Review

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Ultra rapid biosensors for detecting carcinoma biomarkers

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ABSTRACT

Preliminary screening and successful therapy are the two biggest challenges in the battle against cancer prognosis and diagnosis. This life-threatening disease can be profoundly imagined and identified based on the tracing of widely eliciting/expressing protein biomarkers in the biological fluids of an individual. These protein biomarker may fall into broad categories based on the types of tissues or organs being involved and are commonly known as onco-biomarkers or tumor markers. Indicators of cancer are routinely detected using a myriad of developing bio-sensing tools for point of care diagnostics that have really been proven to be an effective solution for early-stage cancer. Modern and advanced approaches offer great advantages over the use of traditional assessment therapies, such as chemotherapy, radiation, surgery, and combinations, of all but display several noticeable drawbacks in that they are time-consuming, hard to use, skilled labour-intensive, costly, less efficient, less sensitive, require more analysis time, and are often not up to the mark. Recently, various bio-sensing approaches have drawn special attention toward on-site screening and real-time detection of critical protein biomarkers. This review provides an insightful overview of current advances made in sensing techniques used for detecting biomarkers for various types of carcinomas. Various sensing methodologies and tools used for the detection of each biomarker have been thoroughly reviewed. Every one of these processes indicates that multifaceted innovation-based tumor diagnostics are becoming an indisputable substitute for conventional strategies.

Keywords: Carcinomas, Cancer Biomarkers, Biosensors.

CONTENTS

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1. INTRODUCTION

Right now, Cancer is one of the significant reasons and major causes of death across the world. It is a serious universal health concern affecting more than half of the world's larger population [1–3]. It is assessed that over 11 million individuals are diagnosed having cancer and there will bean incidence of 16 million new cases each year by the end of 2020 [2, 3]. According to the latest report of the

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American Cancer Society, approximately 606,520 US individuals will die from cancer in the annual year 2020, comparable to more than 1,600 deaths per day. A large number of death cases have been reported in men mostly due to lung, prostate, and colorectum cancer and the lung, breast, and colorectum cancer in women. Practically, one-fourth part of all tumor deaths is because of lung cancer. Cancer is the second most common reason for death among youngsters matured 1 to 14 years in the United States. By the year 2020, an expected 11,050 children of age group (birth to 14 years) and 5,800 adolescents of age (from 15–19 years) will be identified with cancer, and 1,190 and 540, apart, will die from the infection [3, 4]. To reduce the risk of high mortality rate, it is mandatory to detect and diagnose cancer accurately and in the premature phase which is certainly the best solution to boost triumphant medical treatment of the ailment, bring about increased chances of survival rate of the patient [3, 5–8]. Initial prognosis and diagnosis of a malignant tumor are many times conventionally lacking because of the absence of symptoms or side effects during the beginning phases of the illness. All types of a malignant growth are frequently analyzed by the amalgamation of clinical imaging procedures and the identification of serological tumor markers in the real samples [9, 10]. Detection and analysis of tumor biomarkers through standard immunological assays and emerging molecular techniques are already in practice at the clinical forefront. These includes commercial enzyme-linked immunoassay [2, 11–15], radioimmunoassay [2], western blotting, quantitative real-time polymerase chain reaction [2], Immuno histochemistry, fluorescence immunoassay [2], electro-chemiluminescence (ECL) assay [1], chemiluminescence (CL) assay [1, 16, 17] etc. Undoubtedly, all these serological and diagnostics assays performed in clinical and research laboratories displaying the advantage of highly satisfying and effective detection as they largely depend on the utility of specific antibody as the bio-recognition component but still the individual method do not incorporate all the attributes singly due to several drawbacks as they are time-consuming, high cost, lengthy experiments, inconvenient, complicated, pre-treatment of the sample and other handling and operational errors [11, 13, 14, 18–22]. Due to these unavoidable limitations, there is a high demand for the development of selective, sensitive and specific tools which can be able to overcome the outdated methods. Figure 1 clearly depicts the working principle of several types of biosensors for the detection of biomarkers associated with deadly cancer onset and progression.

2. BIOMARKER: REHABILITATION OF UNTIMELY MALIGNANT TUMOR

As stated by National Cancer Institute, a biomarker might be utilized to perceive how well the body reacts to treatment for an illness or condition. Likewise called molecular and signature particle. All the more explicitly as far as clinical utility, a malignancy biomarker may quantify the danger of creating disease in a particular tissue or, on the other hand, may gauge hazard of malignancy movement or expected reaction to treatment.

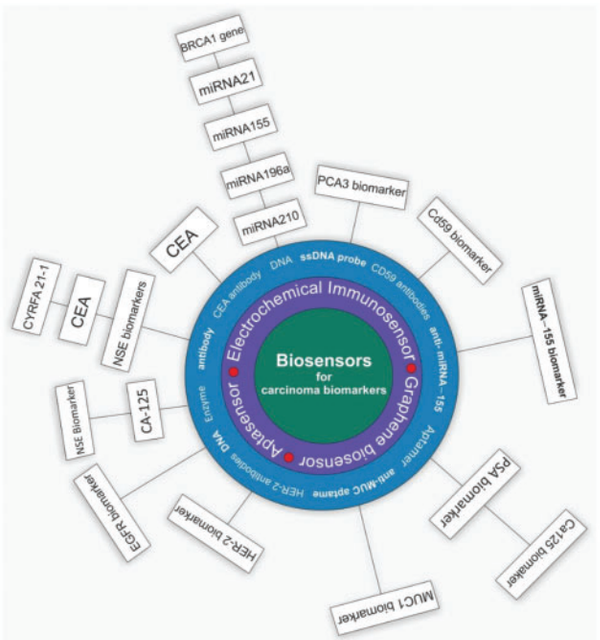

Fig. 1. Schematic portrayal of the concept of working of biosensors for the identification and determination of potential carcinoma biomarkers.

Disease biomarkers can be ordered into the accompanying classes dependent on their utilization. Predictive biomarkers anticipate reaction to explicit helpful mediations. Prognostic biomarkers, then again, may not be straightforwardly connected to or trigger explicit restorative choices. Another class of biomarkers, the indicative biomarker, is utilized to recognize whether a patient has a particular sickness condition. A large population around the world are encountering countless types of alarming tumors due to variegated symptoms on an immediate basis or for other reasons. At present, markers are the only choice and hope since they are an indication of disease emergence due to elevated amounts exhibited by cancer affected cells rather than non-cancerous cells. Also, there are two principle kinds of tumor markers: circulating markers and tissue markers. The majority of biomarkers could be successfully discovered, developed and validated for the screening of many cancer types. In the present review, several traditional and modern biosensing techniques for cancer type brought into the picture for recording the elevated levels of biomarkers, which could be traditionally proteins and other molecules secreted in higher aliquot by the damaged cells and tissues to lessen the anxiety of the prevalent situation arose.

