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NEOTERIC ADVANCES IN GRAPHENE NANOMATERIAL-BASED ELECTROCHEMICAL BIOSENSORS FOR CANCER DIAGNOSIS: A REVIEW

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Abstract— Early diagnosis of the disease can appreciably improve the survival rate or facilitate effective treatment with different modalities. In the field of disease diagnosis; nanomaterial-based bio-sensing and bioimaging techniques are lifting hopes for point-of-care cancer diagnosis with ultra-high selectivity and sensitivity. Graphene, including two-dimensional (2D) graphene films, three-dimensional (3D) graphene architectures, Graphene dots, and graphene hybrids (GHs) nanostructures have attracted the researcher`s interest in the field of biosensing and bioimaging owing to their properties. Versatile platforms of graphene nanomaterials make it as germane to detect the biomarkers at the early stage of cancer. This review selectively summarizes the recent progress in using graphene-based nanomaterials for detecting lung cancer biomarkers**.** Explicitly, graphene-electrochemical biosensors, which are classified according to sensing mechanisms and targets (CEA, NSE, hTERT, CYFRA21-1), are thoroughly discussed**.** Herewith, future scopes and challenges with other matrices, nano-scaffolds have also discoursed in the conclusion and future perspective.

Index Terms— Biosensors; Graphene; Graphene Oxide; Lung cancer; Nano-materials; Biomarkers.

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I. INTRODUCTION

In this going era, cancer is an eye-catching malignancy that jeopardizes human life in various ways and more than 60 human organs could be influenced by different types of cancer [1]. Cancer ranked top 2nd (after cardiovascular diseases: CVD) cause of human deaths with the proportion of global deaths 16% estimated in 2016 [2] by World Health Organization (WHO). In 2014 about 1.7 million new cases of all cancers were diagnosed among which 6 lakh people will die of this disease in the United States [3]. However, in Europe, there were just over 3.4 million new cases of cancer in 2012 of which 1.75 million people were dead. Most cases concerned men (56%) and 44% were women [4]. The prevalence of this abnormality is soaring as a result of variable factors such as the modern lifestyle and the presence of carcinogens in the environment and foods. Moreover, the incidence of different types of cancers is variable in the world considering heterogeneity between gender, genetic and histological types, geographical and ethnic patterns [5]. The current diagnostic methods and long-lasting care practices do not fulfill patient requirements. On the other hand, high treatment costs are a great concern for patients [6]. Therefore, demands grow increasingly for early, precise and cost-effective detection of cancer biomarkers and subsequently prevention of malignancies. Early diagnosis of cancer is crucial for the successful treatment of the disease [52]. Highly sensitive methods are urgently needed for measuring cancer diagnosis markers present at ultra-low levels during the early stages of the disease. Such methods should facilitate early detection and an adequate selection of the treatment of diseases and should lead to increased patient survival rates. Existing diagnostic tests (e.g., ELISA) are not sensitive enough and detect proteins at levels corresponding to advanced stages of the disease. Besides, the detection of low biomarker concentrations that exist in the early stages of cancer so

false negatives could be obtained [51-52]. Recently, liquid chromatography-mass spectrometry (LC-MS) based studies have been taking attention for biomarker discovery but these methods are very high-cost and technically complex for routine clinical diagnostics [7]. The biological challenges for cancer biomarker detection such as the diversity of cancer and the limited ability of a single biomarker to detect all cancers of particular organs with high specificity have encouraged researchers to spend a great deal of effort in this field. To replacing a time-consuming laboratory; smaller, faster devices are highly desired. The monitoring of cancer progress and patient therapy will be improved profoundly if the analytical results available at the patient bedside within few minutes. Biological markers (biomarkers) are interpreted as _a characteristic that is objectively measured and evaluated identically as an indicator of normal biological/pathogenic. In other words, biomarkers are quantifiable measurements of biologic homeostasis that define what is normal, thereby providing a frame of reference for predicting or detecting what is abnormal [8,9]. Biomarkers can be specific cells, molecules, genes and gene products, enzymes or hormones. The alterations/ abnormalities can be due to several factors, including germline or somatic mutations, transcriptional changes and post-translational modifications [10]. They indicate the changes in the expression of a certain protein that can be measured in tissues, body fluids such as urine, sputum, and blood. Also, body temperature (a marker of fever) and blood pressure (a marker of the risk of stroke) have been used in clinical diagnostics for a long time. Table-I bellow contains common biomarkers of the different types of cancer in the human body, while Table-II summarizes the biomarker`s sources, categories, sub-categories and classes [11]. In the literature, there are various cancer biomarkers, which can be categorized into the protein, hormone and co-gene related cancer markers, embryonic and carbohydrate antigens and enzymatic tumor markers. Biomarkers could be used in cancer staging (grading), prognosis, risk assessment and selection of treatment. Sometimes also for the determination of an individual's risk of developing cancer such as the risk of having breast and/or ovarian cancer in women could be increased by a germline mutation (BRCA1) [51].

