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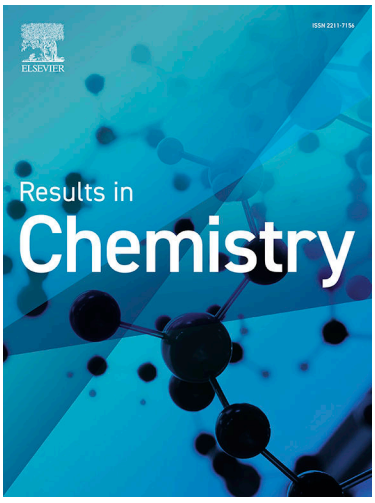

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Graphene oxide-based biosensors for detection of lung cancer: A review

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Abstract

Lung cancer is one of the most common diseases worldwide today and has the highest mortality rate among cancers. Therefore, early diagnosis of this disease is of special importance. Due to the fact that common methods for detecting lung cancer are costly and time-consuming, providing cheaper and faster methods has received special attention. With the significant development of nanotechnology in recent years and the development of various nanomaterials, activities have been carried out in this field. Recent studies show that graphene oxide nanomaterials, due to their unique properties, have a high potential in the design of biosensors to detect lung cancer.

Keywords: Lung cancer, Nano Biosensor, Graphene oxide, Nanohybrid, DNA, nanotechnology.

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1. Introduction

Cancer is a genetic disease that results from the uncontrolled growth and division of cells in a part of the body that results from environmental factors and genetic disorders [1-5]. In other words, cancer occurs as a result of a series of mutations in human genes [6-10]. There are more than 200 types of cancer today, one of the most common of which is lung cancer [11-15]. Lung cancer is the second most common cancer in men and women and is one of the most preventable cancers. There are generally two types of lung cancer [16-21]:

1)Small cell lung cancer (SCLC)
2) Non-small cell lung cancer (NSCLC)
How both grow and spread in the body and how to treat them are different. Lung cancers are classified under the microscope based on the appearance of the cells. Non-small cell lung cancer (NSCLC) is also divided into three categories [22-25]:
1) superficial tissue cancer, 2) mucosal and lymph node carcinoma (glandular epithelium), and 3) large cell lung cancer [26-30]. Among people with this type of cancer, about 85-90% of cases are non-small cell lung cancer and about 10-15% of cases are small cell lung cancer.
The most common clinical symptoms of lung cancer include persistent and chronic cough, chest pain, anorexia, weight loss, sputum, shortness of breath, respiratory infections such as bronchitis, the onset of wheezing and ... Is, which usually does not appear in the early stages of the disease. Therefore, the mortality rate of this type of cancer is very high [31-34]. The most prevalent symptoms of lung cancer include continuous and chronic cough, thoracic pain, anorexia, weight loss, hemoptysis, dyspnea, and respiratory infections such as bronchitis, the onset of wheezing, etc., which do not typically appear in the early stages of the disease, leading to a high mortality rate in this type of cancer [35-38].
In the field of medicine, lung cancer has so far been detected using various methods, including chest radiography (x-ray), computed tomography (CT) scan, magnetic resonance imaging (MRI), bone scan, bronchoscopy, and sputum cytology [39-43]. In recent years, the dramatic advances in nanotechnology and the development of various nanomaterials have facilitated the detection of cancer biomarkers with high accuracy and sensitivity [44-47]. Nanotechnology has provided faster, less expensive, and easier methods with lower detection limits for lung cancer detection. Nanomaterials used in these methods include silica nanowires, gold nanoparticles, carbon nanotubes, quantum dots, magnetic nanoparticles, etc. [48-52,102-106].
Biomarkers are an indicator of the disease biological condition that are used to detect the disease. These biomarkers are also used to study cellular processes and to recognize and control cessation, or changes in the cellular processes of cancer cells [53-57,107-111].

Biomarkers can be proteins, mutated DNA, RNA, lipids, carbohydrates, and small molecules resulting from cellular metabolism [58-60,112-118]. Table 1 exemplifies some of these biomarkers [14].

Table 1. Biomarkers used in the detection of various cancers [14].

Biomarker	Type of cancer
PSA, PSMA	prostate
CEA, NSE	Lung
CA 19-9, BTA	Pancreas
NMP22, BTA	Bladder
CA 15-3, 27, 29, Her-2/neu	Breasts

Since graphene oxide (GO) is readily available and exhibits exceptional optical, electrical, mechanical and chemical properties, it has attracted increasing interests for use in GO-DNA based sensors [60-65]. This paper reviews the advances in GO-DNA based sensors using DNA as recognition elements. In solution, GO is as an excellent acceptor of fluorescence resonance energy transfer (FRET) to quench the fluorescence in dye labeled DNA sequences. This review discusses the emerging GO-DNA based sensors related to FRET for use in the detection of DNA, proteins, metal ions, cysteine (Cys), and others [66-70]. The application of the electrochemical GO-DNA based sensors is also summarized because GO possesses exceptional electrochemical properties. The detection mechanisms and the advantages of GO are also revealed and discussed. GO-DNA based sensors perform well at low cost, and high sensitivity, and provide low detection limits. Additionally, GO-DNA based sensors should appear in the near future as scientists explore their usefulness and properties. Finally, future perspectives and possible challenges in this area are outlined [71-75]. Table 2 show Summary of graphene oxide -based biosensors using various identification techniques.

Table 2. Summary of graphene oxide -based biosensors using various identification techniques [191].

identification techniques	Characteristics of graphene oxide	aim

fluorescence resonance energy transfer	(1) Strong binding with biomolecules through pi-pi stacking and/or hydrogen bonding interactions (2) the fluorescence-quenching capability of nearby fluorescent dye	signal amplification. Biomolecule identification. enzyme assay. cell/tissue imaging. high-throughput screening.
laser desorption/ionization mass spectrometry	(1) Strong absorbance at the excitation laser wavelength of 337 or 355 nm, (2) high affinity toward various amphiphilic biomolecules (electrostatic/hydrophobic/pi-pi stacking interaction), (3) easy protonation of analytes by functional groups on graphene oxide	Biomolecule identification. high-throughput screening enzyme assay. cell/tissue imaging.
Electrochemistry	(1) Outstanding electrocatalytic ability, (2) low charge-transfer resistance	signal amplification. Biomolecule identification. enzyme assay. cell/tissue imaging.
surface-enhanced Raman spectroscopy	(1) Quenching the background fluorescence signal, (2) chemical enhancement in surface-enhanced Raman	Biomolecule identification.

	spectroscopy induced by electron transfer	cell/tissue imaging.

The purpose of this review article, Lung cancer is one of the most important and common types of cancer worldwide. More than 80% of cases are diagnosed in the advanced stages of the disease. Since the diagnosis of this type of cancer in the early stages is very useful in the treatment of the disease, so its early diagnosis is of particular importance. The aim of the present study was to fabricate a biosensor based on graphene oxide-DNA nanohybrid to detect lung cancer. During which the elimination mutations in the cancer cells are detected. The advantages of the proposed method include its high speed, simplicity and cheapness, and the possibility of detecting the disease in the early stages.

2. Risk factors

Tobacco use, particularly smoking, is the crucial risk factor for lung cancer development [76-81] as almost 90% of patients with lung cancer are smokers, and lung cancer development risk is about 20-40 times higher in smokers than in non-smokers. Tobacco use is reported to be the leading cause of lung cancer development in 79% of women and 90% of men, and 90% of mortalities caused with lung cancer occur due to tobacco use [82-87]. After tobacco use, the second leading cause of lung cancer is radon gas [6], which is radioactive, odorless, tasteless, and colorless and is naturally formed from the breakdown of uranium in soils and rocks. According to figures, the annual exposure to this gas is involved in over 20,000 deaths caused with lung cancer in the USA [88-92]. Polycyclic aromatic hydrocarbons, arsenic, asbestos, cadmium, beryllium, compounds containing nickel and chromium, chloromethyl ethers, etc. are among the other factors that increase the risk of this cancer and exist in the workplace [93-98]. Other causes of lung cancer include air pollution, family history of lung cancer, lung radiation therapy, poor diet, old age, and inherited and acquired genetic changes, etc[99-103].

3. Genetic changes in the lung cancer cause

In recent years, scientists have made considerable advances in detecting the effect of risk factors of=n changes in DNA and genes that result in the carcinogenesis of cells [104-109]. They have determined the stages of cancer generation with the involvement of several mutated genes; these genetic changes disrupt the natural order of cell division and differentiation [110-115]. Lung cancer often results from a series of genetic changes, including the activation of proto-oncogenes and their conversion to oncogenes, inactivation of tumor suppressor genes (TSGs), etc[116-121]. Proto-oncogenes are genes responsible for cell division and growth in their natural state and are called oncogenes upon a genetic mutation, with very high gene expression levels [122-126]. TSGs are genes that decelerate cell division and determine the death time of cells. The lack of TSGs leads to uncontrollable cell division [127-131]. Oncogenes involved in lung cancer include c-myc, mutated kras (observed in none of SCLC cases but observable in 15-20% of NSCLC cases and most cases of adenocarcinoma), overexpressed egfr gene, cyclin D1, BCL2, etc[132-

137]. TSGs responsible for most cases of lung cancer include p53 (observed in 90 and 50% of SCLC and NSCLC cases, respectively), Rb (present in 90 and 20% of SCLC and NSCLC cases, respectively), p16 (detected in > 50% and < 1% of NSCLC and SCLC cases, respectively), etc. The hTR and hTERT genes are expressed as a perpetual mechanism in all types of lung cancer [138-143].

3.1. The importance of lung cancer detection

Lung cancer is the most important and widespread cause of cancer-related death among men and women. The high mortality of the cancer is caused with high disease development and a low chance of survival [144-150]. The American Cancer Society has recently reported over 226,000 new cases of lung cancer and more than 160,000 deaths caused with this cancer in the USA, which account for about 27% of deaths induced with various cancers [151-155]. According to global figures, more than 1,000,000 people lose their lives annually due to this type of cancer [156-162]. More than 80% of lung cancer patients lose their lives within less than 5 years after detecting the disease [163-164] as most of them perceive the disease during the advanced stages of the disease when its treatment is hardly possible. Hence, the early detection of lung cancer is of paramount importance [165-170].

3.2. Lung cancer detection methods

In the field of medicine, lung cancer has so far been detected using various methods, including chest radiography (x-ray), computed tomography (CT) scan, magnetic resonance imaging (MRI), bone scan, bronchoscopy, and sputum cytology [171-176]. In recent years, the dramatic advances in nanotechnology and the development of various nanomaterials have facilitated the detection of cancer biomarkers with high accuracy and sensitivity [177-183]. Nanotechnology has provided faster, less expensive, and easier methods with lower detection limits for lung cancer detection. Nanomaterials used in these methods include silica nanowires, gold nanoparticles, carbon nanotubes, quantum dots, magnetic nanoparticles, etc. [184-189].

Biomarkers are an indicator of the disease biological condition that are used to detect the disease. These biomarkers are also used to study cellular processes and to recognize and control cessation, or changes in the cellular processes of cancer cells [190-194]. Biomarkers can be proteins, mutated DNA, RNA, lipids, carbohydrates, and small molecules resulting from cellular metabolism [195-199].

According to database generated by International Agency of Research on Cancer (IARC) Global Cancer Observatory in 2018, they quoted the incidence and mortality rates across 185 countries and 36 types of cancers, in which lung cancer ranked at the top for the deaths in men and third most causes for the deaths in women. Almost 9.6 million cancer deaths reported in 2018 in which approximately 1.8 million deaths, about 18.4% of the total deaths due to lung cancer [200-205]. The worrying rise in deaths due to lung cancer and excessively high prevalence in nature, various cancer control research, and early detection methods have been introduced to control the mortality [206-211]. Typically, lung cancer cure depends upon detection of disease at the initial stage, and effective diagnosing methods result in decrease incidence rates for lung cancers. Currently, the treatment of lung cancer can be done through seven techniques such as chest radiographies (CXRs), computed tomography (CT) scans, magnetic resonance imaging (MRI),

positron emission tomography (PET), and cytology sputum and breath analysis [212-216]. All the available detection techniques of lung cancer have different detection levels and various markers as shown in Table 3. These methods have certain demerits as well, e.g., CXR, septum, and CT are prone to radiation, whereas MRI and PET have limitations in detection and staging lung cancer. Moreover, serum is an invasive technique and the sensitivity and specificity of this technique not high enough for early detection that leads to unacceptability [217-221]. In contrast, sputum had enough potential to detect lung cancer at the initial stage due to gene promoter methylation but still needed more evaluation [222-225]. Besides, VOC in urine showed high sensitivity and specificity but required a more significant number of study samples whereas CXR has low sensitivity and causes high false negative values rate. The most effective technique nowadays in lung cancer detection is CT imaging that contains detailed information about locations and size of nodules. The low-dose CT screening detected the tumors at the early stage of cancer. It resulted in the reduction of mortality by 20.0% and the significant increase in the rate of positive screening tests compared with traditional radiography techniques. Lung cancer disease can be diagnosed when the nodule is small and localized. Mostly the lung nodule size is tiny, approximately 3 mm in diameter. Based on CTs, radiologists classified nodules as malignant and benign. This involves the meticulous examination of 3D-lung voxels by slicing into the number of 2D slices. Due to the incorporation of a huge amount of information in CTs, the analysis must be more precise and accurate to classify nodules into malignant and benign. Typically, examining 3D lung voxels slice by slice from 2D CTs by radiologists is a rigorous task as a CT scan contains an enormous amount of information about lung nodules and gave rise to difficulty in interpretation and identification of cells from CTs images. The detection of lung nodules suffers a lot due to human error. Hence, the diagnosis of lung cancer has been automated using CAD systems as radiologists' assistance for the accurate detection and classification of malignancies. It has been shown that it is a promising approach as a radiologists' assistance.