3. SIGNIFICANCE OF CARCINOMA BIOMARKERS IN CANCER PROGNOSIS AND DIAGNOSTICS

According to the definition given by the National Cancer Institute, a 'Biomarker' is a substance or a biomolecule present in the blood serum, plasma fluids, or tissues that can be fairly quantified and assessed as an indication of a usual or unusual biological activity and a pathogenic condition/infection [3]. A cancer biomarker mentions an exceptional change of a protein, small molecules, DNA, RNA, or hormone in tumor cells. Such changes are portrayed as analytical and prognostic, which can be enlightening in tumor diagnosis and its course of recurrence, separately [23]. These biomarkers refer to the amount that can be evaluated in a specific biological state or illness condition. Cancer-related markers are the substances that can be recognized and fulfil the criteria as signals of pathogenic procedures or pharmacological reaction to treatment. Distinctive on co-markers may be utilized to differentiate general and abnormal pathogenic processes. A perfect biomarker can be originated from neoplastic cells and is usually indistinguishable in healthy and tumor tissues, and can be recognized by simple basic techniques in the accessible biological sample (biological fluids). It should be delicate, specific, and inexpensive. Tumor biomarkers are classified into many types based on the alterations occurring at the genetic level such as mutations, RNA expression profiling, change in copy number, at an epigenetic level like change in the DNA methylation process, at the proteomic level as changes in protein expression profile, at the metabolic level as changes in level and range of low sub-atomic weight metabolites, exosomal microRNAs, flowing tumor cells (CTCs), RNAs and DNAs coursing in blood plasma, blood protein biomarkers. By and large, since proteins are directly involved in cellular processes, thus, they are the most suitable biomarkers for the diagnosis of cancer [24].

4. BIOSENSING APPROACHES FOR DETECTING TUMOR RELATED BIOMARKERS

Several potential sensing techniques have emerged in the last few decades keeping in view the drawbacks exhibited by traditional, inefficient and bulky methods and are still in the pipeline for the rapid and sensitive detection of various blood-based protein biomarkers to examine deadly tumor before spreading to other normal tissues and organs of the body. In this queue, nanotechnology is playing a crucial role in the development of miniaturized devices which are extremely reliable, technology-dependent, fast, sensitive, low cost, label-free, easy to operate, and highly reproducible. Immunosensors are one of the most critical groups of biosensors that have been comprehensively utilized broadly employed to detect cancer protein biomarkers rely upon the association of Ag–Ab with uncommon sensitivity and especially splendid selectivity. It very well may be classified broadly into two kinds (a) labelled immunosensor and (b) label-free immunosensor. In a label-free immunosensor are straightforwardly measured Ag–Ab complex interaction, on the other hand, the labelled immunosensor, used a tag or a label along with a primary or a secondary Ab to amplify the signal [25, 26]. The phenomena of CL uses chemical energy produced from biochemical reactions that give an illuminating discharge of light due to the low energy of the atoms while coming back from the energised state to the ground state. The response among the immobilized biomolecule set apart with CL species and analyte brings about creating light due to this response [27]. ECL is characterized as CL actuated by electrochemical technique. In ECL response reaction, the radiated light is recognised in presence of an optimum required potential. Paper-based biosensors have attracted a lot of considerable attention on account of their many advantages like economical, throw away, faster response time and, biologically acceptable. Different methodologies like UV photolithography, wax printing, and screen printing have been constructed for the manufacture of paper-based biosensors [28]. Microfluidic lab-on-a-chip-based sensor is one of the amazing and potent miniaturized tools that facilitate the joining of complex functionalities in one or more sensing platforms on a single system, with less volume of sample. Microfluidic gadgets encourage viable acknowledgement of biomolecules effective at the expense of little volume of reagents, thus clearing another way for the manufacturing of the point-of-care devices [29]. Giant magneto resistive (GMR) method has been created for biomolecular detection on the parameter of magnetically labelled molecules because it shows various attributes like excellent sensitivity, amicable with the integrated circuits, and the possibility for measuring concentrations of multiple complex biomarkers all at once (viz., multiplex) in a portable device [30]. Molecular imprinting (MIP) is a method to develop accurate sites of activity in polymers via a template having a higher affinity against the target molecule. Molecularly imprinted polymers (MIPs), which are also defined as cross-connected polymers, are customised materials. Determination of different target molecules with the help of field effect transistor (FET) or chemiresistive based biosensors have achieved wide consideration among research networks since they do not involve pre-sample preparation steps and are appropriate for on-site monitoring due to faster and accurate transduction procedure. The mobility and whole analytical performances of the sensors can be upgraded by utilizing nanostructures as alluring channel substances. The electrochemical methods have been discovered encouraging in bio-sensing applications as a result of different benefits including high sensitivity, explicitness, cheaper, low detection limit, great dependability, and easiness in dealing with Refs. [25, 31–36].

4.1. Biosensors for Detecting Hepatic Carcinoma Biomarkers

Lung cancer also additionally characterized as pulmonary carcinoma is doubtlessly the common one among the most fatal type of health affairs of the 21st century. Among men, it is the first driving cause of cancer-related deaths and in women the second driving reason for death around the world, representing about 1.5 million passing yearly. Early and precise screening and examination utilizing practical and cheaper methods are needed on a top priority to accurately diagnose the onset of the disease in an early stage, to decline the number of deaths by improving the chance of survival and to decrease mortality and morbidity rate related with such patients [23]. Chest infections, Cough, coughing up blood, constant breathlessness, lack of energy, emptysis, dyspnea, wheezing, weight loss, fatigue, loss of appetite and distaste are the prominent symptoms of lung cancer and can only be detected in the later stages of the disease [23, 24]. Alas, the early stages of this illnesss typically detected only accidentally. Chest radiography and computer tomography (CT) are the maxima usually used techniques for most lung carcinoma diagnosis. However, as they can solely determine visible and irreversible changes in the lung, there is a necessity for added ways for early identification. To beat this challenge, it is far vital to find out novel, exceptionally sensitive, and specific tumor biomarkers [24]. Aydın et al. [37] developed a novel ultrasensitive and label-free immunosensor for the detection of neuron-specific enolase (NSE) antigen in human serum. The immunosensor was fabricated by using an immobilization surface of the disposable ITO electrode made by epoxy-substituted-polypyrrole P (Pyr-Epx) polymer. NSE is a standard, sensitive, and reliable small cell lung cancer (SCLC) biomarker for lung cancer. Neuron-specific enolase is a glycolytic enzyme released from central, peripheral neurons as well as from the neuroendocrine tissues [37, 38]. The maximum normal range of NSE concentration in the human serum sample is 12.5 $ng \cdot mL^{-1}$. Patients having SCLC bear NSE levels mostly somewhere in the range of 12.5 and 25 $ng \cdot mL^{-1}$. Quick and precise determination of NSE biomarker is significant in clinical diagnosis and ailment treatment [37, 39]. Multiple strategies including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to characterize the alterations of the immunosensor. Besides, scanning electron microscopy (SEM) and atomic force microscopy (AFM) methods were taken to screen the morphology of the electrode surface. The designed sensor showed a linear range of detection of 0.02–7.5 $pg \cdot mL^{-1}$, with minimum limit of detection (LOD) of 6.1 $fg \cdot mL^{-1}$. Furthermore, the constructed immunosensor permit for selective, highly sensitive, and exact detection biomarkers in serum samples after simple dilution in phosphate buffer saline [37].

