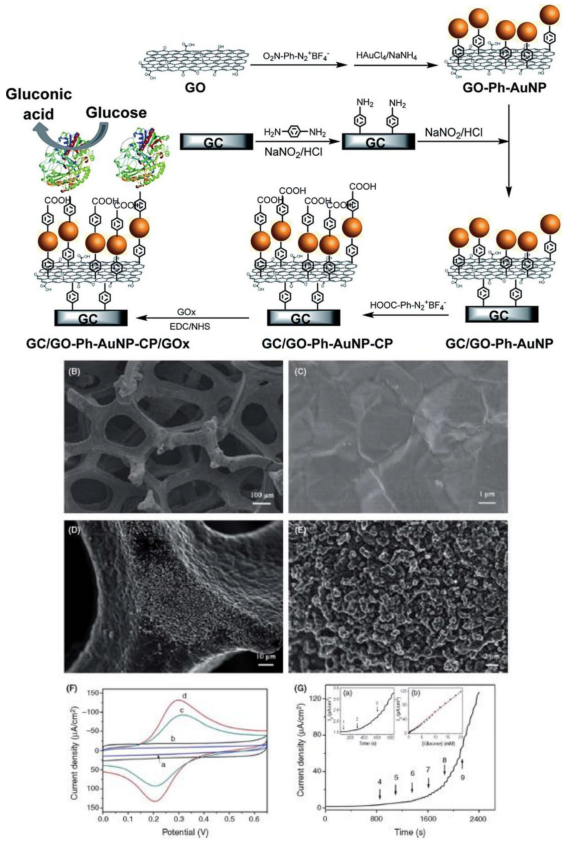

Fig. 6 (A) The schematic fabrication of glucose biosensor using AuNP-decorated GO nanosheet. AuNPs were decorated onto GO nanosheet via a benzene bridge using aryldiazonium salt chemistry (GO-Ph-AuNPs) which was thereafter attached to 4-aminophenyl modified GC electrode. The GC/GO-Ph-AuNPs was further functionalized with 4-carboxyphenyl (CP) before covalently attaching GOx via amide bonds to form GC/GOPh- AuNPs-CP/GOx based glucose sensor.
Schematic illustration of the preparation of 3D-GR-based enzymatic glucose biosensors using (B and C) SEM images of 3D-GR foam with low and high magnification. (D and E) SEM images of Fc-CS/SWCNTs/GOx composite film electrodeposited on 3D graphene with low and high magnification. (F) CV curves of the (a) 3D-GR, (b) CS/GOx/3D-GR, (c) Fc-CS/GOx/3D-GR and (d) Fc-CS/SWCNTs/GOx/3D-GR electrodes in PBS (0.1 M, pH 7.0) at a scan rate of 100 mV s^{-1}. (G) Amperometric response of the Fc-CS/SWCNTs/GOx/3D-GR electrode upon the successively added glucose to stirred PBS (0.1 M, pH 7.0) at 0.4 V. Inset (a) shows the magnified curve from 50 to 850 s. Inset (b) shows the calibration plot of the current as a function of the glucose concentration. [79]

Biosensor Devices Using Graphene-Based Materials and Current Progress

Previously, the structures and outstanding properties of graphene-based materials using different synthesis methods have been exploited in bio sensing applications since graphene is a semi-metal with ultra-high charge mobility giving excellent electronic properties, having large surface area, being capable of being functionalized on its surface. There are many possible approaches to engineer the receptor for targeting biomolecules. In the biomedical field, pristine graphene is not only referred to as an oxide-free graphene presenting stacking, non-covalent interactions and high electrostatic force, but it also offers an infinite surface at a molecular level. Therefore, graphene provides for a high possibility of active sites for charge-bimolecular interactions due to the large specific surface area leading to a sensing enhancement as well as supporting the desired functionalization to target biomolecules to improve the selectivity. Figure 7 illustrates the points of view of the possible interactions of the graphene-based material system. Figure 8 Schematic illustrations of graphene-based biosensors. For example, the pure graphene area as shown in the figure can provide a charged area to absorb any charged molecules or metal ions as well as interactions at a vacancy defect. The functionalized graphene area is able to directly detect the biomolecules by its own oxide components due to the synthesis in which lots of epoxide, hydroxyl and carboxyl groups are formed on the edge and surface sites. In addition, the functionalized graphene allows binding of heteroatoms, nanoparticles (NPS), quantum dots (QDs), DNA, enzymes, proteins, antigens, antibodies, and other specific molecules [78]