Lung cancer is one of the most lethal cancer forms and the second most prevalent cancer in adult men and women around the world [48]. Moreover, it is also the deadliest in both sexes, because a large part of the development of the disease may occur in an asymptomatic form, and the disease is typically manifested at advanced stages [12]. Thus, early detection is a key issue in lung cancer. Low-dose chest computed tomography (CT) scanning has been suggested as a screening tool, especially in the presence of risk factors for lung cancer [13]. This type of procedure, carried out every year, is expected to increase greatly the possibility of early-stage tumor diagnosis, contributing to the increase of the survival rate [14]. However, the technique is affected by a large rate of false positives [15]. Furthermore, the exposure to the ionizing radiation of low-dose CT might increase the risk of developing cancers. Thus, large population screening, even if limited to people considered at risk, requires an alternative solution that should be non-invasive and low cost. It is necessary to have high sensitivity along with the capability of operating in complex media. The simplicity of use should also be considered to ensure that large strata of health operators can adequately utilize the instrumentation. By the same token, materials with good attributes are also needed to be used in, to design electrodes, substrates, transducers, etc. For improved selectivity and sensitivity, bioreceptor immobilization and successful recognition of biological samples development of highly effective materials for biosensor are essential.

TABLE I

GRAPHICAL REPRESENTATION OF BIOMARKER`S SOURCES, CATEGORIES, SUB-CATEGORIES AND CLASSES.

Classes	Sub_categories	Categories	Sources
Antecedent Biomarker	Peptides, fiber proteins, membrane proteins	protein	Blood
Screening Biomarker	Genes, mutations, DNA quality, micro RNA	Genomic	Saliva
Diagnostic Biomarker	Glucose, sucrose, glycans	Carbo-hydrate	Urine
Staging biomarker	Cholesterol, phospholipids,Acylglycerols	Lipid	Tissue /cells
Prognostic biomarker	Relactive oxygen species, Dicarboxylic acid	Metabolities	Faeces

TABLE II

COMMON BIOMARKERS OF THE DIFFERENT TYPES OF CANCER

TYPE OF CANCER	BIOMARKERS
LUNG	NSE, CYFRA21-1, CEA, SCCA, CA 125, VEGF, TPA, MIR-106A-5P, MIR-141-3P, KRAS, ALK, CK19, HTERT
PROSTATE	miR-103a, miR-106a, miR-107, PSA, Pro2PSA, GSTP1, P63, PCA3
LIVER	MIR-100-5P, MIR-122, A-FETOPROTEIN, HCCR-1
BREAST	miR-155, miR-261, CA15-3, EGFR, VEGF165, BRCA L, ERBB2, HER2, MUCIN-1
Gastric	miR-29c, miR-148a, CA19-9, CEA
Ovarian	miR-92, miR-93, miR-126, HE4, Mesothelin
Brain	miR-10b, MGMT, COX-2, p14arf