Kalkal et al. [40] developed a somehow similar biosensor for the detection of small cell lung cancer biomarkers by utilizing graphene quantum dots and gold nanoparticles where the former behaves as an energy donor and later works as an energy acceptor. In the proposed fluorescent biosensor, the NSE monoclonal antibodies were covalently fixed to the amine-N-GQDs platform to get the anti-NSE/amine-N-GQDs bio-functionalized surface. Here, fluorescence response studies were conducted using an anti-NSE/amine-N-GQDs@AuNPs nanoprobe- and nano-surface energy transfer (NSET) mechanism was adopted for the development of label-free, efficient, and affordable biosensor for NSE detection. The present biosensor displays the wide linear detection range of 0.1 $pg \cdot mL^{-1}$ to 1000 $ng \cdot mL^{-1}$ with a wonderful low detection limit of 0.09 $pg \cdot mL^{-1}$. Apart from this, the biosensor shows outstanding performance in real samples with an average value of 94.69%. Wu et al. [41] demonstrated a multiplexed fluoro-immunoassay for the quantification of threesome lung cancer biomarkers CYRFA 21-1, CEA, and NSE present in serum sample using multicolour quantum dots as reporter probe with labelled antibodies and micro-magnetic beads as an immobilization substrate in a single format. The assay was designed in sandwich-type layout employing 2 monoclonal antibodies concurrently capturing dual sites on the target molecule. The fragments of the three cancer biomarkers were altogether analyzed in a single reaction attaining LOD of 1.0 $ng \cdot mL^{-1}$ (364 $pg \cdot mL^{-1}$ for CYRFA21-1, 38 $pg \cdot mL^{-1}$ for CEA, 370 $pg \cdot mL^{-1}$ for NSE). The advantages of the assay are simple, low cost, reproducible and require low sample volume for the easy, simultaneous detection of multiple proteins in blood serum. Nguyen et al. [42–45] proposed a simple, sensitive aptamer-based sensing platform based on amine-terminated aptamer modified-gold electrodes for the detection of lung carcinoma cell line A549 using the microfluidic channel with a flow rate kept below 10 $\mu L \cdot min^{-1}$. For the effective and selective identification of A549 cells, the response of the fabricated sensor was measured by EIS and an optical microscope. The measured outcomes were also shown by making use of a similar circuit model developed for impedance-based analysis. The proposed sensor was found to be highly efficient for detecting target A549 cancerous cells when compared to control cells i.e., MKN45 cells, Hela cells, Caco-2 cells, and Red Blood Cells. Even though, the recognition limit of the current sensor was as yet restricted, the aptamer biochip showed numerous appealing attributes, to be specific, effortlessness, fast, cheap, minimal effort, biocompatible, selectivity, and affectability towards the determination of lung cancer cells. Kovalska et al. [46, 47] shows a label-less, cheaper, highly sensitive, non-invasive electrochemical immunosensor for the identification of CD59, a clinically important biomarker of lung cancer. This platform was based on graphene oxide nanoparticles incorporated into the pencil graphite electrode. The experimental framework of the immunosensor was produced

through immobilization of the counter CD59 antibodies on a graphene oxide (GO) nanoparticle integrated into a pencil graphite electrode. The purpose of the usage of GO nanoparticles was to improve the conductivity performance of the immunosensor. SEM, EIS, and CV were used for the characterization of the fabricated probe. The detection of CD59 was accompanied by measuring the current, which uncovered a linear range of 1 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$ with a minimum limit of detection 1 $fg \cdot mL^{-1}$. The potential of the immunosensor was evident by its good storage stability and specificity. The immunosensor shows no limitations in overall performance and is highly recommended for the on the spot clinical diagnosis due to the capability of miniaturisation into a compact device.

Khatoon et al. [48] developed an electrochemical sensor for the detection of ethyl acetate, a lung cancer biomarker. The synthesis of undoped and doped SnO_2 nanomaterial was made by the sol–gel method for checking the doping effect of copper and nickel. The average limit of sensitivity was detected for three metal oxides as SnO_2 is 0.3 μA/ppb, for $CuSnO_2$ as 4.8 μA/ppb and for $NiSnO_2$ as 2.3 μA/ppb over a wide linear range of 1 ppb to 20 ppb. Zhou et al. [49] demonstrated a newer *in-situ*, robust colourimetric aptamer modified gold nanoparticles aptasensor for the monitoring of a nucleoside, adenosine as a lung cancer biomarker. The performance of the aptasensor was found excellent for the AuNPs-aptamer-biotin system when compared with the rest of the three-aptamer platform. The sensor utilized an in-house biomimetic electronic eye system for real-time detection of the change in colour intensity of the gold nanoparticles upon binding with the aptamer nanoparticles. The detection was lie in the range of 5.0 μM–60.0 μM with LOD of 0.17 μM. The performance of the sensor for the detection of adenosine was further proved in the samples of artificial urine and water and a linear range was detected from 5.0 to 50.0 μM with a LOD of 0.48 μM. Zeng et al. [50] demonstrated an electrochemical immunosensor based on the sandwich-type strategy for the determination of CYFRA21-1, generally associated with non-small cell lung cancer (NSCLC). The sensor was fabricated for the enhancement and detection of a signal produced by the molecule when immobilised on the GA/3D-G/GCE framework. The developed electrochemical response was identified by DPV in a buffer and clinical samples for CYFRA21-1 with a wide range from 0.1 to 150 $ng \cdot mL^{-1}$ and a lower limit of detection 43 $pg \cdot mL^{-1}$. Singh et al. [51] reported the atomically thin layer of graphene synthesized on the copper substrate using a CVD method for constructing a biosensing surface for CEA detection. PBSE/graphene/Cu electrode have been used for the covalent attachment of the anti-CEA antibody. The specific detection of carcinoembryonic antigens, a composite of anti-CEA/PBSE/graphene/Cu was used as an electrode following which the electrical measurements were accomplished using the EIS technique. The linear range of detection was physiologically between 1.0–25.0 ng mL^{-1} with a LOD of 0.23 $ng \cdot mL^{-1}$. Chiu et al. [52] demonstrated an SPR based biosensing platform by utilizing GO-COOH as an immobilization material for the quick and reliable detection of CK19 protein biomarker present in plasma containing non-small lung carcinoma cells. The detection limit of CK19 was 0.05 $pg \cdot mL^{-1}$ with a linear range of 0.001 to 100 $pg \cdot mL^{-1}$. Shoja et al. [53] fabricated an electrochemical genobiosensor based on rGO/f-OMC nanocomposite modified pencil graphite electrode for the detection of lung cancer biomarker. This was one of the best electrochemical biosensors reported for the identification of EGFR exon21 L858R point mutation. The constructed sensor was undergone CV, FESEM, FTIR and EDS for their characterization profiling. It was observed that the developed sensor displayed longer firmness with a lowest detection limit of 120 nM and a sensitive linear range from 0.1 μM to 3 μM. Tian et al. [54] developed a paper-based DNA sensor for the noninvasive identification of mutations associated with non-small cell lung cancer. EGFR biomarker was detected on the phenomena of DNA hybridization technique. A polypyrrole membrane was initially modified by an ssDNA molecule via Coulomb force and fabricated over the gold-coated electrode surface by weak bonds. A linear relationship was established among target DNA concentrations with respect to the current produced which was detected as 0.5 nM to 500.0 nM with an LOD as low as 0.167 nM.

4.2. Biosensors for Detecting Prostate Cancer Biomarkers

Prostate cancer is the second most common cancer in the United States pass by lung cancer [2, 55, 56]. In the case of prostate cancer testing, biomarkers perform a major role in the detection, screening, diagnosis, prognosis, and management in clinical settings. These biomarkers are biological molecules that can indicate disease conditions and can have multiple sources, such as ribonucleic acid transcripts (RNA), proteins, deoxyribonucleic acid (DNA), or epigenetic modifications of DNA, metabolic products or other associated changes. The biomarkers can be detected either invasively such as tumor tissue samples or non-invasively from biological materials by separating cells or tissues from human blood, serum, plasma, or urine samples [3]. Tracking of prostate diseases is mainly accomplished with the aid of determining the amount of prostate-specific antigen in blood samples. Blood serum PSA assessments are very sensitive, but sadly PSA is not always especially precise for prostate tumors because excessive PSA concentrations won't be most cancers related, at the same time as even worse in most cases, tumors may additionally develop earlier prior to this concentration increases. So, early treatment requires screening and malignant prostate tissue biopsy based on PSA [56, 57]. An electrochemical biosensor was currently reported which can simultaneously detect

vascular endothelial growth factor and PSA based on the nanocomposite of graphene oxide/ssDNA/PLLA nanoparticles [37]. Osteopontin (OPN), a 33–34 kDa single-chain glycoprotein consists of 261 amino acid residues synthesized by the normal and abnormal prostate cells, the concentration of which is increased in men having prostate cancer. OPN is practically associated with all means of tumor development and is being examined as a potent biomarker for the diagnosis and early forecast of prostate cancer. Serum PSA levels are clinically utilized as a marker of prostate cancer screening, observing the effectiveness of treatment, and assessing the likelihood of revocation post-treatment. It is produced by both the normal and the diseased prostate cells, but the levels of which are elevated in men with prostate cancer [1].