Table 3. Early detection methods of lung cancer [122].

Method	Detection level	Markers
Breath analysis	Molecular level	Volatile and non-volatile-organic compounds
CTs	Tissular level	Neoplastic tissue
CXR	Tissular level	Neoplastic tissue
PET	Tissular level	Neoplastic tissue
Serum test	Cellular or molecular level	Circulating tumor cells, circulating DNA, plasma proteins, telomerase, etc

Serum test	Cellular or molecular level	Abnormal cells and methylated gene promoters
Urine test	Molecular level	Urine volatile odorants

3.2.1. engineered biomimetic membranes

There are plenty of bio membranes in the human body with different functions such as preserving cells and tissues from foreign microorganisms, determining and choosing various biological compounds to penetrate, transporting nutrition and wastes, communicating between the intra- and extra-cellular matrix, and so on. The bio membranes are composed of chemical and/or biological components to adjust and regulate the concentrations and interactions of small molecules, enzymes, proteins, genetic materials, and even tissues and organs. There are two major types of barrier tissues in the human body. The first type, including skin, lung, and gut, provides the interface to the outside of the human body. The second type separates the chambers inside the human body, for instance, the blood− brain barrier and kidney. Barrier tissues manage homeostasis and prevent the occurrence of dangerous impacts on organs. In vitro barrier models are used to assess whether a substance can reach a particular compartment to show intentional or unintentional effects and examine its impact on the barrier integrity. Most of the artificial and engineered membranes are inspired by the natural cell membranes for various applications. The unique capabilities of cell membranes include mass transfer, energy conversion, and signal transduction. The cell membrane is the boundary that intercepts the inner space of the cell from the outside environment and plays a critical role in all cellular activities and functionalities. Cell membranes are mainly based on the bilayer of lipid amphipathic including phospholipids, glycolipids, cholesterols, and cholesterol esters, and embedded proteins such as anchored lipid proteins, integral proteins, and peripheral proteins, plus carbohydrates like polysaccharides and oligosaccharides. Pathological changes in the chemistry, structure, and functions of bio membranes like fluid or gas permeability, charge potential, and fluidity lead to many kinds of diseases. Natural human biomembranes are complex structures that have intra- and extra-cellular connections and intricate functionalities. Artificial biomimetic membranes or engineered bio membrane systems mimic the natural bio membrane properties allowing to understand various functionalities and opening a new avenue toward tissue engineering. Bio membrane engineering is a strategy to generate biological membranes in the laboratory for understanding the mechanism of natural bio membranes like cell membranes and developing more efficient treatments for diseased tissues. The previous studies mainly have taken place on natural membranes originating from various pre-existing tissues. Due to the shortage of natural tissues, researchers have introduced artificial or engineered biomembranes. Artificial biomembranes can mimic biological constructions with a dissipative particle dynamic. Artificial membranes can be applied as a model to assess potential drugs for transdermal delivery. They appeared to be useful in tracking the bioavailability of chemicals from various vehicles. Biomimetic and biochemical membranes have a great potential for the design of artificial tissue-engineered organs.

3.2.2. Silica nanowires (SNWs)

SNWs are one-dimensional structures measuring below 100 nm in two dimensions and more in one dimension [185-190]. These nanostructures are synthesized using the vapor–liquid–solid (VLS) growth procedure and have received much attention with researchers owing to their specific physical, optical, and electronic properties, photoluminescence, high surface-to-volume ratio, and biocompatibility [191-195]. A SEM image of SNWs with an average diameter of 200 nm is shown in Fig 1 [17]. To detect lung cancer using SNWs, free NH_2 groups should be first produced through the functionalization of SNWs with 3-aminopropyltrimethoxysilane (APTMS). Then, capture antibodies of specific cancer antigens are then stabilized on the surface of SNWs (Fig.2). A blocking protein is used to control non-specific bindings. Cancer antigens in the sample attach to those on the surface of nanowires, and then detector antibodies containing alkaline phosphatase (AP) attach to cancer antigens [55-57]. This binding causes the conversion of para-nitrophenyl phosphate (PNPP) to para-nitrophenol (PNP) influenced with AP (fig.3). As an electro-active compound, PNP participates in electrochemical reactions and, finally, the amount of the cancer antigen in the sample is determined using the voltammetry technique [196-200]. A sample voltammogram recorded using this method (Fig. 4) shows no peaks in the absence of a cancer antigen. In this detection method, interleukin-10 (IL-10) and osteopontin (OPN) cancer antigens are used as lung cancer biomarkers, with limits of detection (LOD) of < 1 $fgml^{-1}$ and 1 $pgml^{-1}$ for ideally pure and clinical samples, respectively [18].

Figure 1. A SEM image of SNWs [17].

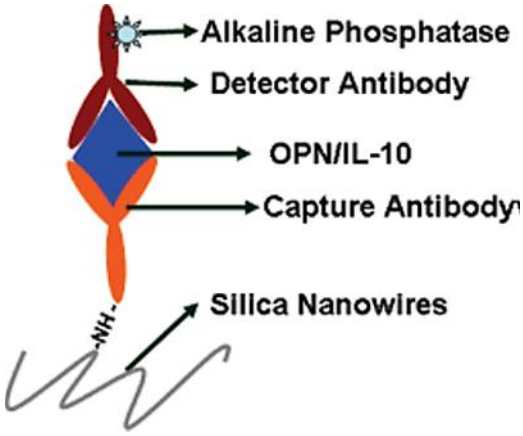


Figure 2. The protocol used in the present method [17].

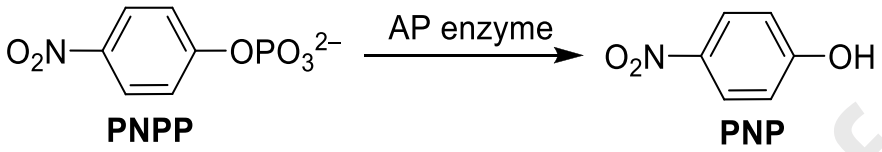


Figure 3. The enzymatic conversion of PNPP to PNP in the presence of AP [17].

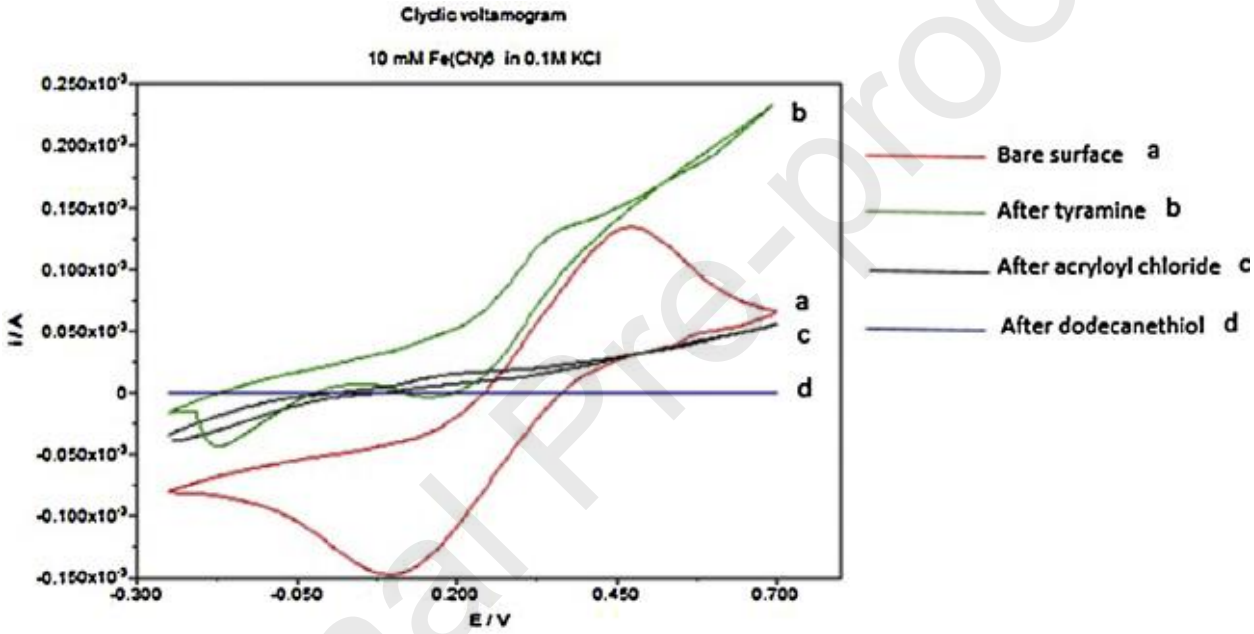


Figure4. Recorded voltammograms for samples without cancer antigen [17].

3.2.3. Gold nanoparticles (AuNPs)

Lung cancer is detected using AuNPs in several steps. In the first step, the exhaled air is collected from cancer patients and, in the second step, volatile organic compounds (VOCs) in the exhaled air of cancer patients, considered as lung cancer biomarkers and also the reactive oxygen species are known as one of the important agents resulting in lung cancer, are identified using specific techniques. In the next steps, sensors are designed based on AuNPs in which AuNPs measuring 5 nm are functionalized with various organic groups, such as decanethiol, 1-butanethiol, 2-ethyl hexanethiol, hexanethiol, 2-mercaptobenzoxazole, etc. Then, the functionalized AuNPs are placed on the surface of ID electrodes. A TEM image of the functionalized AuNPs in these sensors is depicted in Figure 5 AuNPs and their surrounding organic groups appear as dark and bright spots, respectively. Metal NPs cause electrical

conductivity, and organic molecules provide sites for the adsorption of VOCs. In the final steps, designed sensors are installed in a circuit inside a chamber where the exhaled air is directed, and the exposure of sensors to the exhaled air causes a reversible change in the circuit impedance. The response of sensors to exhaled air samples of cancerous and healthy individuals is represented with drawing diagrams, an example of which is shown in Figure 6 These diagrams show different impedance changes for cancerous and healthy samples, allowing the detection of cancer.

Figure 6 illustrates the response of sensors containing AuNPs functionalized with 2-mercaptobenzoxazole (red rhombi) and tert-dodecanethiol (black triangles). Exposure to exhaled air samples of cancerous (blank shapes) and healthy (solid shapes) individuals increases the impedance, with a greater elevation for cancerous samples. It is noteworthy that the gray and green parts of the diagram indicate the exposure of sensors to vacuum and the samples, respectively.

Since the sensitivity of sensors to lung cancer biomarkers is rarely affected in the presence of water molecules, and people's exhaled air contains almost 80% relative humidity, the sensors have good efficiency, as confirmed in Figure 7. LODs of 1-5 ppb have been determined for most sensors; for example, LODs of about 2-10 ppb are reported for sensors containing NPs functionalized with 2-mercaptobenzoxazole and 4-methoxy-toluene-thiol [20].

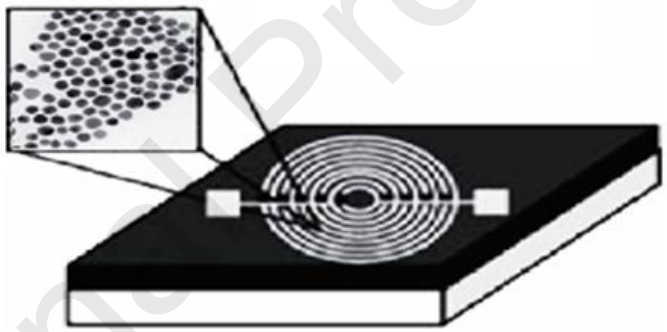


Figure 5. Sensors based on AuNPs [19].

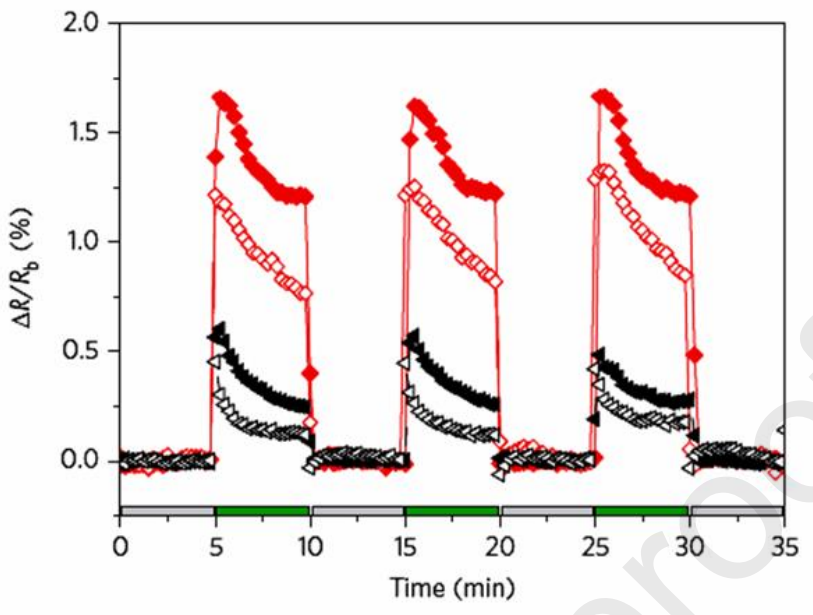


Figure6. The response of sensors to cancerous and healthy samples [20].