As a one-atom-thick planar sheet of sp2-bonded carbon atoms, graphene has received much attention in recent years in materials science due to its unusual properties, such as half-integer quantum Hall effect and ballistic and extraordinary electron transport [16,53-55]**.** Besides the electronic spectra, graphene also exhibits unique mechanical properties such as high mechanical strength, tunable bandgap [49]; a large surface area, high elasticity, superior in heat transfer [17] and also having the hydrophobic characteristic, chemical and mechanical stability [18]. Stoichiometric process (chemical reactions) offers a great potential to use the graphene in various applications and it enables researches to control the electronic properties as well as to enhance the electrochemical activities [19]. Oxidizing graphite can alter its chemical structure, forming functional groups at the edges and basal plane of the sheets show a better biocompatibility and transform into hydrophilic characteristics [20]. GO is defined as an oxygenated monolayer of carbon atoms (2D network of sp2 and sp3 bonded atoms), produced through the oxidation process [21, 22]. It is really important to address and to standardize the nomenclature for graphite oxide as GTO [23] is appropriate rather than GO. This is because of the terminology usage itself indicates the graphene-based materials having completely different characteristics from its physical structure to physicochemical properties. The presence of oxygenated groups such as hydroxyl and epoxy on the basal plane and carboxyl groups at the edges have their advantages and disadvantages [24]. On one hand, the oxygen-containing groups are really useful to develop the chemical and electrochemical biosensors which promote to the attachment of biological recognition element and ease for surface functionalization. On the other hand, the oxygenated groups also limit the electrical conductivity due to its insulating nature compared to the graphene sheet [49]. Various approaches have been employed to reduce the oxygen-containing groups by chemically, thermally, hydrothermally, electrochemically to introduced to mitigate the known fact of GO that it suffers from electrical conductivity (insulating behavior) because of the high degree of oxygenated groups In general, rGO is a monolayer (2D) composed of carbon atoms where a large number of oxygenated groups in it have been removed. This article reviews recent progress in the development of electrochemical biosensors for lung cancer biomarkers based on graphene nanomaterials.

II. STRATEGY OF ELECTROCHEMICAL SENSORS:

Amore sensitive technology platform is urgently needed to fulfill the rapid diagnosis requirements in cancer marker detection during the early stages of the disease [25]. As the classical methods (such as ELISA) for diagnosis of cancer may take several hours or even days from when tests are ordered to when results are received. These methods can be tedious, time-consuming and often require extra care and expensive instruments [50]. This especially makes an early diagnosis of cancer more difficult for cancer patients who are admitted to an emergency department. One of the key challenges in detecting biomarkers in cancer is the lack of sensitivity. The electrochemical analysis is one of the most sensitive methods for detecting inorganic, organic and even biologic substances. It is also suitable when used in the assay of cancer biomarkers [26, 27].

An ‘electrochemical biosensor’ is a self-contained integrated device which is capable of providing a specific quantitative or semi-quantitative analytical information using a bio-receptor retained in direct spatial contact and it can be classified based on the transduction modes [28]. The transduction mode is a type of measurement for the electrochemical biosensor and it could be based on potentiometric, amperometric, conductometric, impedimetric or field effects transistor [29]. Each type of measurements have a specific role[49], for instance, potentiometric is based on potential difference measurement, amperometric is based on current measurement from the electrochemical redox activity, impedimetric is based on impedance measurement of analyte/target binding event, conductometric is based on based on current conduction measurement during bio-recognition event

and the field effects transistor operates based on carrier mobility between source and drain due to electric field effect controls from the biasing gate terminal [30,31].
The application of electrochemical immunoassay is likely the most promising way to solve some of the problems concerning sensitivity, speed, selectivity and economic (one-step) measurements because an effective combination of immunochemistry coupled with electrochemistry could provide the basis of direct electrical detection for a wide range of analytes with specificity and great sensitivity [32]. In consideration its portability, low cost and high sensitivity, electrochemical sensors have become an attractive alternative to help in rapid diagnosis, providing better intervention and reducing the test-time of dissemination, which is highly beneficial in reducing patient stress [33].

III. DIAGNOSING CANCER VIA GRAPHENE-BASED ELECTROCHEMICAL BIOSENSORS

Due to the extremely low concentration of cancer biomarkers in tissue or blood, the sensitivity of the sensors is the key parameter for the newly developed for cancer biosensor [50]. Continuously improving the sensitivity of the sensor is the requirement of analysis testing and is currently the focus of this study. The methods for improvement of the sensitivity of immune-sensors were adopted by the measurement signal amplification via enzymatic reaction, magnetic microspheres, application of nanomaterials, controlled release of electro-active regents and polymer in the film of the sensors.
Electrochemical analysis method widely used in bioassay and medical testing has high sensitivity, low detection limit, and a wide linear detection range. It is simple, rapid, convenient and utilizes minimal instrumentation [34]. Electrochemical biosensors provide the combined characterizations of the high sensitivity of the electrochemical analysis. In this genre, the integration of graphene composites with transducers have shown a remarkable improvement in physicochemical properties such as it enhances the electrical conductivity, electron transfer rate and electro-active surface area to volume ratio owing to their ultra-thin 2D characteristic, which provide an important solution for higher sensing sensitivity [35]. The high surface area of graphene ≈ 2630 m2 g-1 (see Table 1) extremely increases biosensor's sensing areas, analytes loading and their 2D nanostructure flat surface becomes vigorously sensitive to any changes on its surface which is important to capture a biological event at nano-scale environments [36]. The technology to integrate 2D nanomaterials with transducers have brought a great opportunity in medical fields for disease detection because of their end-product exhibits high selectivity and sensitivity to detect a variety of biological analytes [37]. The analytical summary of graphene-based materials that bridges transducers for heart disease, lung cancer, asthma, and diabetes detection in term of the target molecule, immobilization matrixes, deposition technique, the electrochemical principle as well as their performances [49]. Further elucidation of each disease and the development of transducers using graphene-based materials as well as their performance can be found below sub-sections.