Soares et al. [56] developed an electrochemical biosensor utilizing PCA3 ssDNA probe molecule adhered on a matrix layer of chitosan and multi-walled carbon nanotubes for the detection of PCA3. PCA3 biomarker is a newly discovered prostate cancer biomarker which is a specific, non-coding RNA having 3922 nucleotide sequences in a fully matured form. The concerned biomarker is found to be only expressed in prostate cancer and showing no relation to other prostate cancer-related ailments. Detection of PCA3 has also been done by several methods like a polymerase chain reaction, fluorescence emission, optical methods, and transcription-mediated amplification which are high priced, require trained personnel and complicated devices. In this study, the employment and desire of the chitosan polysaccharide and carbon nanomaterial became influenced by previous studies in which these sorts of fabric succeeded in serving the good performance of the biological molecules with MWCNTs additionally enhancing the electrical signal output. The fabricated biosensor showed high selectivity and sensitivity with a better-optimised performance for the detection of PCA3 synthetic controls. This very first type of electrochemical and impedance-based biosensor was able to detect PCA3 with a limit of detection traceable to 0.128 $nmol \cdot L^{-1}$. Heydari-Bafrooei and Shamszadeh [1] successfully demonstrated an ultrasensitive, marker-free, newer aptasensor for fast, sensitive detection of PSA in blood serum samples. The fabrication and modification of the electrode surface were done by forming a nanocomposite of gold nanoparticles over the layer of rGO multi-walled carbon nanotube. The formation of the aptamer-PSA complex was corresponding to the changes in the electron transfer resistance and differential pulse voltammetry current. The fabricated biosensor has many advantages since the blending of two different nanomaterials explicit excellent properties. The use of AuNPs on rGO-MWCNT produce effective signal amplification also the integration of gold nanoparticles improved the conductivity between the graphene nanosheets. Compared with other nanomaterials surfaces like AuNPs, MWCNT/AuNPs, rGO-MWCNT/AuNPs and rGO-MWCNT, blended nano-mixture modified electrode proving to be the best sensitive platform for the analysis of PSA. The designed aptasensor had an incredibly low detection limit of 1.0 $pg \cdot mL^{-1}$ with the detection range of 0.005–20 $ng \cdot mL^{-1}$ and 0.005–100 $ng \cdot mL^{-1}$ for DPV and EIS curves. The proposed tool could be a good candidate for the PSA analysis at the clinical level.

Tang et al. [8] proposed a peptide-based electrochemical biosensor for ultrasensitive PSA detection. Here, Bovine serum albumin could be successfully implemented the first time to enhance the sensitivity of the method. The substrate of Chitosan-$Pb_2[Fe(CN)_6]$-PDDA-GO was used as a novel redox species for the fabrication of the glassy carbon electrode via the drop coating process. Electrochemical studies were performed using a triple electrode system. Chitosan works by joining with peptide, adsorb Pb^{2+} ionic particles by coordination effect for ionic current signal intensification. The framework of GO-PDDA helps in enlarging the specific area along with the conductivity of the sensing surface, thereby resulting in more signal amplification. The developed amperometric biosensor showed a lower detection limit of 0.01 $fg \cdot mL^{-1}$ with a broad linear range of 100 $ng \cdot mL^{-1} \sim 1$ $fg \cdot mL^{-1}$ respectively. Sana et al. [59] introduced a label-free photonic crystal based double nanocavity type resonator biosensor for the detection of a very small volume of PSA biomarker in the human blood [58]. After adjusting the process of immobilization of the target biomarker protein, the authors were able to detect the PSA in the concentration range as low as 0.5 $ng \cdot mL^{-1}$. The activity of the sensor was checked by Sucrose and PSA together. They reported on the real PSA biomarker detection those employed in clinical diagnosis utilizing highly sensitive antigen-antibody reaction assay. Wu et al. [60] proposed Pt@AuNPs assembled cheap, transferable quantitative capillary biosensor for the point of care diagnostics applications. This novel biosensor is devoted to biomarker detection with the naked eye. The developed biosensor utilised simple sandwich immunoassay with antibody covered magnetic beads and antibody-coated Pt@AuNPs to recognize prostate-specific antigen and carcinoembryonic antigen. For detection of positive samples, mAb1-Pt@AuNPs was linked to mAb_2-MBs via antigen bridge effect, rinsed and added hydrogen peroxide upon which an enormous volume of oxygen gas was produced. For negative samples detection, no mAb_1-Pt@AuNPs composite was bound to mAb_2-MBs in absence of antigen, rinsed and added hydrogen peroxide upon which no oxygen gas was released. The concentrations of these biomarkers were determined by estimating the movement distance of blue ink as instigated by the evolution of O_2 gas by means of Pt@AuNPs-catalyzed dissociation of H_2O_2. The LDR and LOD shown by the sensor for PSA were 0.02–2.5 $ng \cdot mL^{-1}$ and 0.017 $ng \cdot mL^{-1}$ and for CEA were 0.063–16 $ng \cdot mL^{-1}$ and 0.044 $ng \cdot mL^{-1}$.

This biosensor has good applicability towards clinical diagnosis, personalized medicine and food safety and environmental monitoring. When compared to other conventional detection methods, it was found to be affordable, sensitive, robust, ease of operation, less need of types of equipment, simple design, easy to assemble and above all these properties, provide better detection capability.

Presnova et al. [61] demonstrated a field-effect transistor-based biosensor for quantitative detection of PSA biomarker in human serum and buffer samples in real-time. This technique made use of gold nanoparticles and 3-glycidopropyltrimethox-ysilane bearing –SH groups for the functionalization of silicon nanowire-based transistor. The concentration range of prostate-specific antigen lies in the order of 23 $fg \cdot mL^{-1}$–500 $ng \cdot mL^{-1}$ with a lower LOD as 23 $fg \cdot mL^{-1}$. Sana et al. [59] developed a resonating biosensor based on photonic crystal for the determination of PSA. The proposed double nanocavity sensor was optimised for two different analytes (sucrose and real PSA biomarker) simultaneously. The detection was purely based on antigen-antibody complex formation with a low detected limit observed as 0.5 $ng \cdot mL^{-1}$. Shin et al. [62] demonstrated a magnetic bead-based biosensor using EIS for the selective detection of PSA. This BEIS system incorporated a microwell array for immunoassay purpose in human serum, plasma and buffer samples and represented an enhanced sensitivity with a detection limit up to 100 $fg \cdot mL^{-1}$ and a wide linear response from 100 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$.