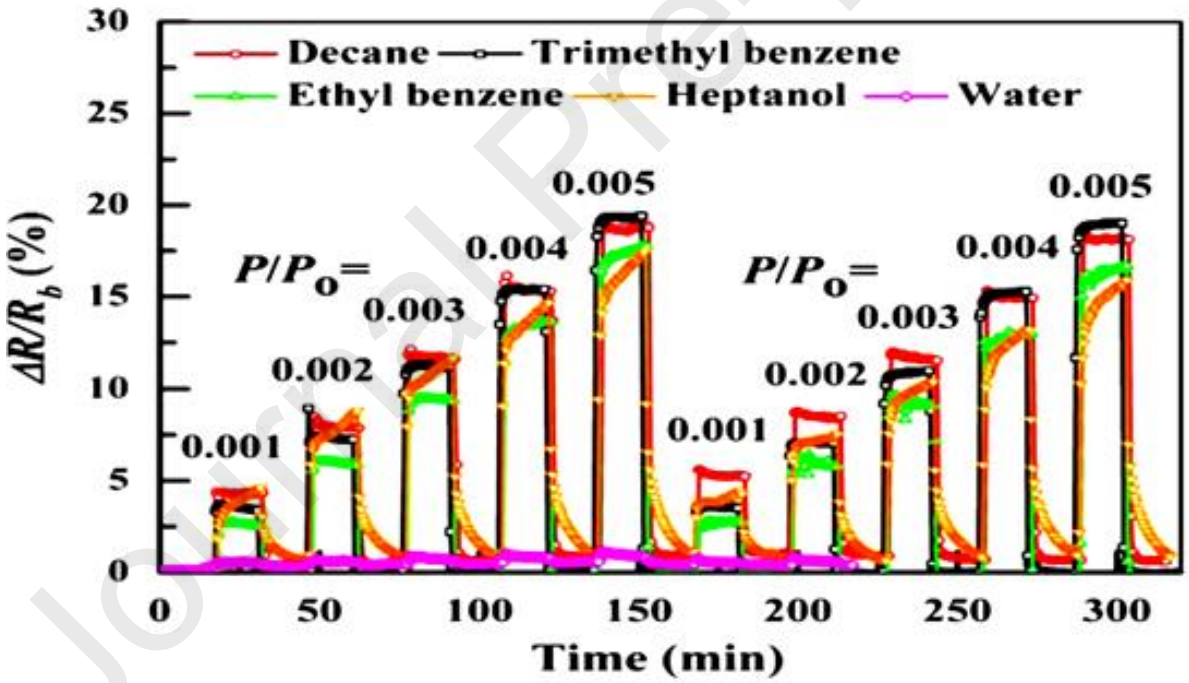
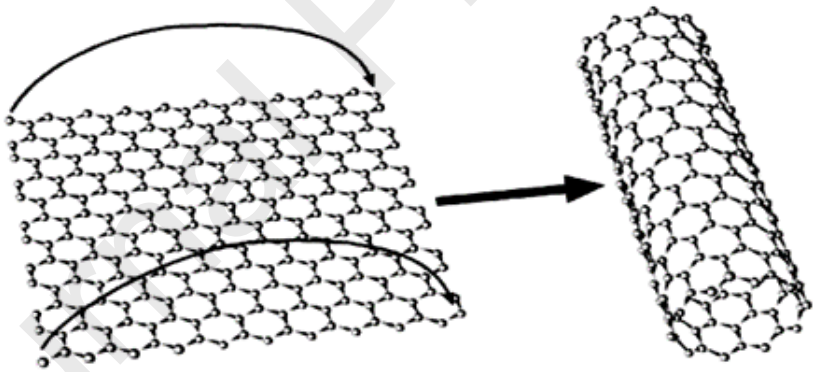


Figure 7. The response of sensors to four lung cancer biomarkers at different concentrations and water [20].

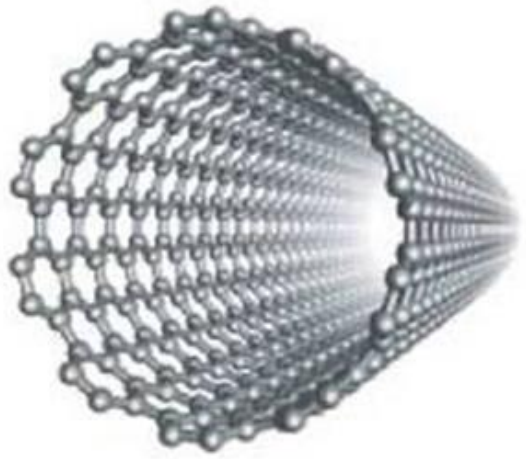
3.2.4. Carbon nanotubes (CNTs)

First discovered by Iijima (1991), CNTs consist of graphene sheets enrolled in cylindrical structures. The formation procedure of CNTs from graphene sheets is shown in Figure 8(a) [21]. These nanotubes are structurally classified into two groups, SWCNTs and MWCNTs, with the former and the latter consisting of a single cylindrical graphene sheet and multiple concentric cylindrical graphene sheets, respectively. The structures of SWCNTs and MWCNTs are depicted in Figures 8(b-c) respectively [24]. SWCNTs have an internal diameter of 1-2 nm, with a corresponding value of 2-25 nm in MWCNTs having an interlayer distance of 0.36 nm. The length of these one-dimensional nanostructures varies from 1 µm to a few hundred µm. Owing to important features, such as a high surface area, high mechanical strength, low weight, high thermochemical stability, specific electronic properties, etc., CNTs are widely used in the biomedicine field. A kind of biosensor has been designed to detect lung cancer using SWCNTs, which are functionalized using a non-polymer organic substance, pentadecane ($C_{15}H_{32}$) or trioxane ($C_{23}H_{48}$), to increase the biosensor selectivity (Fig8(d). These functionalized nanotubes are then placed on the surface of ID electrodes (Fig8(e), which as biosensors are placed in the test device (Fig8(h).

In this method, VOCs in the exhaled air of cancer patients are also considered as lung cancer biomarkers [83-86]. Due to high absorbability, CNTs adsorb VOCs in the exhaled air of cancer patients, there with changing the impedance in the system. Similar to the previous technique, the response of biosensors to the exhaled air of cancer patients and healthy individuals is represented with diagrams, which differently show changes in the impedance for cancer and healthy samples, allowing the detection of lung cancer. It is noteworthy that these biosensors are reported to be more sensitive to the polar 1,2,4-trimethylbenzene VOC than to the nonpolar decane VOC [27].



(a)



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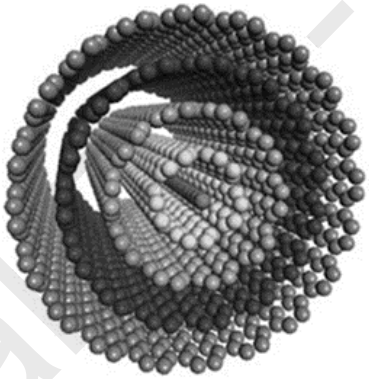

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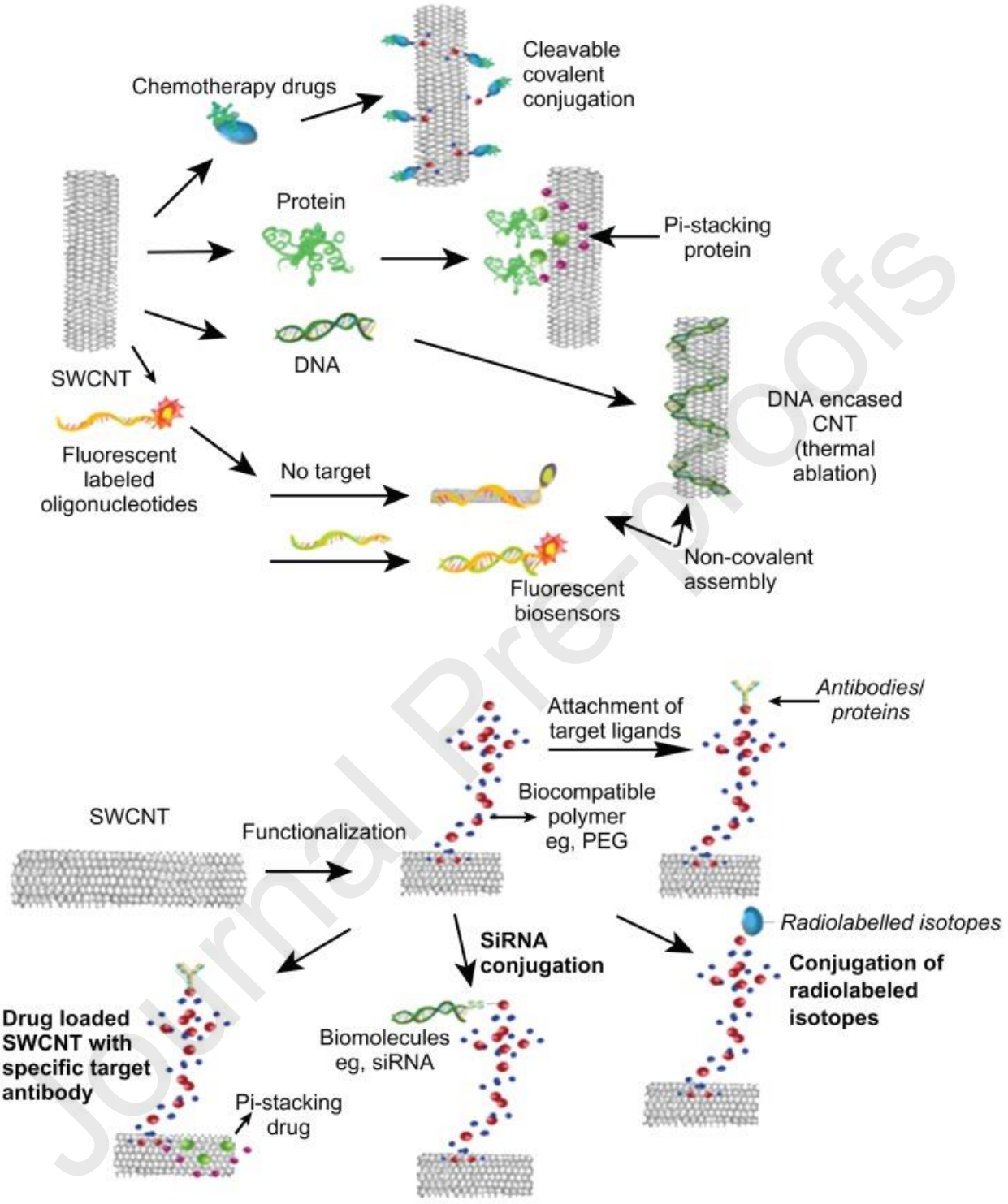
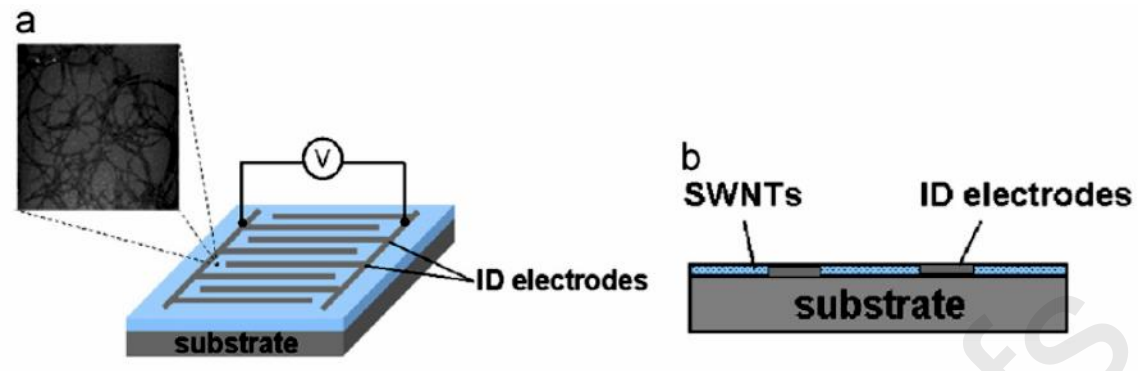

Chemotherapy drugs
Cleavable covalent conjugation
Protein
Pi-stacking protein
DNA
SWCNT
DNA encased CNT (thermal ablation)
Fluorescent labeled oligonucleotides
No target
Non-covalent assembly
Fluorescent biosensors
Antibodies/ proteins
Attachment of target ligands
Biocompatible polymer eg, PEG
SWCNT
Functionalization
Radiolabelled isotopes
SiRNA conjugation
Conjugation of radiolabeled isotopes
Drug loaded SWCNT with specific target antibody
Biomolecules eg, siRNA
Pi-stacking drug

(d)



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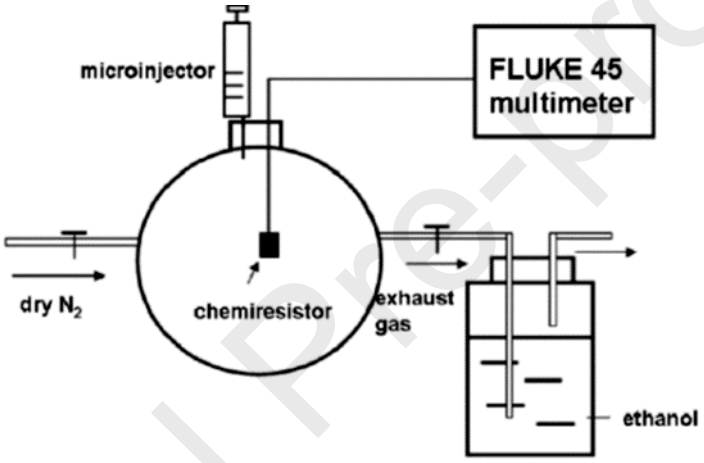

(h)

Figure 8(a). The formation procedure of CNTs from graphene sheets [27]. (b). Single-walled carbon nanotubes [27]. (c). Multi-walled carbon nanotubes [27]. (d). The functionalization process of SWCNTs [27]. (e). a) A SEM image of functionalized SWCNTs; b) the cross-section of the ID electrode [27]. (h). A schematic of the test device [27].