IV. LUNG CANCER DETECTION

According to the world cancer report by WHO, in 2012, the estimated numbers of new cases for all types of cancer reached more than 14 million and 8 million alone for cancer-related deaths including males and females around the globe. Among all types of new cases, lung cancer is the top rank holder with 13 % where breast cancer takes second place with 11.9% of the total new cases [38]. Lung cancer is defined as an uncontrollable growth of abnormal cells in one or both lungs. The dysfunction cells in the lung will lead to the formation of a lump like tissue/mass that generally known as tumor and it is called metastasis when the cancer cells spread to other parts of the body. The highly related lung cancer biomarkers are shown in Figure 1. SCLC (small cell lung cancer) and NSCLC (non-small cell lung cancer) are the two most common types of lung cancer that appear in the human body according to the American Cancer Society. In comparison with, NSCLC is more malignant accounts for approximately 80%- 85% meanwhile SCLC represents about 10% to 15% malignancies [39, 40]. The carcinoembryonic antigen (CEA) is widely accepted as a tumor biomarker and the marker is highly associated with diseases such as lung cancer, ovarian cancer, colon cancer, breast cancer, and pancreatic cancer. Where CEA is a glycoprotein with a molecular mass of ~200 kDa and categorized as SCLC [41-43].

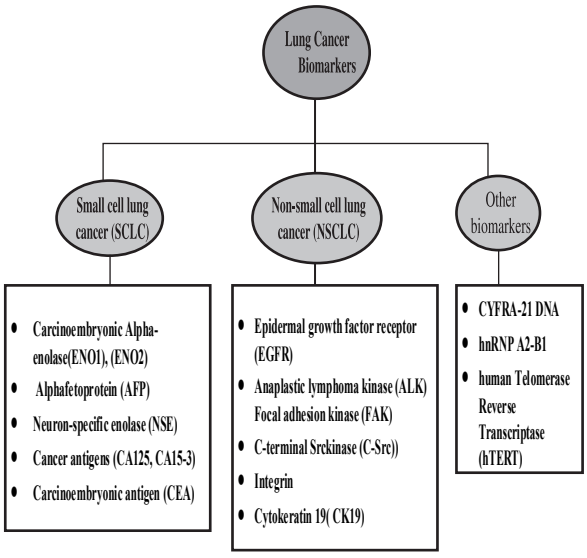

Figure 01: Highly related biomarkers for lung cancer detection.

In this section, we rundown the recent developments of electrochemical sensors based on graphene-based transducers for carcinoembryonic antigen (CEA) detection associated with lung cancer. A flexible electrochemical paper-based biosensor was developed by using poly (4 ethylene dioxythiophene): poly (styrene sulfonate) (PEDOT: PSS) and reduced graphene oxide (rGO) composite for CEA detection [41]. They prepared (PEDOT: PSS + ethylene glycol + rGO) aqueous suspension and dipped the Whatman paper into the solution for 1 h and then dried at 100 °C in a hot air oven followed by the treatment with ethylene glycol for 20 minutes and drying the paper at 100 °C for about 1 h. Eventually, through physical absorption onto PEDOT: PSS/rGO based electro-active paper the CEA antibodies are immobilized which is the affable biomarker for lung cancer. They found the sensor has a high sensitivity of 25.8 μAng- 1 mL cm-2 with a detection range of 2-8 ng/mL. The hybridization between the polymer and rGO enhances the electrical conductivity and also improve electrochemical performance along with the signal stability.