4.3. Biosensors for Detecting Ovarian Cancer Biomarkers

Ovarian cancer being one of the most lethal types of cancer among every gynaecological [63] disease and the fifth driving reason for death because of cancer in women. It is the cancer of the female reproductive system. This is to a great extent because this cancer is asymptomatic and diagnosed in later stages. Its late prognosis is mainly related to the advanced level of the disease, also the high return rate linked with the development of chemoresistance. Survival stats have not modified notably over the past three decades, underlining the fact that existing therapeutic methods and premature detection demands considerable refinements. In the USA, an expected 22,280 new ovarian cancer patients were evaluated to surrender to ovarian cancer and approximately 14,240 deaths are expected in 2016 [64]. Several reported biomarkers such as CA-125 (MUC16), mucin 1 (MUC1), HE4 (Human epididymis protein-4), prostasin, osteopontin and lysophosphatidic acid (LPA) and can be normally determined in the blood of the patient, are currently being employed for the detection of different stages, treatment and diagnosis of ovarian cancer and its progression, but no single biomarker is expected to show contradicting diagnostic accuracy reliability, and sensitivity [65, 66]. Among these, the most completely evaluated marker for screening of ovarian malignant growth is CA-125. Traditional diagnostic techniques like ELISA, mass spectrometry, PCR, immunoassay, and radioimmunoassay [67] have been utilised for biomarker assurance. Be that as it may, these methods include complicated pre-treatment of sample, costly, refined instrumentation, need of employing proficient workforce, long information analysis time, wary washing, and intricate separation stages. Hereafter, it is basic to manufacture savvy, delicate sensors with fast response time [66, 67]. Jin et al. [68] exhibited the development of photoluminescence biosensor employing the aptamer of CA125 antigen and 5-fluorouracil loaded Ag_2S quantum dots. The outside surface of as-arranged QDs was coated with polyethyleneimine, trailed by blending the aptamer/5-Fu complex to form Ag_2S QDs/aptamer/5-Fu hybrid. During the blend of Ag_2S QDs with aptamer/5-Fu complex, near-infrared (NIR) photoluminescence of Quantum Dots (peak at 850 nm) was sharply reduced, assigned to photo-induced electron move from QDs to 5-Fu. There is a strong binding affinity of CA125 with its aptamer which made the recovery of NIR PL recognizable. Henceforth, the authors developed this hybrid (Ag_2S QDs/aptamer/5-Fu complex) as a new NIR PL turn of a sensitive label-free probe for the accurate detection of CA125 marker protein. The aptamer modified PL based biosensor uncovered a low LOD of 0.07 $ng \cdot mL^{-1}$ over a wide linear concentration range from 0.1 to 106 $ng \cdot mL^{-1}$ with exceptionally high selectivity, sensitivity and a fair correlation coefficient (R^2) of 0.9961. The applicability of the developed NIR PL probe for CA125 determination was checked out by inspecting its performance in genuine human serum, urine, and gastric juice. Hamd-Ghadareh et al. [69] developed a highly sensitive fluorescence immunosensor based on fluorescence resonance energy transfer mechanism (FRET) for CA125. For enhancing the amplification signal output carbon dots were coated with aptamer which then used selection probe and PAMAM-dendrimers/gold nanoparticle mixture was employed for covalent immobilization of CA125 Antibodies. The measurement of FRET signals upon formation of sandwich type complex between anti-CA125, CA125 and CDs-Aptamer is a direct indication of the increase in the concentration of CA125 whereas weak signals obtained due to diminished level of the biomarker. Under most suitable conditions, the immunosensor presented a really low limit of detection (0.5 $fg \cdot mL^{-1}$) with a linear range of 1.0 $fg \cdot mL^{-1}$–1.0 $ng \cdot mL^{-1}$ of CA125 with enhanced sensitivity and specificity. The proposed test is appropriate for the detection of OVCAR-3 cancer cells in the zone of 2500–20,000 cells.

Li et al. [70] described a fluorometricturn on aptasensor method for the detection of MUC1 ovarian cancer biomarker. This assay was purelylaid on the interplay among a luminescent ruthenium (II) complex and CdZnTeS quantum dots and signal increase through

the hybridization chain reaction. The obtained fluorescence signal linearly expresses a low detection limit of 0.13 $ng \cdot mL^{-1}$ and direct detection of MUC1 concentration in the range of 0.2–100 $ng \cdot mL^{-1}$. The current method was too employed for the resolution of MUC1 in spiked serum samples. Wang et al. [71] demonstrated the aptamer fluorometric method utilizing a nanomaterial platform. The Carbon Dots and AuNPs composite were adopted towards the specific detection of MUC1 by binding with anti-MUC1 aptamer. This system uses the inner filter effect deployed by the gold nanoparticles over the blue fluorescence producing property of the carbon dots. The method exhibits a linearity range of 5.3–200 $ng \cdot mL^{-1}$ and a low detection limit of 5.3 $ng \cdot mL^{-1}$ with good selectivity. Hasanzadeh et al. [27, 72] proposed a fluorescent-based method using CdTe QDs and a rolling mediated cascade extension approach to improve the envision of MUC1 portray on cellular surfaces. Quantum dots were taken as fluorescent labels for surface glycans. Surface plasmon resonance (SPR) based sensors in amalgamation with the use of a variety of nanomaterials are also increasing intense attention towards the determination of ovarian cancer biomarkers. These sensors utilize surface plasmon waves to characterize changes that occurred in the course of the interrelation of the biological receptor and the target analyte molecule over the sensor surface. This strategy is very sensitive with regard to the change in the refractive index of the media surrounding thereof. Wu et al. [73] developed a new, throwaway, tag-less ECL biosensor fabricated on the multi-functionalised g-C_3N_4 electrode for the one-step determination of carbohydrate antigen 125 (CA125) graphitic carbon nitride (g-C_3N_4) was successfully coated on the surface of screen-printed carbon electrodes for achieving the sensitive recognition of the biomarker. This disposable sensor showed a broad linear range of detection for CA125 (0.001–5 $U \cdot mL^{-1}$) and LOD of 0.4 $mU \cdot mL^{-1}$. Likewise, the immunosensor additionally displayed high specificity, great reproducibility and prolong stability. Every one of these properties offers extraordinary promise for quick, easy, selective screening of CA125 in clinical and practical applications. Tan et al. [74] proposed an ECL colour-selective immunoassay utilizing CdSe nanocrystals as ECL tags for CA125 target determination. Multi-colour selective ECL sensor unveils the limit of detection of 5×10^{-5} $U \cdot mL^{-1}$ with a linear array of 10^{-4} to 1 $U \cdot mL^{-1}$. Babamiri et al. [75] suggested a strategy for the simultaneous detection of cancer antigen 125 and cancer antigen 15-3. The multi-fold sensitive immune-test was accomplished by making the aid of PAMAM-sulfanilic acid-$Ru(bpy)_3^{2+}$ and polyamidoamine dendrimer-quantum dots (PAMAM-QDs), both as the signal probes and Fe_3O_4–SiO_2 dendrimer as an immunosensing interface as well as a magnetic bead. This sensor showed low LOD for CA 125 and CA 15-3 markers as 0.1 $\mu U \cdot mL^{-1}$ and 10 $\mu U \cdot mL^{-1}$ over the linear range of 1 $\mu U \cdot mL^{-1}$ to 1 $U \cdot mL^{-1}$ and 0.1 $mU \cdot mL^{-1}$ to 100 $U \cdot mL^{-1}$. The applicability of the sensor for concurrent detection of the CA125 and CA15-3 markers in human serum tests was assessed and got outcomes were seen as adequate concurrence with those acquired with an ELISA taking as reference method. Qu [76] fabricated an ultrasensitive electrochemical bio immune sensor based on an AuNPs/N-doped GNs/FTO nanocomposite. This design was used for the recognition of ovarian tumor by detecting the level of human epididymis protein-4 (HE4). HE-4 is overexpressed in malignant ovary cancer as well as in the normal cells and tissues of the reproductive system. One limitation of the usage of this biomarker is the lower concentration in blood serum which restrict the early cancer detection. In the proposed study, the nanocomposite modified electrode was seen as exceptionally delicate and specific towards the bio-sensing of HE4, with the limit of detection determined at 1.21 $\mu g \cdot mL^{-1}$ along with a linear range of 10–65 $\mu g \cdot mL^{-1}$.