3.2.5. Quantum dots (QTs)

QTs are semiconductor nanocrystals of 2-10 nm in diameter that emit light upon excitation. These zero-dimensional structures consist of 100-100,000 atoms and have received much attention in two recent decades owing to their unique optical-physical properties. The properties of these nanomaterials, including wide excitability range, emission spectrum with a narrow and adjustable width from the UV to the near-IR range, high photoluminescence quantum yield (60-85%), high luminescence, and specific stability against light, have led to their wide medical applications.

For lung cancer detection using QDs, an electrochemiluminescence (ECL) sensor has been designed to detect the carcinoembryonic antigen (CEA) as a lung cancer biomarker. After the synthesis of Fe_3O_4/CdSe–CdS/APS nanocomposites, a poly (alylamine hydrochloride) (PAH) layer and then CdSe–CdS QDs are placed on the surface of Fe_3O_4 magnetic NPs. Ultimately, 3-aminopropyltriethoxysilane (APS) forms a layer on the surface of Fe_3O_4/CdSe–CdS NPs (Fig. 9). APS is an effective factor in binding to biomolecules and stabilizes nanocomposites on the surface of the electrode. In the next step, a kind of magnetic electrode is designed with stabilizing a magnet inside a gold electrode. Fe_3O_4/CdSe–CdS/APS nanocomposites are stabilized on the surface of this electrode with magnetic force, and then AuNPs are accumulated on the electrode. After binding of CEA antibodies to AuNPs and the electrode placement in $K_2S_2O_8$-containing phosphate buffer, bovine serum albumin (BSA) is finally used to block all non-specific binding sites of the sensor. The design procedure of the ECL sensor is exhibited in Figure 10. This detection method is based on ECL, which is a kind of luminescence produced during electrochemical reactions; in other words, ECL is a light generation procedure using electrochemical reactions to produce reactive species on the electrode surface. The possible mechanism of this procedure is presented in Equations 1 - 4:

$$CdSe\text{-}CdS + e^- \rightarrow CdSe\text{-}CdS^- \quad (1)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^- \quad (2)$$

$$CdSe\text{–}CdS^- + SO_4^- \rightarrow CdSe\text{-}CdS^* + SO_4^{2-} \quad (3)$$

$$CdSe\text{-}CdS^* \rightarrow CdSe\text{-}CdS + h\nu \quad (4)$$

The measured intensity of ECL peaks during the sensor manufacturing steps is depicted in Figure 11. The b peak is stabilized when Fe_3O_4/CdSe–CdS/APS nanocomposites are stabilized on the surface of the electrode, and this increase in the peak intensity is attributed to the catalyst role of APS. The stabilization of AuNPs on the surface of the electrode further increases the ECL peak intensity (peak c) as AuNPs accelerate the electron transfer rate in ECL reactions. After the stabilization of antibodies on the electrode surface in the final step, this protein layer prevents the electron transfer, penetration of the S2O82- reactor to the electrode surface, and finally the ECL reaction, there with reducing the ECL peak intensity (peak d). The examination of samples containing the CEA antigen revealed that the ECL peak intensity decreased gradually with increasing the CEA antigen concentration. This is because the complex formation increased steric hindrance and prevented electron and K2S2O8 transfer to the electrode surface during the ECL reaction. Figure 12 represents the standard calibration curve for detecting the CEA antigen, in which the linear relationship between the ECL signal and CEA concentration allows for the quantification of CEA. With an LOD of 32 fml^{-1}, this cancer detection method features high sensitivity and selectivity. The LOD of this method is reportedly lower than other techniques, including the ESEIA method. It should be noted that this method is not specific to lung cancer detection and is used to detect other types of cancer.

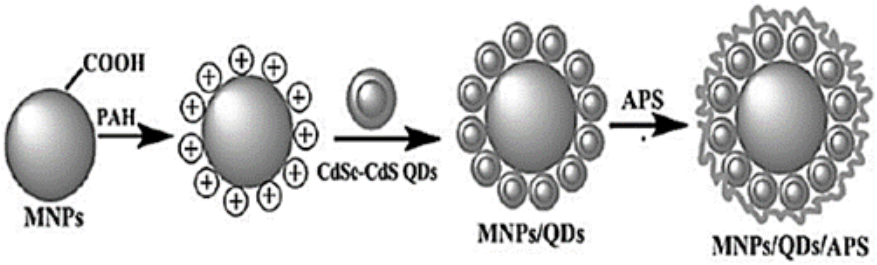

Figure9. The formation procedure of Fe_3O_4/CdSe–CdS/APS nanocomposites [32].

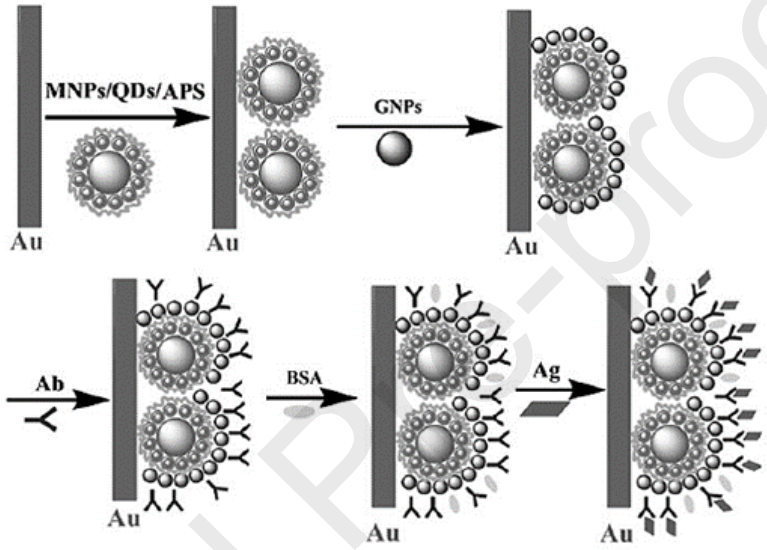

Figure 10. The design procedure of the ECL sensor [32].

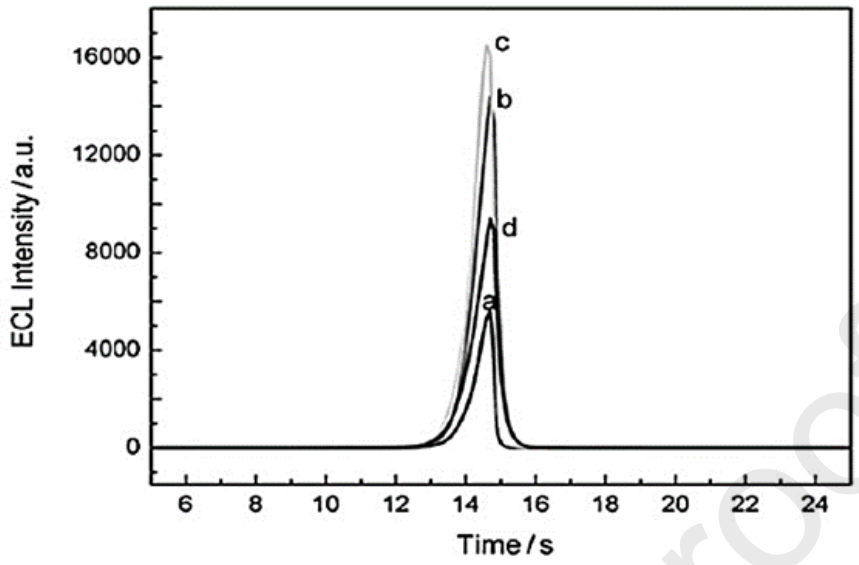

Figure 11. a) MNP/CdSe–CdS, b) MNP/CdSe–CdS/APS, c) MNP/CdSe–CdS/APS/Au NPs, and d) MNP/CdSe–CdS/APS/Au NP/Ab[32].

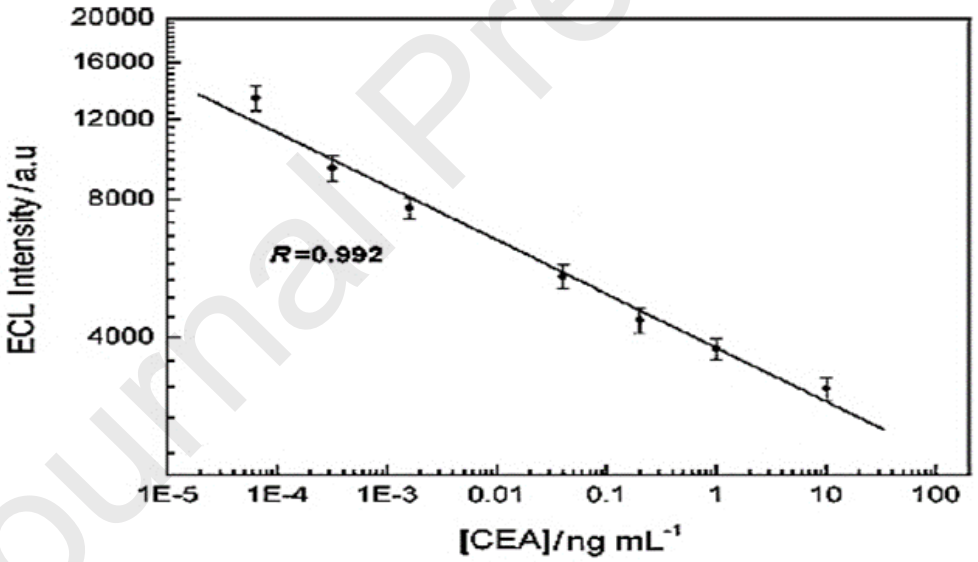

Figure 12. The standard calibration curve for detecting the CEA antigen [32].

4. Graphene

Graphene is a 2D sheet of sp2 hybrid-bonded carbon atoms in a hexagonal lattice [33]. Graphene sheets are formed with placing carbon atoms side with side, and each tetravalent carbon atom is bonded to three other carbon atoms with three covalent bonds in a graphene sheet. These three bonds are in a single sheet, with equal angles of 120° between them. The structure of graphene is shown in Figure 13(a) [34]. In 2004, a group of physicists headed with Andre Geim and Kostya Novoselov used a simple and very different method to produce graphene in Manchester University, resulting in a revolution in this field. They produced a single sheet (a monolayer of atoms) using 3D graphite through the micromechanical lamination method. This method could easily produce high-quality graphene crystals in dimensions of more than 100 µm [35].

This 2D nanomaterial, graphene, is the latest member of the family of multi-dimensional carbon materials, including fullerenes as 0D nanomaterials, SWNTs as 1D nanomaterials, and graphite as a 3D material (Fig. 13(b) [33]. Graphene is a 2D carbon allotrope (with one-atom thickness) structured as a honeycomb lattice, which is the strongest material known hitherto. This compound is the fundamental constituent of CNTs and large fullerenes [36].

The 2D structure of graphene has rendered this material some unique physicochemical properties, including high Young's modulus (about 1100 GPa), high fracture toughness (125 GPa), good thermal conductivity (about 5000 W/mK), high charge carrier mobility or, in other words, high electrical conductivity (200 m2/Vm), high specific surface area (calculated value: 2630 m2/g), high light transmissibility (about 97.7%), and remarkable transmittance phenomena such as the quantum Hall effect [37].

In recent years, different methods for graphene synthesis have been investigated in many studies. High-quality graphene is now synthesized using various techniques, such as chemical vapor deposition (CVD), epitaxial growth on electrically insulating surfaces, formation of colloidal suspensions, and micromechanical lamination of graphite. In most cases, lamination of graphite is chosen owing to its simplicity, high efficiency, and low cost [37 - 38].