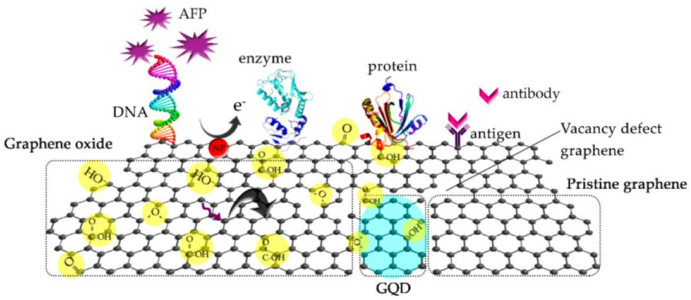

Figure 7. Schematic illustration of the graphene-based materials that can be immobilized with biomolecules as the receptor. [78]

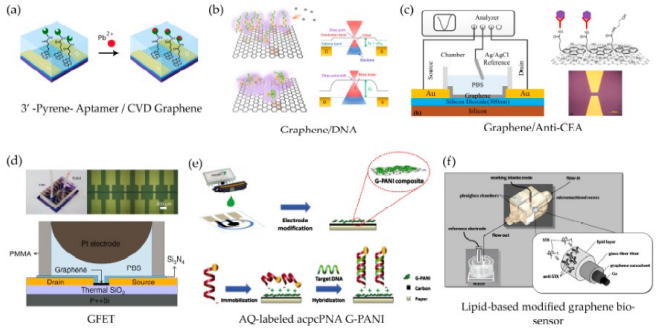

Figure 8. Schematic illustrations of graphene-based biosensors: (**a**) Pb2+ in blood biosensor based on GFET (**b**) Pb2+ biosensor based on graphene/DNA (**c**) CEA protein biosensor based on graphene/anti-CEA (**d**) real-time binding kinetics and affinity of DNA hybridization based on GFET (**e**) paper-based biosensor for human papillomavirus (HPV) detection and (**f**) a lipid-based modified graphene electrochemical biosensor [78].

Conclusions

In this review, we discuss our points of view on the intrinsic properties of graphene and its surface functionalization concerned with the transduction mechanisms in biomedical applications. We also explain several well-known techniques used for the synthesis of graphene-based materials and their properties. A variety of graphene-based materials have been made consisting of pristine graphene and the functionalization of graphene oxide, reduced graphene oxide and graphene quantum dot. The mechanisms are discussed with respect to the most recent bio sensing devices for drug delivery, biosensors, healthcare sensors, bio imaging, and other novel techniques. Graphene is one of the most well-known 2D materials. The major characteristic and properties of graphene are outstanding, i.e., zero-bandgap semiconductor, linear-like at the Dirac point, relativistic-like charge velocity, ultra-high charge mobility, transparency, large surface area, non-toxicity, having proximity induced ability, high tensile strength and high thermal conductivity, etc. However, to maintain those properties, graphene has to be perfectly proper. Alternative techniques by chemical synthesis that serve for ambient circumstances are also presented. In the synthetic procedures, the graphite is modified and functionalized by various oxygen-containing groups. In addition, the functionalized graphene is always contaminated by impurities, defects, and disorder. Hence, the structure of graphene would be importantly changed, especially the electronic properties will be distorted. On the other hand, the presence of the oxygen-containing groups and the ability to functionalize onto the graphene-based structure are important in the electrochemistry of the bio sensing applications such as for labeling biomolecule recognition, for enhancing the sensing signal, for increasing the number of active sites and active area, and for probing biomolecules in an imaging application. A novel variety of the graphene-based biosensors is presented in the last section. The engineering of bio sensing platforms, the mechanisms and techniques are discussed. Many new approaches are discussed in detail in this review. Since graphene material has been very well established, other 2D materials have now been explored. This opens up a wide range of possibilities and plays a crucial role in sensor and biosensor applications utilizing the largest surface area. In the upcoming future, these 2D novel materials will be further developed and tailored for specificity of bio receptors. These materials can be employed and integrated in different sensor and biosensor platforms giving an ultra-high sensitivity and may provide a solution to some challenges, such as early stage cancer detection.

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