Gao et al. [77] proposed a current, badge-free, and characteristic electrically active redox electrochemical immunosensor in view of gold nanoparticles (AuNPs) and Nile blue A (NB) hybridized electrochemically diminished GO nanocomposite for the identification of carcinoembryonic antigen (CEA). The sensor was functionalized by immobilizing anti-CEA on the nanocomposite fabricated electrode surface. The elevated concentration of CEA was directly found proportional to the declined NB current response where this phenomenon was totally based on the formation of antigen-antibody immunocomplex. Under identical conditions, the linear detection range of the immunosensor was evaluated to be from 0.001 to 40 $ng \cdot mL^{-1}$ and the detection limit was found to be 0.00045 $ng \cdot mL^{-1}$. The recently developed strategy was further employed to determine CEA levels in clinical samples with palatable outcomes and may give promising likely potential clinical applications with the properties of the effortless procedure, high affectability, stability, and selectivity. Filippidou et al. [78] developed a novel reduced graphene oxide-based biosensor reduced at a lower temperature i.e., 180° Crestoring its sp^2 lattice with silver paint congregated on a substrate of silicon oxide. After heat treatment rGO bears multiple functional groups over its surface permitting the proper attachment of the bioprobe. The proposed biosensor was subjected to the detection of 157 bp DNA fragments labelled with biotin. This DNA has a BRCA1 gene containing an exon 20, mainly related to the expression of ovarian cancer. The Concentrations of DNA was distinguished as 0.2 Nm demonstrating that the sensor is sufficiently sensitive enough to identify the amplified DNA obtained after PCR. This plan has been conceived with the goal that the dual methods are executed successively. Wang et al. [79] reported the novel MOF-on-MOF strategy to detect CA125 and live cancer cells. By incorporating the distinct properties of

each MOF fragment to produce a synergistic hetero structure unit, it was displaying amazing biocompatibility, good endocytosis and solid fluorescence signal of Tb-MOF-on-Fe-MOF. This TbFe-bimetallic (MOF) composite-based aptamer gave a low limit of detection of 58 $\mu U \cdot mL^{-1}$ over a wider range of 100 $\mu U \cdot mL^{-1}$ to 200 $U \cdot mL^{-1}$ with outstanding stability, selectivity, superb repeatability, and fine relevancy in serum samples. Torati et al. [80] presented gold nanomaterial-based label-free, one step electrochemical immunosensor for CA125 identification. The electrode based on hierarchical gold nanostructures was fabricated by cysteamine hydrochloride producing amine functional groups, succeeded by further stimulation with EDC-NHS cross-linkers. The characterization of the properties of the GNs modified electrode was accomplished by CV, EIS and DPV, respectively by using $Fe(CN)_6^{3-/4-}$ as an electrochemical redox indicator. This proposed immunosensor showed linear narrow range varies from 10 to 100 $U \cdot mL^{-1}$ with lowest limit of detection of 5.5 $U \cdot mL^{-1}$, therefore, making it extremely good candidate for real-time applications. Zheng et al. [81] designed the label-free multiamplification strategy integrated voltammetric immunosensor based on Prussian Blue-Platinum-NP-polyaniline composite electrode for the sensing of Cancer antigen-125 that disclose fair linearity concentration range from 0.01–5000 $U \cdot mL^{-1}$ with a detection limit of 4.4 $mU \cdot mL^{-1}$.

Gasparotto et al. [82] fabricated an immunosensor based on ZnO nanorods/Au nanoparticles nanocomposite for the determination of CA125/MUC126. A biosensing platform was developed by using gold nanoparticles adsorbed on the zinc oxide surface for the immobilization of anti-CA125 antibody upon modification by cystamine thiol and glutaraldehyde cross-linker. The proposed electrochemical sensor worked on the principle of antigen-antibody interactions displayed a specific response with a LOD of detection of 2.5 $ng \cdot mL^{-1}$ and a wide linear range 2.5 $ng \cdot \mu L^{-1}$ to 1 $\mu g \cdot \mu L^{-1}$. Gazze et al. [83] demonstrated the most sensitive graphene-based biosensor for the early diagnosis of ovarian cancer marker CA 125 found in serum samples. The sensor modification was achieved via. polyaniline layer on the surface of graphene screen-printed electrode. The fabricated surface was undergone characterization by CV and EIS measurements. The lower limit of detection obtained was 0.923 ng/μL along with a linear range of 0.92 $pg \cdot mL^{-1}$–15.20 $ng \cdot mL^{-1}$ depicting the excellent results produced till date. Jafari et al. [84] proposed a newer immunoassay strategy based on the interplay of Ag NPs-GQDs conductive nano-ink for the detection of CA 125 in plasma samples of patients. The anti-CEA antibodies were immobilized on the constructed interface of Ag NPs-GQDs which results in the increment of surface area thereby attaching a higher concentration of the CA 125 antibody. The whole arrangement was measured by electrochemical tools like DPV and SWV. Under standard conditions, the sensor showed a detection limit of 0.01 $U \cdot mL^{-1}$ and a linear detection range of 0.01–400 $U \cdot mL^{-1}$. Samadi Pakchin et al. [85] developed an electrochemical immunoassay for the detection of carcinoma antigen 125. Lactate oxidase enzyme was utilised as a labelled probe and CS-AuNP/MWCNT/GO was employed as the electrode substrate. The planned immunosensor displayed two direct ranges from 0.01 to 0.5 $U \cdot mL^{-1}$ and 0.5 to 100 $U \cdot mL^{-1}$ and the limit of recognition was assessed to be 0.002 $U \cdot mL^{-1}$. The designed immunosensor showed magnificent repeatability and steadiness with exceptional selectivity regarding the identification of CA125 when contrasted with the ELISA technique. Liang et al. [86] demonstrated a pH-responsive amperometric immunoassay for analysis of CA125 antigens. Zeoliticimidazolate system (ZIF-8) was used to shape empty MB-polydopamine (MB-PDA), over which antibodies were covalently immobilized. Under identical situations, the current immunosensor displayed better reproducibility with greater sensitivity and specificity and showed a very low detection limit of 0.336 $\mu U \cdot mL^{-1}$ (S/N = 3) and widespread response from 0.0001 to 100 $U \cdot mL^{-1}$ with regard to CA125. Fan et al. [87] devised a powerful tool for the detection of CA 125 based on the reduced graphene oxide/thionine/gold nanoparticles nanohybrid assisted electrochemical sensor. The system was devoted to the fact that after binding of immunocomplexes the current value of thionine was sharply decreased since it was a sign of direct measurement of the concentration of CA125 antigens. The results indicated the detection limit of 0.01 $U \cdot mL^{-1}$ with a wide linear range from 0.1 $U \cdot mL^{-1}$ to 200 $U \cdot mL^{-1}$. Wang et al. [88] proposed an ECL biosensor based on $NiFe_2O_4$ nanotubes as an enzymatically favoured biosensing framework been devised for the rapid and sensitive detection of human epididymis protein 4 (HE4). This novel ECL based ratio metric sensing platform could be able to detect the HE4 in the range of 10 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$ with a LOD of 3.3 $fg \cdot mL^{-1}$.