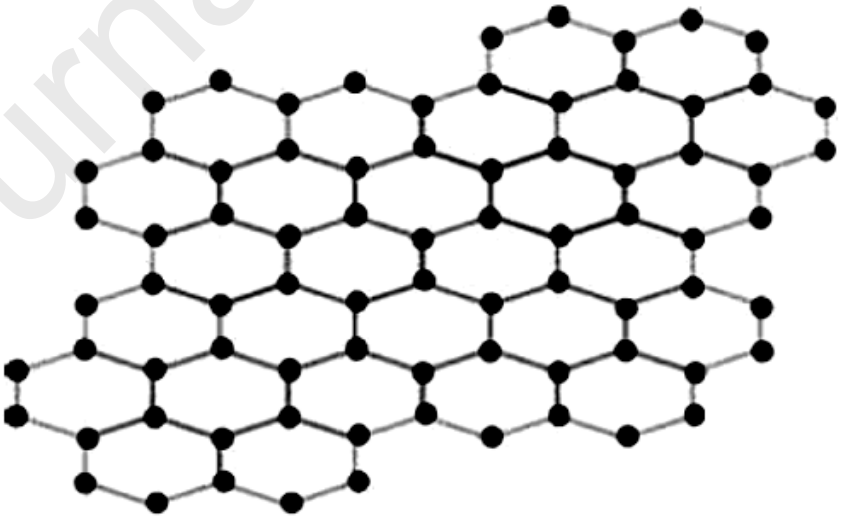

Figure 13(a). The structure of grapheme [37].

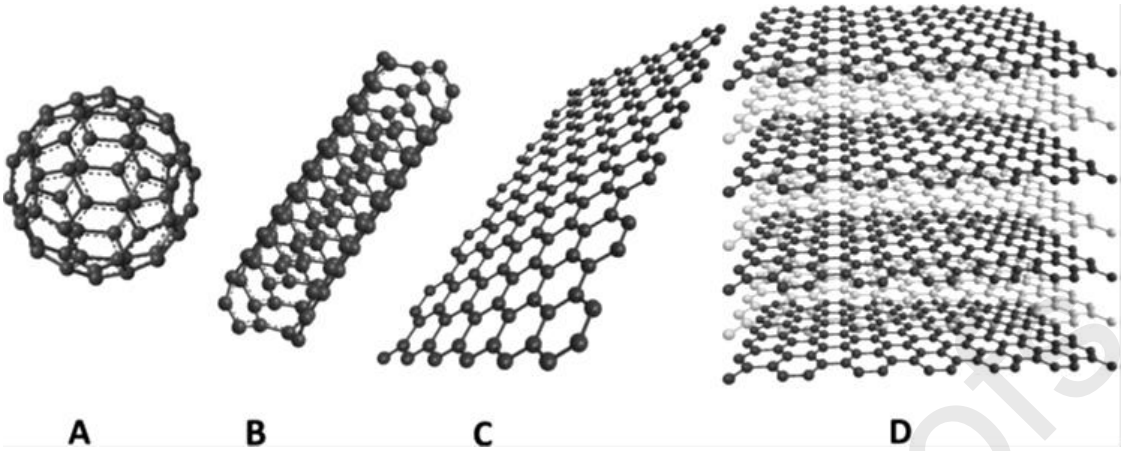

Figure 13(b). A. Fullerene, B. Single-walled carbon nanotubes, C. graphene, and D. graphite [37].

4.1. Graphene oxide (GO)

As a derivative of graphene, GO consists of a 2D layer of sp2-hybridized carbon atoms in a hexagonal lattice structure. It contains carbon atoms with sp3 hybridization connected to oxygenated functional groups. The use of strong oxidants is the most widely used method for the lamination of graphite to synthesize GO in which graphite oxide is obtained with incorporating oxygenated functional groups in the graphite structure. The two-layer distance increases from 0.335 nm in graphite to about 0.625 nm in graphite oxide [40], which is basically laminated with ultrasonication, yielding monolayer GO sheets (measuring a few hundred nm to several μm) [41].

It is difficult to accurately determine the GO structure, but GO is obviously the contiguous aromatic lattice of graphene connected to functional groups of alcohols, epoxides, ketones, aldehydes, and carboxylic acids.

The accurate chemical structure of GO has been controversial for many years, but no unambiguous model has ever been proposed for various reasons. Regular lattices consisting of discrete repetitive units were proposed in most preliminary structural models of GO. Hofmann and Holst's structure is composed of epoxy functional groups dispersed on the whole plane of graphite with pure CO_2 molecular formula. Instead of the sp2 hybridization of Hofmann and Holst, Ruess's model (1946) changed the basal plane structure of GO into a sp^3 hybridized structure. In this model, a quarter of epoxide-containing cyclohexenes were in positions 1 and 3, and those containing the hydroxyl group were in position 4.

The ether and epoxide groups were eliminated in a model introduced by Scholz and Boehm (1969). Later, Nakajima and Matsuo offered an interesting model based on a reticulate framework similar to poly (dicarbon monofluoride) $(C_2F)n$. The structural models proposed for GO are listed in Figure 14(a) [42]. The most recent model proposed for GO rejects the reticulate

model and focuses on an amorphous non-stoichiometric structure, which is the most known GO structural model presented by Lerf and Klinowski (1998)[42] THIS structural model is shown in Figure 14(b) [42].

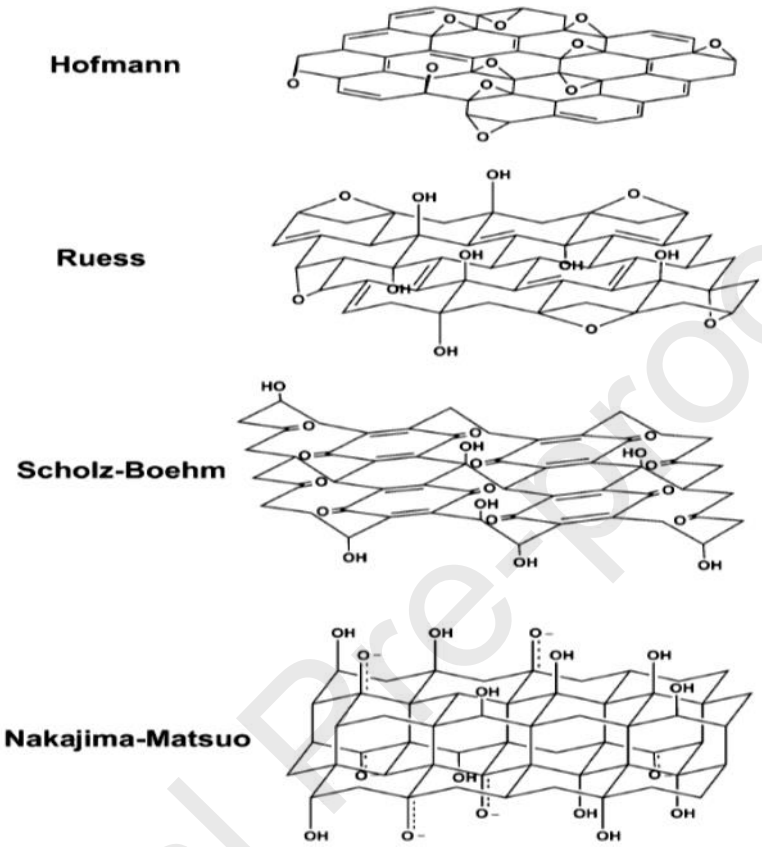

Figure 14(a). Structural models proposed for GO [39].

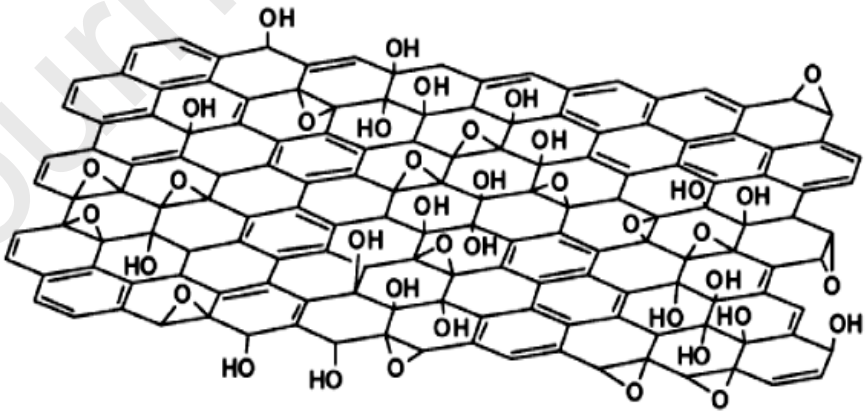

Figure 14(b). Lerf and Klinowski's structural model[42].

4.2. Applications of GO

Graphene and chemically modified graphene (CMG) are excellent options for various applications such as energy-storage materials [109], paper-like materials [110], polymeric composites [111], smart touch screens [112], liquid crystal devices [113], mechanical oscillators [114], sensors [115], and biomedical applications [38,116]. GO is mainly important owing to its fundamental physicochemical structure, resulting in its extraordinary chemical adaptability and unusual physical properties. GO chemical adaptability [104-105] results from oxygenated functional groups on its carbon structure, allowing for relatively easy functionalization with organic molecules of biological structures through covalent or non-covalent bonds under mild conditions. The adaptive effects resulting from the entire structures defined on the GO surface, along with its optical, mechanical, and electronic properties have led to the development of new versatile hybrid structures with a high potential in cancer treatment [37].

Omid Akhavan etal [101] Remote controllable graphite micro/nano-swimmers moving based on the graphene jet nanomotor has been designed and investigated for the first time. The swimmers showed high self-exfoliations in water (resulting in jet ejection of the graphene flakes), due to production, accumulation and explosion of H2 gas (observed as micro/nanobubbles in the track of the swimmers) between the already Na intercalated graphene sheet constituents. Such graphene jet nanomotors (with ejection speeds of as high as ~7000 m/s) provided the thrust forces of at least ~0.7 L (pN) (in which L (μm) is swimmer size) and average forward speeds of ~177–400 μm/s. As a high advantage, the graphene nanomotors require no chemical additives as fuels in water. Meanwhile, the direction of motion of the swimmers equipped with TiO2 NPs was remotely controllable by electromagnetic waves (combination of a magnetostatic field and UV irradiation), providing nanorobotics in faraway and/or inaccessible regions. Although the rate of self exfoliation and speed of TiO_2-grafted swimmers were significantly enhanced by UV irradiation (because of further production of H_2 nanobubbles), the total distance of the self-exfoliated swimmers (with the same initial sizes) was unchanged. This reconfirmed the mass ejection-based propulsion mechanism. The graphene-based swimmers were highly sensitive to the presence of water in the solution (no significant propulsion was observed for the swimmers floated in organic solutions). Such selective sensitivity provides applications in which the water content of the environment can affect (e.g., can trigger) the motion and self-exfoliation of the swimmers. These swimmers can open up designing the remote controllable environmentally-responsive micro/nano-machines which are highly desirable in upcoming nanorobotics, nanomedicine (drug delivery and other nanotechnology-based therapeutic purposes), and green environmental remediation.

Priscilla Kailian Ang etal [102] We have demonstrated an approach to obtain conducting graphene sheets based on the intercalation and exfoliation of Graphene oxide sediments with tetrabutylammonium ions. A homogeneous colloidal suspension of mildly oxidized graphene sheets, coupled with a high monolayer yield (90%), allows it to be used for device fabrication. We demonstrated that impurity doping by the substrate reduces the intrinsic field-effect transistor carrier mobility of such chemically processed graphene film by at least an order. Using NaF for

ionic screening, the mobility of the graphene can be improved by one order from the unscreened situation in dry air. From electrical transport measurement and in situ Raman spectroscopy, we found that the dopant concentration is reduced from 1.5 $*10^{12}$ to 2.8 * 10^{11} cm^2, and as a consequence, the hole mobility is increased from 59 to 460 $cm^2/ (V \cdot s)$ and electron mobility is increased from 17 to 310 cm^2 / (V · s). We also show that the effect of the ionic electrolyte depends on the specific property of the ions. In the case of KCl, there is a concentration-dependent interplay of ionic screening and chemical doping, which may arise from specific adsorption of the Cl^- at the graphene water interface.

Negative differential resistance (NDR) devices with intrinsic unique quantum characteristics can realize fascinating features in the next generations of memory saving, highly quick switching, and THz high-frequency oscillating nanodevices as well as multivalued logic gates with low-power consumptions. Although traditional NDR devices (which work based on two-terminal quantum tunneling or intervalley charge transfer between conventional n−p semiconductors) are known historically as Esaki diodes (and then Gunn diodes and/or atomic-scale as well as molecular-based devices), the development of two-dimensional - Negative differential resistance nanodevices has recently attracted much attention for the design and fabrication of ultrathin quantum tunneling diodes applicable in upcoming integrated circuits[103].