A water-dispersible graphene/amphiphilic pyrene (PPYGR) derivative modified with gold nanoparticles (AuNPs) nano-composite for sensitive detection of CEA which is a label-free impedimetric immunosensor [42]. They functionalized the graphene through non-covalent attachment by using water-soluble 4-armed poly (ethylene glycol)-NH2 (PEG) and pyrene butyric acid (PY) to manufacture PPYGR nano-composite. The produced PPYGR nano-composite exhibits an improvement in graphene hydrophilicity and loading capacity of AuNPs. This strategic also shows efficiency in immobilizing the CEA antibodies onto the AuNPs/PPYGR nanocomposite. Thus, the monoclonal CEA antibody (anti-CEA) was immobilized onto the AuNPs/ PPYGR nanocomposite using drop casts technique which takes 12 h for incubation at 35 ˚C as for the sensing platform. The as-fabricated sensor exhibits an excellent detection performance with a a wider range of response (0.1 - 1000 ng/mL) to detect CEA with a detection limit of0.06 ng/mL.

Wang et al. [43] (2017) fabricated an electrochemical immunosensor based on bimetallic (AgPt) nanorings modified with rGO forming (AgPt NRs-rGO) nano-composite for CEA detection. Using drop a casting technique and followed by drying it in the air, the AgPt NRs-rGO suspension deposited onto a glassy carbon electrode. Then, they coated anti-CEA on the electrode surface followed by drying the electrode at 4°C in the refrigerator. Finally, immersion into the bovine serum album (BSA) solution after washing the electrode with a phosphate buffer solution (PSA) and. The developed immunosensor shows very good performance having a wide linear range of 5 fg/mL–50ng/mL for the detection of CEA and the low detection limit of 1.43 fg/mL, improved stability, enhanced reproducibility, and selectivity. Where the BSA works as blocking agent molecules to fill undesired spaces where no antibody is occupied.

Chen et al. [44] (2018) reported a three-dimensional electrochemical DNA biosensor based on 3D graphene functionalized with Ag nanoparticle for sensitive DNA detection. Where the highly conductive nano-composite layer was characterized by using scanning and transmission electron microscopy, contact angle analysis and cyclic voltammetry. They combined 3D graphene (3D GF) and Ag NPs for CYFRA21-1 DNA detection as *CYFRA21-1* DNA is a sensitive and specific marker for non-small cell lung cancer (NSCLC). Hence, the 3D GF/Ag NPs improved immobilization of DNA on the sensor and reduced the detection limit. Moreover, the 3D GF/AgNPs provide a favorable microenvironment to retain the bioactivity of immobilized probe ssDNA and effectively promote electron transfer owing to their excellent biocompatibility and good conductivity. Under optimal conditions, the proposed biosensor could detect target DNA down to 1.0×10^{-14} M with high sensitivity and the peak currents were linear with the logarithm of the concentration of target DNA from 1.0×10^{-14} to 1.0×10^{-7} M. Furthermore, in clinical trials, this 3D biosensing system detected *CYFRA21-1* DNA in real lung cancer samples with satisfactory results.

An ultrasensitive electrochemical immunoassay strategy to detect neuron-specific enolase (NSE) with a triple signal amplification strategy biomarker was proposed, which combined 3D-GNS/CS as the matrix, OMCSi-Au materials as labels, and AuNPs-induced silver deposition on the immunosensor surface Fang et al. [46] (2019). The proposed immunosensor showed great performance for NSE with acceptable reproducibility, stability, reliable detection, and high sensitivity, it provides great potential in clinical application. Utilization of porous three-dimensional graphene-starch architecture (3D-GNS) on the immunosensor surface to construct a unique 3D immune-electrode, which would greatly accelerate electron transfer and capture more protein molecules and thus it enhances the sensitivity of the device. 3D-GNS was prepared with starch as a crosslinking agent and stabilizer, which is biocompatible and environmentally friendly. After a sandwich-type immunoreaction, the OMCSi-Au labeled Ab2 was trapped on the surface of immunosensor, the high concentration of AuNPs with high dispersion greatly catalyze the deposition of silver nanoparticles. The deposited silver nanoparticles (AgNPs) could be tested directly with anodic stripping voltammetric analysis (ASV) in potassium chloride solution to monitor the immunoreactions. The linear detection range for the proposed sensor reported as 0.02 pg/mL to 35 ng/mL for neuron-specific enolase antigen and the detection limit of 0.008 pg/mL. The satisfactory results obtained with high stability and sensitivity confirmed the

practicality and promising applicability of this three-dimensional graphene-starch architecture (3D-GNS) based immunosensor for clinical applications to allow for the early detection of cancer.