4.4. Biosensors for Detecting Pancreatic Cancer Biomarkers

Pancreatic cancer is an exceptionally dangerous malignant cancer, having a mean survival rate of 5-years of less than 9% in the state of the US [55, 89]. In accordance with the past records, ~227,000 deaths every year worldwide is due to the high asymptomatic beginning, higher reoccurrence rate, and <5% respectable limited cancers in the case of PC [90]. Regrettably, early indicative and successful methodologies are missing for PC and there is an unrest demand to investigate symptomatic instruments with high sensitivity, specificity and repeatability [91]. Consequently, there is a dire need to research the fundamental molecular methods of PC and recognize novel analytic biomarkers for the illness. Distinguishing an insignificantly invasive/non-invasive and

touchy diagnostic method has recently become a popular topic of research and there has been some progression in the study of serum-based biomarkers [92–94]. Different protein biomarkers, like CA 19-9, CEACAM1, MUC4, MUC1, macrophage inhibitory cytokine, MMP-9, Osteoprotegerin (OPG), ICAM-1 [95], osteopontin (OPN), thrombospondin-2 [96], carboxypeptidase A, IGFBP2/3 [97], AGR2 [98] and HE4 [99] have been screened for pancreatic ductal adenocarcinoma. Amidst these biomarkers, CA 19-9 is the main biomarker that has been approved by the US Food and Drug Administration for better prediction and diagnosis after the course of the infection. Hao [100] suggested an electrochemical method based on the graphene polydopamine nanocomposite platform for the analysis and sensitive detection of Ki-67 protein, a pancreatic cancer biomarker. This biosensor follows a two-amplification strategy shown by the multienzyme-antibody functionalized AuNP-PDA@graphene and the AuNP-PDA composite. The amperometric immunosensor exhibits a linear response region from 4–800 $pg \cdot mL^{-1}$ with the lowest detection limit of 1.7 $pg \cdot mL^{-1}$. Soares et al. [101] fabricated a generic immunosensor for the detection of pancreatic cancer biomarker CA 19-9 in the buffer solutions. The sensor was based on the electro-spun nanofibers of polyamide 6 and poly(allylamine hydrochloride). A 3D matrix was formed by coating the nanofibers nanostructured network by both MWCNTs and AuNPs. This structure was good for the attachment of anti-CA 19-9 antibodies. Utilizing impedance spectroscopy data, it was confirmed that the proposed electrospun immunosensor was capable to analyze and detect CA 19-9 with a detection limit of 1.84 $U \cdot mL^{-1}$ and 1.57 $U \cdot mL^{-1}$ for the nanostructured platform bearing MWCNTs and AuNPs, respectively. Ibáñez-Redin et al. [102] demonstrated a nanostructured capacitative cost-effective, one-use biosensor for early detection of clinically applicable glycoprotein biomarker carbohydrate antigen 19-9 merged with an advanced fabrication technology involving the usage of cheaper interdigitated Ag screen-printed electrodes for the sensitive and specific detection. The modification of electrodes was done via graphene oxide and carbon nano-onions due to which analytical performance of the biosensor was manifold increased. This device displayed high repeatability low detection limit of 0.12 $U \cdot mL^{-1}$ with a linear dynamic region from 0.3–100 $U \cdot mL^{-1}$ normally relevant within the range of other diseases. Dackson Gudagunti et al. [103] developed a marker free biosensor for the detection of the same biomarker discussed above i.e., CA 19-9. This method was based upon negative dielectrophoretic spectroscopy (DEP) utlilizing an interdigitated gold electrode. DEP is a type of transduction process of a particular biosensor. This mechanism is employed for the generation of negative force on the modified polystyrene microspheres which in turn produces a substantial change with regard to the electric field created by the electrode and the amount of the biomarker. The speed of repulsion for PM attached to a Mab, then to CA 19-9 analyte was estimated for many concentration levels as 0 $U \cdot mL^{-1}$ and 37 $U \cdot mL^{-1}$, at the frequency range from 0.5 to 2 MHz.

Zeng et al. [104] developed a multiplexed electrochemical microRNA biosensor platform built by using a DNA tetrahedral nanostructure probe for the spontaneous detection of four serum-based pancreatic cancer-associated miRNA biomarkers such as miRNA21, miRNA155, miRNA196a and miRNA210 respectively at one time. The nano capture probe was gathered in the form of a sandwich assay onto the one-use screen-printed gold-coated electrode. Cyclic voltammetry was chosen for recording the data of the electrochemical method used. The wide linear region of 10 fM∼1 nM was calculated and the low limit of sensitive detection was noted as 10 fM which was found quite sensitive and efficient. Joshi and Waghmode [105] developed another delicate sensor dependent on graphene quantum dots on-chip which was used to identify p16 gene transformations in pancreatic malignancy. Electrochemical techniques, such as DPV and EIS, were employed for constructing a unique DNA hybridization sensor for pancreatic cancer. The proposed sensor is explicit detection ability of 0.10 pM. This investigation builds up the use of GQDs as a fabrication matrix and a novel system for identifying genetic alterations through the chip, which can be additionally evolved as a coordinated transportable detection strategy. Yu et al. [106] developed a powerful, electrochemical sensing system based on 3-D surface molecular imprinting technique for the real-time detection of CEA from pancreatic cancer cyst fluid. In addition to MIP, potentiometric sensing and ELISA assay were also used to evaluate pancreatic cyst fluid samples in a certified clinical path lab. A limit of detection of 0.5 $ng \cdot mL^{-1}$ was shown by the developed MI sensor with good sensitivity. Soares et al. [107] the irreversible adsorption method with the acceptability of the Langmuir-Freundlich model was presented for the diagnosis of pancreatic antigen p53 to differentiate among MCF7 cells. Such irreversible adsorption method was enough sensitive showing a limit of detection of 1.4 $pg \cdot mL^{-1}$.

Xu et al. [108] developed a composite film based on Chitosan-MWCNTs-Thionine for the easy detection of CEA tumor biomarker. This methodology used a microelectrode array system upon which the detection phenomenon was entirely based. The response of current was found to be linear with respect to the concentration of CEA antigens as more and more antigen-antibody events would lead to the reduction in the value of current. The produced output gave the value of detection of CEA as 0.5 $pg \cdot mL^{-1}$ and a concentration of CEA in the linear range from 1 $pg \cdot mL^{-1}$ to 100 $ng \cdot mL^{-1}$. Prasad et al. [109] demonstrated another paper-based microfluidic electrochemical immunosensor utilizing gold nanoparticles and graphene

oxide for the ultra-selective detection of pseudopodium-enriched atypical kinase 1 (PEAK1) cancer marker. A simple approach was adopted for the immobilization of potent GO layers on the paper-based electrodes over which no further modification step was needed to functionalize the surface with anti-PEAK1 antibodies. The proposed immunosensor display a LOD of 10 $pg \cdot mL^{-1}$ with a wide response range in between 10 $pg \cdot mL^{-1}$ to 10^6 $pg \cdot mL^{-1}$. The immunosensor was highly suitable and robust for the low cost, point of care detection under less resource availability. Moccia et al. [110] demonstrated the development of peptide nucleic acid biosensor for the detection of miRNA-492, a biomarker identified for pancreatic ductal adenocarcinoma. The electrochemical biosensor used PNA as a recognition material for detecting biomarker in undiluted serum samples. The paper biosensor showed a linear range of 100 nM and an LOD of 6 nM. Baryeh et al. [111] developed an assay based on gold nanoparticles and a portable strip reader for fast and specific quantification of Carbohydrate Antigen 19-9 in pancreatic patient's plasma sample. The immunochromatographic test was based on sandwich like immune-reactions. Here, the sandwich reaction takes place between a capture anti-CA 19-9, CA 19-9 antigen and GNP tagged secondary antibody and CA 19-9 concentration was detected with respect to the intensity of the red colour generated by the assembly of GNP on the testing region of lateral flow strip biosensor. The presence of red line due to gold nanoparticles assembly was detected using strip reader. This assay gave an LOD of 5 $U \cdot mL^{-1}$ with a wide range from 5 $U \cdot mL^{-1}$ to 100 $U \cdot mL^{-1}$. The proposed system presented good results as comparable to ELISA technique and could be a better platform for variety of clinical and medical applications. Ibáñez-Redin et al. [112] proposed a disposable capacitive immunosensor for the rapid and easy identification of CA 19-9 biomarker. Screen printed interdigitated electrodes altered with carbon nano-onions and GO. Further, these electrodes were employed for the immobilization of anti-CA19-9 antibodies. The results obtained with CNO was better when compared with GO. The sensor showed the limit of detection of 0.12 $U \cdot mL^{-1}$ and a linear range from 0.3–100 $U \cdot mL^{-1}$. This was the first endeavour to inspect the use of CNOs in such a type of cost-effective biosensors. Yang et al. [113] developed a pancreatic cancer antigen ULBP2 detection based economical immunosensor. UL16 binding protein 2 is a recently identified marker protein showing the reliability of maximum sensitivity and specificity with regard to pancreatic cancer detection. The developed biosensor reported a LOD of 1 $pg \cdot mL^{-1}$ with higher sensitivity as 332.2 pg/mL, excellent reproducibility 5.03%, better linearity ($R^2 = 0.98$), and long stable time as 28 days. Pang et al. [114] proposed a dual SERS biosensor for microRNAs determination in blood samples and exosome of pancreatic cancer patients. The detection platform was mainly built on the conjugates of Fe_3O_4@Ag-DNAAu@Ag@DTNB. The limit of detection obtained via the developed sensor was 1.8 aM with a wide linear range of 3 aM to 100 pM.