Reza Rahighi etal [103] Heterojunction of the degenerate p-type GNP and rGO could result in the development of a novel nanodevice with a reversible quantum tunneling NDR feature (in the frame of cellulose-based PGFs, having a myriad of aqueous pores with a size of ∼2 nm enclosed by nearly six-layer rGO walls), for the first time. The two-terminal Negative differential resistance peak showed high stability and reversibility at high temperatures ranging from 586 to 592 K. It was found that environmental oxygen can play a critical role in water-based degeneration of the initial p-type regions around the GNPs and subsequently resulting in a sharp quantum tunneling Negative differential resistance peak. The electron-band-structure based mechanism, which could successfully describe the lateral quantum tunneling behavior of the Negative differential resistance device, was also used for the evaluation of the band gap energy of the p-type PGF with the value of ∼1.8 eV. These results not only can encourage more investigations on band gap engineering of graphene-based materials but also can ensure the possibility of applying the imminent quantum-based devices at high temperatures.

4.3. Applications of GO in bioelectrochemistry

Graphene has been successfully applied in bioelectrochemistry and is a useful nanomaterial in nanoelectronics science due to its high conductivity and unique surface properties such as very low thickness (one atom) and reversible adsorption of protein on the surface [43-44]. Graphene-based materials can be used as an ideal surface for protein adaptation and a facilitated electron-protein transfer, in which GO causes an effective electrical binding between the electrode and the oxidation-reduction centers of several heme-containing metalloproteins, including cytochrome C, myoglobin, and horseradish peroxidase (Fig. 15). It is noteworthy that proteins maintain their bioactivity and coherent structure when they are adsorbed on GO. This property predicts the wide application of the GO-protein complex in the development of biosensors [45].

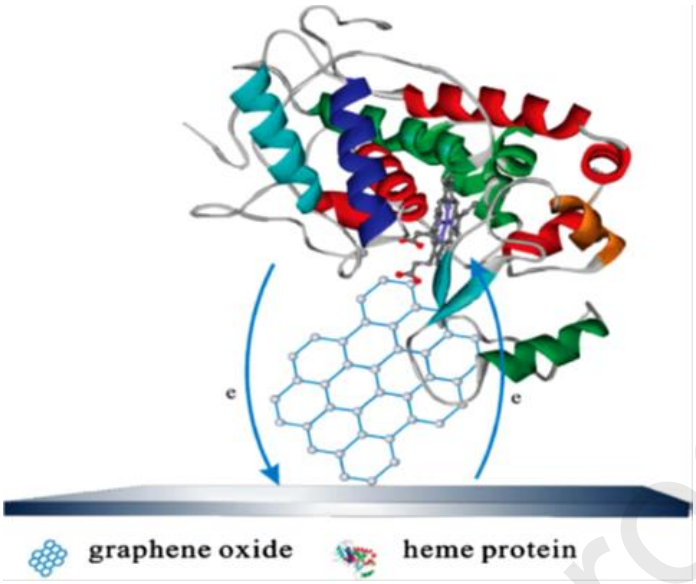

Figure15. Electrical binding of proteins with GO in electrochemistry [43].

Omid Akhavan etal [106] Graphene oxide nanowalls with extremely sharp edges and preferred vertical orientation were deposited on a graphite electrode by using electrophoretic deposition in an Mg(2+)-GO electrolyte. Using differential pulse voltammetry (DPV), reduced graphene nanowalls (RGNWs) were applied for the first time, in developing an ultra-high-resolution electrochemical biosensor for detection of the four bases of DNA (G, A, T, and C) by monitoring the oxidation signals of the individual nucleotide bases. The extremely enhanced electrochemical reactivity of the four free bases of DNA, single-stranded DNA, and double-stranded DNA (dsDNA) at the surface of the RGNW electrode was compared to electrochemical performances of reduced graphene nanosheet (RGNS), graphite, and glassy carbon electrodes. By increasing the number of DPVs up to 100 scans, the RGNW electrode exhibited an excellent stability with only 15% variation in the oxidation signals, while for the RGNS electrode no detectable signals relating to T and C of 0.1 μM dsDNA were observed. The linear dynamic detection range of the RGNW electrode for dsDNA was checked in the wide range of 0.1 fM to 10 mM, while for the RGNS electrode, it was from 2.0 pM to <10 mM. The lower limits of dsDNA detection of the RGNW and RGNS electrodes were estimated as 9.4 zM (~5 dsDNA/mL) and 5.4 fM, respectively. The RGNWs were efficient in label-free detection of single nucleotide polymorphisms of 20 zM oligonucleotides (~10 DNA/mL) having a specific sequence. Therefore, the RGNWs can effectively contribute to the development of ultra-high-sensitive electrochemical biosensors with single-DNA resolutions.

Akhavan etal [107] Mg2+-charged spongy graphene electrodes (SGEs) were fabricated by using electrophoretic deposition of chemically exfoliated graphene oxide sheets on graphite rods. The SGEs were able to present two distinguishable signals (originated from electrochemical oxidation of guanine) in differential pulse voltammetry (DPV) of leukemia and normal blood cells, in contrast to glassy carbon electrodes giving only one overlapped peak. Hence, the SGEs were applied in fast (60 min) and ultra-sensitive detection of leukemia (single abnormal cells in

~109 normal cells) in a blood serum. The sensitivity obtained by the SGEs was three orders of magnitude better than that of the best available and current technologies (e.g., specific mutations by polymerase chain reaction with detection limit of one abnormal cell in ~106 normal cells) which not only are expensive, but also require several days for incubation. Significant variations in DPV signals of the SGEs after the first electrochemical cycle indicated that the best performance of the SGEs can be achieved only at the first cycle. The linear dynamic detection behavior of the SGEs was investigated in wide concentration range of $1.0 \times 105^{-0.1}$ cell/mL [107]. The lower detection limit was estimated ~0.02 cell/mL [107], based on the current resolution obtained by the SGEs.

Saeed et al [108] Two different DNA (ERBB2c and CD24c) modified gold nanoparticles and graphene oxide loaded on glassy carbon electrodes were prepared for early detection of breast cancer markers by electrochemical detection of HER2. Comparative study of ERBB2c and CD24c for the detection was carried out. A "sandwich-type" detection strategy was employed in this electrochemical DNA biosensor and its response was measured by amperometric detection. The electrochemical signal enhancement achieved via gold nanoparticles and grapheme oxide system allowed for sensitive detection of the breast cancer biomarker ERBB2 and the control marker CD24. The modified graphene oxide was characterized using Raman spectroscopy, UV-visible spectroscopy, Fourier transform infrared spectroscopy transmission electron microscopy, scanning electron microscopy and energy-dispersive X-ray spectroscopy. The various steps involved in the modification of a glassy carbon electrode with graphene oxide, gold nanoparticles and DNA probes, target and reporter probe were electrochemically characterized using cyclic voltammetry and electrochemical impedance spectroscopy. Using amperometric detection of a horse radish peroxidase label, detection limits of 0.16nM and 0.23nM were obtained with sensitivity 378nA/nM and 219nA/nM for ERBB2 andCD24 respectively.

Nayak et al [132] Nanotechnology is defined as the branch of science dealing with extremely small-sized particles with a size in the range of 1–100 nm, which are termed nanoparticles. Due to the extremely small size of nanoparticles, they display unique electronic and optical properties, which differentiate them from their bulk form. Thus, due to the unique properties of nanoparticles, they play a crucial role in a variety of fields, including the biomedical, environmental, agricultural, and industrial fields. Selenium belongs to Group 16 of the periodic table with an atomic number of 34 and its nanoparticles have been highlighted as a potential material to alleviate several problems due to the formation of biofilms, production of ROS, low redox activity, etc. These nanoparticles can be synthesized through chemical, physical and biological methods. Since existing reviews mainly concentrated on the individual applications of selenium nanoparticles such as in diagnosis and therapeutics, the present review mainly highlights the potential activity of selenium nanoparticles in the biomedical domain, making them a potential theragnostic agent. Specifically, this review will present detailed information on the bioimaging and therapeutic activity, together with the role of selenium nanoparticles in the current scenario of the ongoing pandemic (SARS-CoV-2). It will also focus on procedures for their synthesis and properties that make them potential candidates for applications in various domains. Finally, we provide a detailed future outlook.

Nayak, V. et al [133] With the successful establishment of biosensors in the bioanalytical field, nowadays, Nano biosensors are also trending and are carving their ways in various domains, but they have gained much attention in the environmental domain as they can easily detect various

pollutants from the environment and can help in remediation of dyes, contaminants, etc. Moreover, Nano biosensors are expected to overcome multiple hindrances faced by conventional biosensors without creating any major drawbacks. The various properties like sensitivity, stability, response time, the limit of detection (LOD), etc., and different fabrication techniques, including chemical, physical, and surface modification techniques, majorly define the utilities of Nano biosensors. Further, Nano biosensors can be broadly classified based on transducers and biorecognition elements.

4.4. Biomedical applications of GO

Applications of nanomaterials are always limited due to their solubility and low stability in biological solutions. However, GO is widely used in biomedical fields owing to its hydrophilic groups (COOH) and a hydrophobic plane sheet [120-121].

Apoptosis is programmed cell death that eliminates aged, damaged, additional, and harmful cells in the body. Any disruption in the apoptosis process results in such diseases as cancer, autoimmune disorders, neurodegenerative disorders, and heart failure. Caspase 3 has been identified as a key mediator of apoptosis.

Wang et al [44] detected its activation in live cells using a GO-peptide nanohybrid as a nano sensor of intracellular protease. The peptide probe was connected to GO using the N-hydroxysuccinimide (EDC-NHS) coupling (Fig. 16) and showed unique solubility and stability in water and the cell growth medium. An LOD of 7.25 ng mL^{-1} (≈ 0.4 nM) was obtained for Caspase 3 with numerous laboratory experiments. The GO-peptide nanohybrid was effectively directed into Hela cells (cervical cancer cells), and Caspase 3 activation stimulated with staurosporine (STS) was detected with the Nano sensor of intracellular protease (Fig. 17) [44]. The development of new biosensors with sensitivity, selectivity, and high speed is of paramount importance in the detection of pathogens, medical diagnoses, immune screening, and preventing environmental pollution. Easy surface modification, high strength, good water dispersibility, and photoluminescence are some unique properties of GO, leading to its high potential for use in biosensors.

Jung et al [45] have reported a GO-based biosensor to identify rotavirus as a pathogen. In this biosensor, GO was precipitated on an amine-modified glassy surface, and the rotavirus antibody was stabilized on the GO surface through the amide formation reaction using carbodiimide. The second rotavirus antibody was connected to AuNPs using a DNA molecule as the mediator. Figure 18 exhibits the connection of the rotavirus antibody to AuNPs.

When the rotavirus cell in the sample is connected to the antibody on the GO surface with the specific antibody-antigen interaction, the connection of the target cell is confirmed with observing fluorescence reduction through the fluorescence resonance energy transfer (FERT) between GO and AuNPs attached to the second antibody. This allows for pathogen detection with the biosensor shown in Figure 18. It is worthy of note that the LOD of the biosensor was 105 pfu mL^{-1} for rotavirus [45].

Zhu et al [46] have recently reported GO-based probes containing fluorescent dyes to identify specific sequences of DNA. They identified three nucleotide sequences of the human

immunodeficiency virus (HIV), variola virus (VV), and Ebola virus (EV) using Alex Fluor 488, ROX, and Cy5 organic dyes. This sensitive multi-DNA detection method is schematically shown in Figure 19.

In the first step, single-stranded DNA probes containing organic dyes are adsorbed on GO, and then the cDNA of each probe (T1, T2, and T3 target DNAs) are added to hybridize probes with target DNAs to be separated from the GO surface. The first and the second steps were respectively associated with a sharp reduction and an increase in the fluorescence intensity of organic dyes (Figure 20).

The relationship between the fluorescence intensities of Alex Fluor 488, ROX, and Cy5 organic dyes and the concentrations of target DNAs was examined in the Tris-HCl buffer under optimal conditions. Figure 21 depicts the fluorescence spectrum of the simultaneous scan at different concentrations of target DNAs (Fig. 21a) and the linear relationship between the fluorescence intensity of organic dyes (ΔI) and the concentrations (C) of target DNAs (Fig. 22 b-d). In this figure, $\Delta I1$, $\Delta I2$, and $\Delta I3$ respectively represent the fluorescence intensities of Alex Fluor 488, ROX, and Cy5 organic dyes, and C1, C2, and C3 respectively indicate the DNA concentrations of HIV, VV, and EV. Besides, LODs of 36, 37, and 250 pM for HIV, VV, and EV were respectively obtained in this method [46].

The FAM-labeled single-stranded DNA probe (P1) attaches to GO sheets via non-covalent interactions, associated with a reduction in the FAM fluorescence intensity (Fig22, step a). The P1 then forms a hybrid with the added target DNA (HIV1), and the resulting double-stranded DNA is separated from the GO surface; this process increases the FAM fluorescence intensity (Fig22, step b).

Figure 23 displays the spectrum of high P fluorescence emission (peak a) due to the presence of FAM and in the absence of GO in different conditions. In the presence of GO, however, the P adsorption on GO reduces the fluorescence emission with about 97% (peak c). The P-GO complex also shows a considerable increase in the fluorescence emission (peak d) upon the addition of HIV.

Proteins were identified in the process shown in Figure 24. In this process, the adsorption of the FAM-labeled aptamer on GO sheets sharply decreased fluorescence emission (about 96%). Besides, thrombin (as the target molecule) connection to the aptamer led to its separation from the GO surface, there with elevating fluorescence emission.