A new window and method for lung cancer detection has paved through with the introduction of a noninvasive salivary biosensor. Choudhary et. [45] reported the fabrication of ultrasensitive Graphene Oxide (GO) based electrochemical immunosensor for the very first time to detect human telomerase reverse transcriptase (hTERT) a lung cancer biomarker. They fabricated immuno-electrode by covalent immobilization of rabbit anti-hTERT antibodies (Ab) onto GO films on ITO coated glass. Moreover, GO-based immunosensor exhibits specificity and low detection up to 10 ag mL-1 (10×10-18 g mL-1) in wide detection range (10 ag mL-1-50 ng mL-1) for hTERT corroborate by the electrochemical differential Pulse Voltammetry (DPV). The proposed immunosensor showed the potentiality for low-level detection of hTERT in biological fluids (lung/oral) samples. The uniform distribution of GO nanosheets; having large surface area enhanced the performance by efficient loading of antibodies.

TABLE III

BIOSENSORS BASED ON GRAPHENE NANOMATERIALS WITH DETECTION SPECIFICATIONS FOR LUNG CANCER

Biomarker (Target)	Method	Linear range	Detection limit
NSE	Electrochemical	20 fg mL-1 to 35 ng mL-1	ng mL-1 8 fgmL-1
hTERT	Electrochemical	10 ag mL-1 to 50 ng mL-1	10 ag mL-1
CYFRA 21-1	Electrochemical	1.0×10^{-14} M to 1.0×10^{-7} M	1.0×10^{-14} M
CEA	Electrochemical	5 fg/mL to 50ng/mL	1.43 fg/mL
CEA	Electrochemical	0.1 to 1000 ng/mL	0.06 ng/mL
CEA	Electrochemical	2 to 8 ng/mL	25.8 µAng- 1 mL cm-2

V. CONCLUSION AND FUTURE PERSPECTIVE

As early-stage detection of lung cancer is arduous so it has the highest mortality rate within all types of cancers. About 70% of lung cancer cases are detected at the advanced stage and it can be detected through high-end equipment or invasive procedure like positron-emission tomography (PET) scan, computed tomography (CT) scan, magnetic resonance imaging (MRI), fluorescence bronchoscopy, mediastinoscopy, sputum cytology, needle biopsy (fine needle aspiration), blood tests, pulmonary function tests, immunological methods, however, the survival chances are often too low. Furthermore, these methods are only available at the selected hospital because of the expensiveness and required trained personnel.

In recent years, great advancements have been seen in the field of the biosensor, based on graphene-nanomaterials aptitudes used in electrochemical biosensors, for the early-stage detection of lung cancer. Different kinds of graphene-based materials such as Graphene Oxide (GO) films, reduced Graphene Oxide (rGO), 3D graphene architectures, graphene-modified Ag, Au nanomaterials have been introduced in this paper. graphene, in turn, makes cancer identification more sensitive, selective and accurate on account of its elegant versatility and outstanding properties. For example, high electrochemical performances are attributed due to the fast electron transfer and efficient immobilization of receptors on a large surface area (synergistic effects of nanocomposite hybridization) supported by the uniform distribution of graphene nanosheets. Though advances made in this area are exciting and encouraging in cancer detection the commercialization of such biosensor is still in its early stage for all aforementioned diseases. The drawbacks and challenges presented with graphene nanomaterials need to be overcome. For example, a universal method to synthesis graphene-based materials with desired size and layers; the production of a high-quality graphene layer with zero defect. However, other quasi-2D nanomaterials such as hexagonal boron nitride (h-BN) [56] and transition metal dichalcogenides like molybdenum disulfide (MoS_2) [58-61], niobium Selenide ($NbSe_2$) [57], tungsten disulfide (WS_2) [62] are widely studied by researchers for the development of biosensors. Recently, the MoS_2 gets large attention among researches to develop electrochemical biosensors. It has additional advantages of the tunable bandgap and an easy exfoliation into a single layer- due to weak Van der Waals bonding force over pristine graphene. The hybridization between graphene and MoS_2 would bring an extra high surface area and also expected to have an excellent electrocatalytic activity that is suitable for electrochemical applications [58-61]. We would like to wind up by stating that there is still much room for the scientific research and technological development of graphene-related theory, material-matrices concerning the applications of graphene-based nanomaterials.

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