Figure 24 depicts the fluorescence emission spectrum of the FAM-labeled aptamer in the presence of GO and different concentrations of human thrombin; the fluorescence emission intensity increases with increasing thrombin concentrations. It is noteworthy that aptamers attach very specifically to proteins, and an LOD of about 2 nM for human thrombin obtained in this method indicates its high sensitivity and selectivity [47].

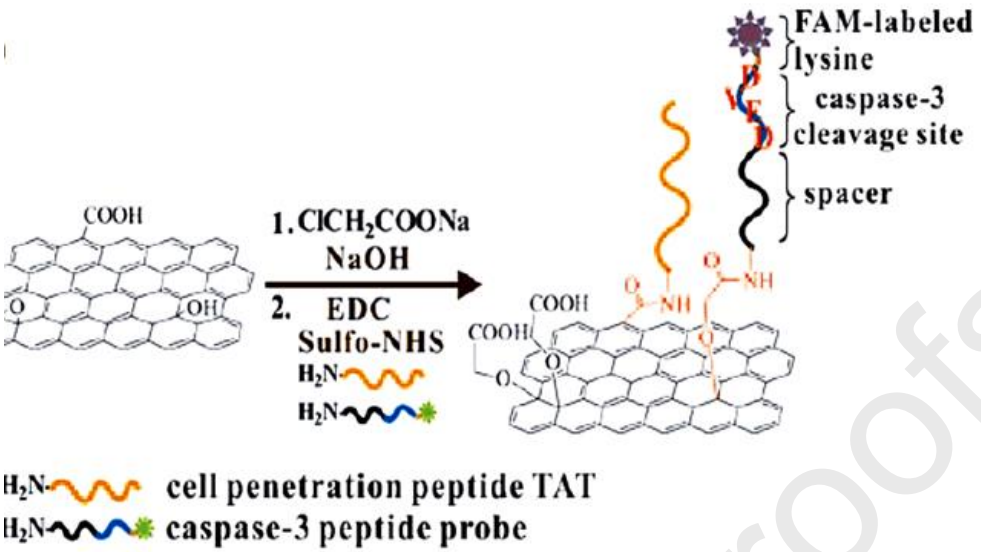

Figure16. Peptide stabilization on the GO surface [43].

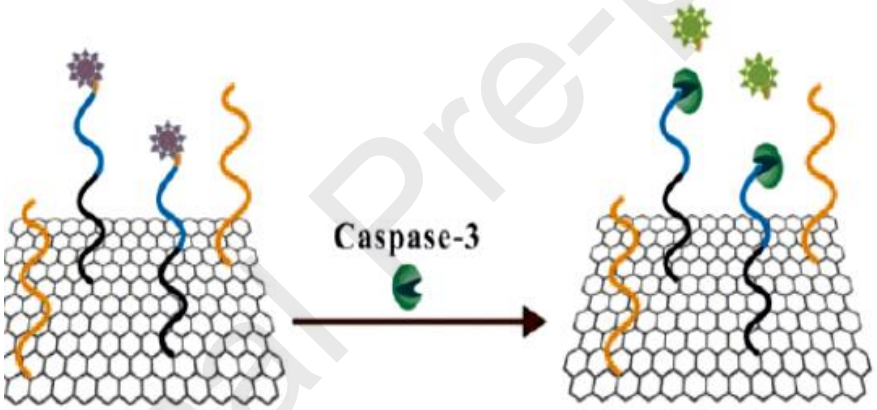

Figure17. Caspase 3 detection using the GO-peptide nanohybrid [44].

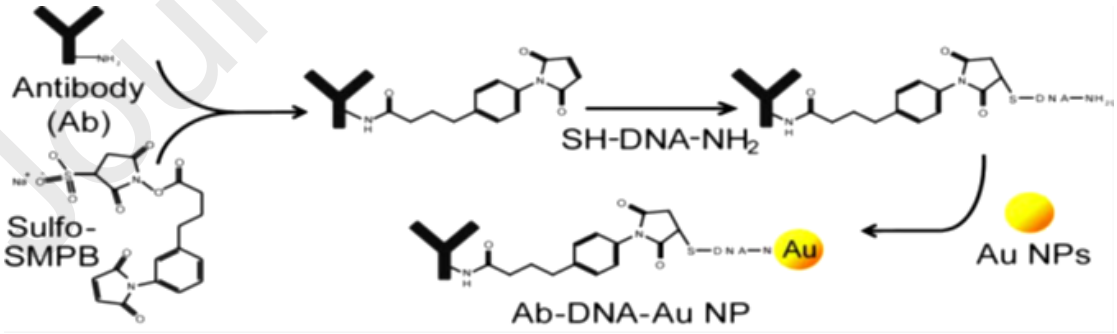

Figure 18. The formation of the Ab-DNA-AuNP complex[45].

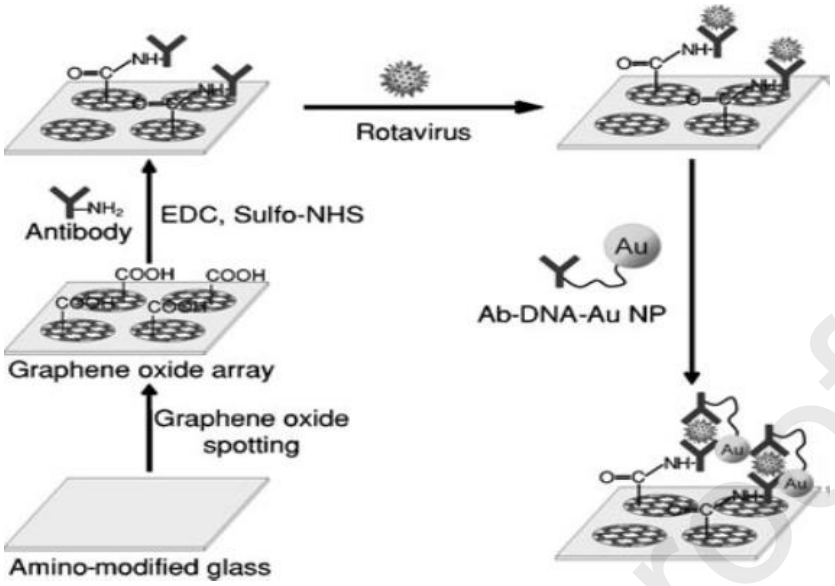

Figure 19. A GO-based biosensor [46].

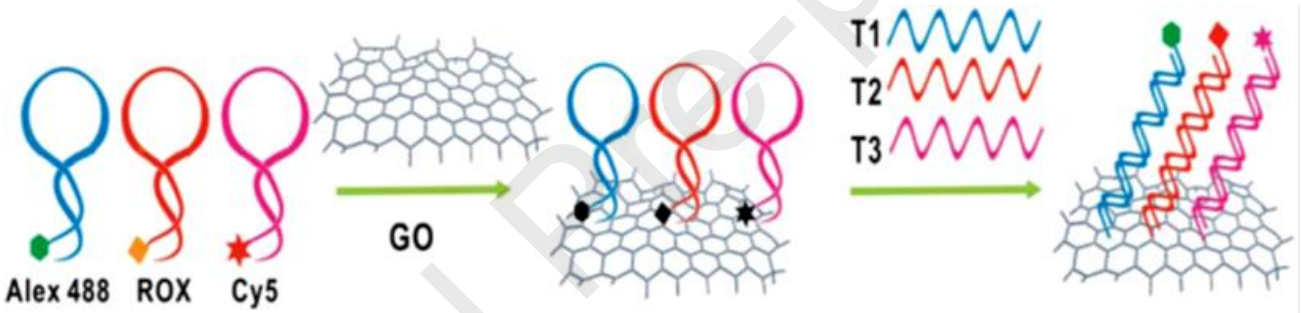

Figure 20. Detection of the target multi-DNA [47].

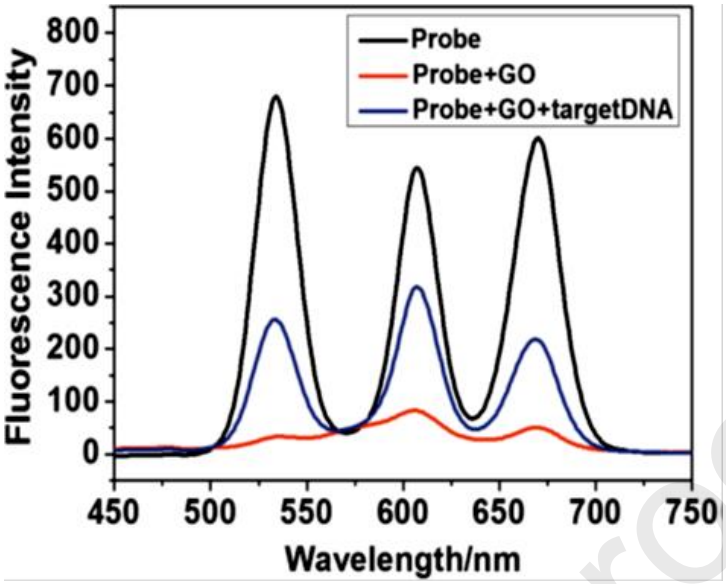

Figure 21. The fluorescence spectrum of the simultaneous scan [48].

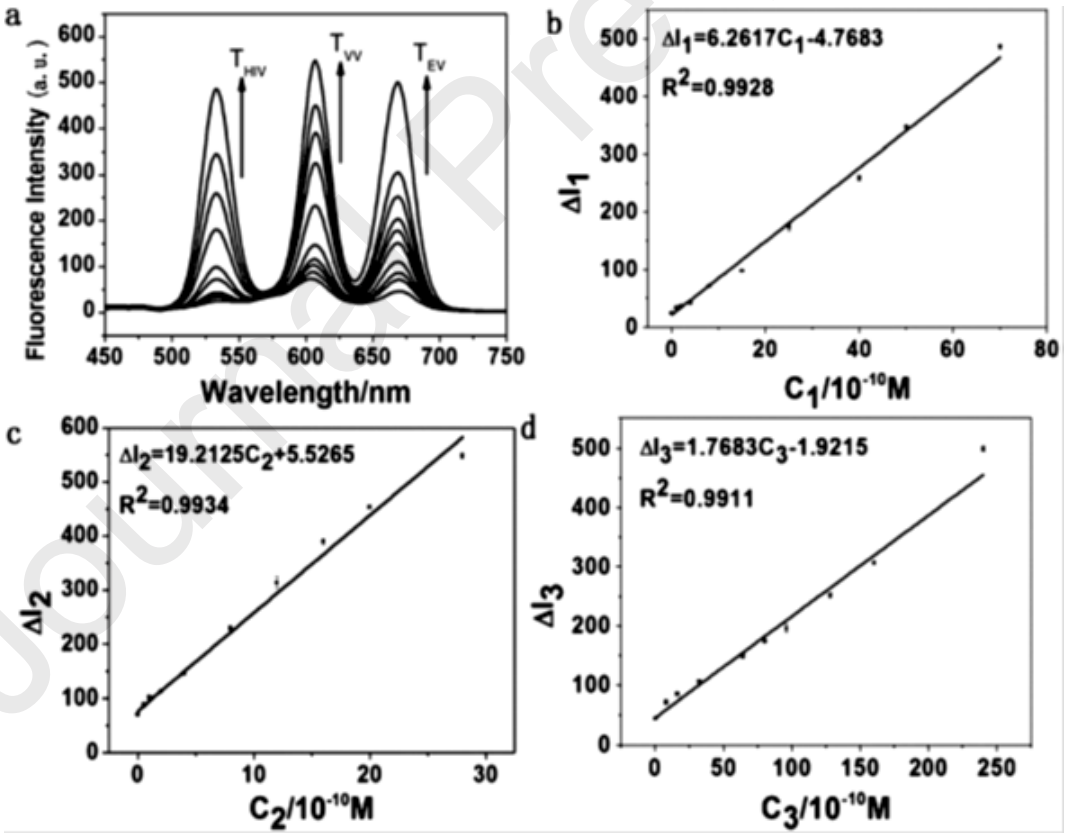

Figure 22. The fluorescence spectrum of the simultaneous scan at different concentrations (C) of target DNAs; calibration curves to quantify the DNA concentrations of b) HIV, c) VV, and d) EV [49].

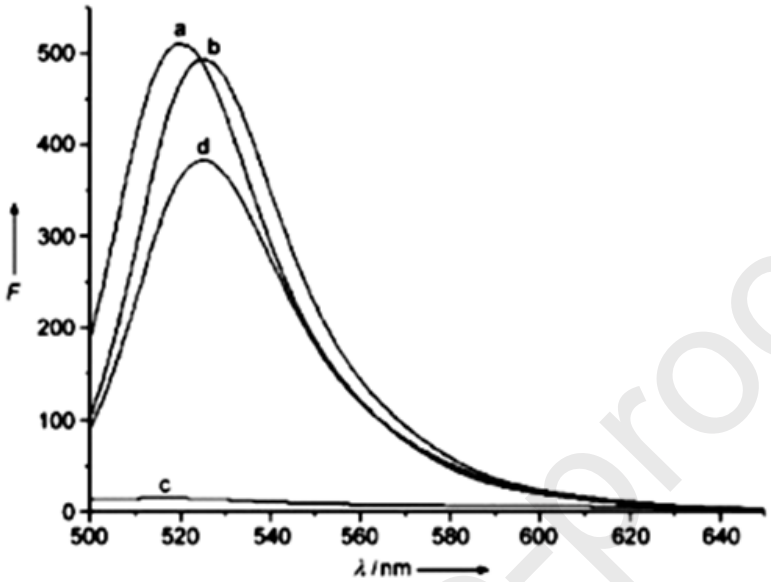

Figure 23. P1 fluorescence emission spectrum in different conditions: a) P in the presence of Tris-HCL buffer, b) P + HIV, c) P1 + GO, and d) P + GO + HIV[49].

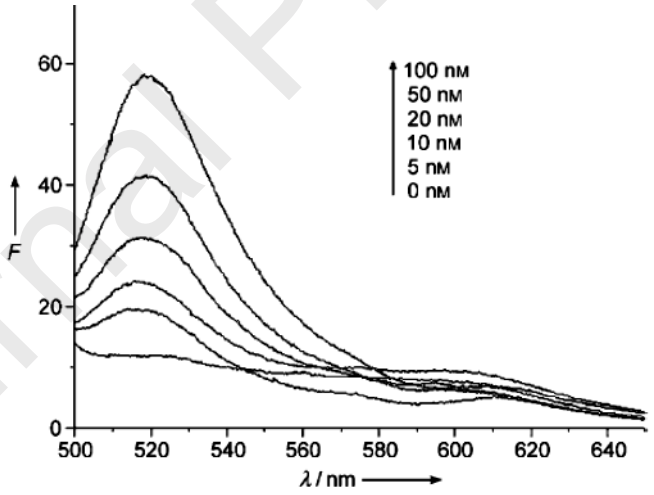

Figure 24. The GO-aptamer fluorescence emission spectrum at different concentrations of thrombin.

Hashmi et al [130] The SARS-CoV-2 pandemic has spread worldwide and is still not stopping, which has led to the loss of several lives, and the country's economy has also suffered. Nevertheless, the spread of the virus is not ended yet and presently, several mutants and variants are appearing, which are more dangerous and harmful than the previous one. There is an urgent need to develop the tremendous diagnostic device and treatment strategy of the evolving and re-emerging diseases to combat SARS-CoV-2 pandemic situations in the present situation.

Graphene and graphene-based derivatives are suitable candidates to fight against coronavirus due to their excellent antibacterial activity, great electrical and thermal conductivity. In this evaluated literature, we have mentioned in detail about graphene and its derivatives, which can be used to prepare medical components, devices, PPEs, effective masks, biosensors, antiviral coating, 3D printed graphene and graphene-based nanofoams. The use of medical components and PPEs based on graphene-based materials are very appropriate because these medical kits and PPEs are very lightweight, easy to use, protect from UV-rays, and show great antibacterial activity, good thermal and electrical conductivity. Despite the excellent properties of graphene and its derivatives, many graphene fabricated fabrics like cotton, polyester, etc., are still not adoptable for large-scale manufacturing. Moreover, graphene is a monoatomic thick nanomaterial and can cause discomfort for the user, so graphene-based clothing should be modified with other materials to ensure sustainability and comfort. Graphene and graphene-based sensors are good alternatives to sense the SARS-CoV-2 virus and other virulence diseases. Further, graphene-based coatings and surfaces assume a critical part in controlling the viral spread over high-contact segments and items because of their remarkable antibacterial activity. GO and its allied derivatives are the new expectation for the battle against these intense sicknesses differently; this demonstrates that the path of graphene and graphene-based materials for medical care applications is as yet distant into what is to come. Indeed, graphene and graphene-based materials hold a great potential compared with other unique materials owing to their unique and tremendous electrical, chemical, and mechanical properties. In the future, properties of graphene-based constituents can be modified through control functionalization for great commercial applications. Further, the increase in the utilities of graphene-based nanomaterials in the biomedical domain will also increase the market opportunities and increase the country's economy. However, major limitations possessed by graphene-based nanomaterials should be preferred and should be resolved for better results. However, the outbreak of SARS-CoV-2 has increased the biomedical pollution like open disposal of PPE kits, diagnosing kits, medical tools, masks, etc., which have led to the spread of contamination in nearby water bodies, soil and air. Similarly, better disposable protocols are needed to be set up for the proper and safe disposal of graphene-based medical wastes. So, graphene and graphene-based materials are used to improve science and technology for medical and healthcare utilities to combat the recent critical scenario of the SARS-CoV-2 pandemic. It will also play a vital role in combating other future pandemics caused via viruses, bacteria, fungi, etc.

4.5. Nanotoxicity of graphene and graphene oxide

Graphene and its derivatives are promising candidates for important biomedical applications because of their versatility [210-215]. The prospective use of graphene-based materials in a biological context requires a detailed comprehension of the toxicity of these materials. Moreover, due to the expanding applications of nanotechnology, human and environmental exposures to graphene-based nanomaterials are likely to increase in the future [216-220]. Because of the potential risk factors associated with the manufacture and use of graphene-related materials, the number of nanotoxicological studies of these compounds has been increasing rapidly in the past decade [221-223]. These studies have researched the effects of the nano structural/biological interactions on different organizational levels of the living system, from biomolecules to animals. This review discusses recent results based on in vitro and in vivo cytotoxicity and genotoxicity studies of graphene-related materials and critically examines the methodologies employed to evaluate their toxicities [224]. The environmental impact from the manipulation and application

of graphene materials is also reported and discussed. Finally, this review presents mechanistic aspects of graphene toxicity in biological systems. More detailed studies aiming to investigate the toxicity of graphene-based materials and to properly associate the biological phenomenon with their chemical, structural, and morphological variations that result from several synthetic and processing possibilities are needed. Knowledge about graphene-based materials could ensure the safe application of this versatile material. Consequently, the focus of this review is to provide a source of inspiration for new nanotoxicological approaches for graphene-based materials [137].

4.6. Nano biosensors in biomedical applications

Advancements in biosensors have opened the way for their utilization in biomedical applications such as drug target, drug discovery, and drug delivery. Carbon nanotubes (CNTs) have emerged as smart materials for the production of biosensors, drug delivery vehicles, and biomolecule carriers in tissue engineering [138]. The biocompatibility of different materials such as metal oxides and graphene oxide holds the responsibility for their convenience in biomedical applications [140]. Figure25 schematic showing some of the biomedical applications of nanoparticles.

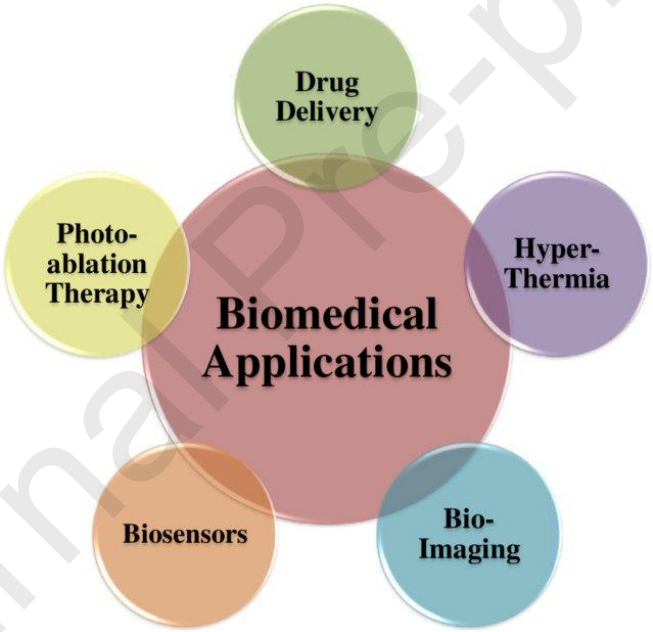

Figure 25. schematic showing some of the biomedical applications of nanoparticles [138].

4.7. Nano biosensors in environmental utilities

Environmental pollution has become a severe universal challenge and has a direct impact on all life forms. Hence, there is an urgent need for fast, reliable, and low-cost biosensors to monitor and detect contaminations in various resources. Conventional methods used for the quantitative analysis of environmental samples require complicated multistep procedures and fail to monitor contaminants in real-time. To overcome these issues, various Nano biosensors are in use. Nanotechnology has evolved very fast in recent years, and biosensors based on nanomaterials (nanobiosensors) are finding a variety of applications in the environmental, clinical, and agricultural domains just due to the versatile properties of the nanomaterials used for designing

nanobiosensors. The major threat to environment is the release of heavy metal ions, pesticides, chemical toxins, etc., into water bodies, food, and the atmosphere. These sources are directly in contact with the living world and these environmental pollutants pose severe global threats to health. Using nanoscale-structured material for improving biosensing technology has brought in a novel and cost-effective technology for environmental analysis and monitoring. Due to the complexity of these pollutants, traditional biosensor methods face some limitations, which are overcome by using nanobiosensors; these types of sensors are very selective and sensitive [132].

4.8. Nano biosensors in food monitoring applications

Biosensors are a powerful analytical tool for the rapid analysis of several chemical molecules. Hence, biosensor development for different applications is growing rapidly owing to new advancements in nanotechnologies. Nanoscale sensors offer the potential of integration in miniaturized and automated devices. This review summarizes the recent trends and advancements in Nano biosensors for biomedical, food monitoring, and environmental applications [192].

The food and agriculture sector controls the economic growth of a developing country. The food industries have practices of growing crops, raising livestock and sea foods, food processing and packaging, regulating production and distribution with quality and safety. The process control and monitoring quality are crucial steps. Here we review Nano sensors and Nano biosensors as alternative of classical quantification methods [192]. Nanoscale dimensions of metal nanoparticles, metal nanoclusters, metal oxide nanoparticles, metal and carbon quantum dots, graphene, carbon nanotubes, and nanocomposites expand the sensitivity by signal amplification and integrate several novel transduction principles such as enhanced electrochemical, optical, Raman, enhanced catalytic activity, and superparamagnetic properties into the nanosensors. The electrochemical nanosensors, optical nanosensors, electronic nose and electronic tongue, Nano barcode technology, and wireless nanosensors have revolutionized the sensing in food and agriculture sectors with multiplex and real-time sensing capabilities [193]. Despite previous success stories of the remunerative health sector, the approaches are transferred subsequently to food and agriculture sector; with potential application in detection of food contaminants such as preservatives, antibiotics, heavy metal ions, toxins, microbial load, and pathogens along with the rapid monitoring of temperature, traceability, humidity, gas, and aroma of the food stuff [227-232].

5.regarding challenges and their possible solutions

Lung cancer is a common and deadly cancer, therefore, the anticipation to revolutionise the detection of lung cancer for early detection and timely treatment lies in the innovation of sensing technologies, particularly biosensors that could provide rapid detection of lung cancer biomarkers. The review of lung cancer biomarkers, transducing techniques and recent graphene-based biosensor implementations have built a foundation for future works to be based upon. Research on novel sensing platforms has enabled the emergence of biosensors for cancer marker detection. Nevertheless, with each step forward, there are arisen challenges and hindrances. The availability of a scalable synthesis route for graphene remains to be addressed.

6. Conclusion

GO–DNA based sensors perform well at low cost, and high sensitivity, and provide low detection limits. Additionally, GO–DNA based sensors should appear in the near future as scientists explore their usefulness and properties. Finally, future perspectives and possible challenges in this area are outlined. The results of these recent research studies exhibit the outstanding performance of GO compared with current techniques. However, some challenges related to DNA sensors-based GO remain and need to be resolved. Because ssDNA is adsorbed on the surface of graphene oxide, not all dsDNA can detach from the surface of GO after the complementary ssDNA, protein or other molecules combine to ssDNA. This hinders the further improvement of the sensitivity of reported DNA sensors based on most of the recent publications reviewed, although a few authors have reported some methods for solving this problem. Currently, most published literature reports that only one target can be detected for one DNA-based sensor using GO in the liquid phase. GO in the solid phase was scarcely explored. If more targets can be detected with one sensor, the throughput of detection will be improved. GO bears oxygen functional groups on its basal planes and edges. Therefore, graphene oxide in the solid phase can also be used to make devices for sensing without chemical modification on the surface of graphene oxide. The devices are made using lithography, thermal evaporation and other micro–nanorelated scientific technology. If one GO–DNA based sensor is like an array with different DNA elements, many targets will be detected. Lung cancer is among the most important and prevalent types of cancer worldwide, and over 80% of the cases are diagnosed in the advanced steps of the disease. Since the early detection of this cancer is very useful in the disease treatment, its early diagnosis is of paramount importance. This study aims to produce a Nano biosensor based on the DNA-GO nanohybrid to detect lung cancer and deletion mutations in cancer cells. The proposed method features such advantages as high speed, simplicity, low cost, and disease detection in its early stages. Biosensors are a powerful analytical tool for the rapid analysis of several chemical molecules.

Author Contributions Statement

H.N.K AL-Salman, Chou-Yi Hsu, Zainab Nizar Jawad, Zaid H. Mahmoud: **reviewing and editing.**

Faraj Mohammed, Abdulnaser Saud, Zuhair I. Al-Mashhadani, Laila Sami Abu hadal: **reviewing and editing.**

ehsan kianfar: **Investigation, writing, reviewing and editing.**

Competing interests

The author declare that they have no competing interests.

Consent for publication

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