4.5. Biosensors for Detecting Breast Cancer Biomarkers

Human epidermal growth factor receptor-2 (HER-2), Estrogen receptor (ER), and progesterone receptor (PR) are the vital protein biomarkers for the medical screening and diagnosis of breast cancer. Sensitive and accurate determination of ER, PR, and HER-2 are of utmost importance for the prognosis and diagnosis of breast cancer. The conventional diagnosis technologies are mammography, X-rays, ultrasounds, Positron emission tomography (PET), CT scan, biopsy and magnetic resonance imaging (MRI) etc. [115]. In common, these traditional diagnostic techniques show very limited triumph from the beginning phase as they are very expensive, monotonous, time-consuming, and might often result in erroneous positive or negative consequences [116]. Thus, it is the need of time to develop such potential devices which are very accurate, less invasive, robust, reproducible, reliable, easy fabrication, specific and sensitive with low-risk factors [116, 117]. Loyez et al. [118] presented a sandwich optical fibre assay SPR configured biosensor for the detection of HER-2 breast cancer biomarker. This sensor was dependent on antibodies and aptamer explicitly integrated into a sandwich format. The HER2 protein was detected in a marker-free environment at 0.6 $\mu g \cdot mL^{-1}$ (5.16 nM) while achieving enhanced amplification response using HER-2 antibodies gave an almost 100-fold signal increase with a limit of 9.3 $ng \cdot mL^{-1}$ (77.4 pM). Arya et al. [121] developed another biomarker detection approach based on a capacitative aptasensor for the detection of breast cancer biomarker, human epidermal growth factor receptor 2 (HER-2). The electrochemical framework was built by the integration of gold electrodes. EIS was used to explore the sensor performance through checking of the alterations in capacitance. Other characterization methods portrayed were CV, nuclear power microscopy EIS and contact point contemplates. The aptasensor displayed recognition of HER-2 from 1 pM to 100 nM both in the buffer and concentrated serum with a cut-off less than 1 pM. The method was quite acceptable and ready to construct another aptamer-based sensor for biomarkers detection in concentrated serum samples. Formation of nanocomposites by employing graphene nanomaterial and its related oxygen containing by-products are also emerging as potential strategies to address the utility of such graphene based electrochemical and fluorescent biosensor surfaces for the sensing of an extensive range of biological molecules specifically detecting certain volatile compounds, chemicals, dangerous gases, heavy metal toxicity, pollutants, and cancer biomarkers exhibiting enhanced sensitivity, selectivity, lower detection limit by extending concentrations of analytes upto nanomolar and picomolar

range [119]. Apart from several attributes and properties of graphene, graphene nanomaterials and its robust advantages are frequently seen in manufacturing and designing certain pliable and resilient technology based chemical and gas sensor devices [120].

Sun et al. [122] experimentally demonstrated a biosensor utilizing silica microfiber interferometry process for the sensitive detection of low concentration of breast cancer biomarker in biological fluids. The fabrication and functionalization of microfiber were done by antibodies via covalent bond for the specific capture of the target analyte i.e., HER-2 marker. The sensor could be able to catch the differences in the refractive index due to the formation of antigen-antibody complexes on the surface of the microfiber and converted it into a huge wavelength shift. The designed label-free optical fiber biosensor displays limit of sensitivity at 0.1 nm/ng $\cdot$ mL^{-1} both in the serum samples and buffers solutions. The measurement was dependent on a refractive index which was the basic sensing method adopted here. The upsides of basic detection strategy and simplicity of scaling down may make the biosensor on a promising stage for tracking or diagnosing the variety of clinical applications. Arora et al. [123] developed a newer approach that uses the physical adsorption method in which thin-film SnO_2 was employed for the formation of the immobilization matrix. The authors use an Ab/SnO_2/Pt/Ti/glass Immunoelectrode composite for the sensitive determination of breast cancer-specific CA 15-3 antigen protein. The whole characterization was done with the aid of CV, EIS and SEM techniques. Ab/SnO_2/Pt/Ti/glass Immunoelectrode showed a sharp increase in detecting a particular Antigen CA15-3 in a wide range from 50 ng $\cdot$ dL^{-1} to 700 ng $\cdot$ dL^{-1} (8 U $\cdot$ mL^{-1} to 120 U $\cdot$ mL^{-1}) with LOD of 38.63 ng $\cdot$ dL^{-1}. Azimzadeh et al. [124] demonstrated an oligo-hybridization dependent nanobiosensor for the detection of circulating plasma miRNA-155 detection. This system uses a dual detection mechanism using electrochemical detection techniques along with the incorporation of carbon and gold nanomaterials. A few layers of GO were coated on the glassy carbon electrode. The electrode was further functionalized with thiol-gold nanorods on which the reduction in signals of the target miRNA detection was accomplished by employing DPV method. The relationship between the electrochemical measurement and the target miRNA level was denoted as 2.0 fM to 138.0 pM with a detection limit of 0.6 fM. Moreover, this platform depicts high specificity and had the option to segregate between complementary and non-complementary target miRNA species. Wang et al. [125] developed a lateral flow through an optofluidic silicon metasurface bio-sensing mechanism for the detection of ErbB2 biomarker for breast cancer diagnosis and screening. This sensor amalgamated nanofluidics and nanophotonics to achieve good sensitivity and higher efficiency. The biosensor identifies the epidermal development factor receptor 2 biomarkers for malignant growth screening, by focusing on the resonance shifts in light of the formation of the ligand-receptor complex at the optofluidic silicon metasurface. The detection limit shown for ErbB2 by the gadget is 0.7 ng $\cdot$ mL^{-1} with the range of detection 0.01–10 nM.

Paimard et al. [126] developed an electrochemical detection method based on modified GCE for the detection of MUC1, a protein expressed in breast cancer biomarker. The fabrication of glassy carbon electrodes was accompanied by multi-walled carbon nanotubes, core–shell nanofibers, and gold nanoparticles respectively. This modified surface was used for covalent attachment of MUC1-aptamer conjugate. EIS and CV are done to characterize the fabricated electrode surface. The analytical detection range of MUC1 was from 5 to 115 nM with a limit of detection as 2.7 nm. Senel et al. [127] proposed a novel DNA biosensor based electrochemical method for the determination of mutations that occurred accidentally in the BRAC1 gene which could be one of the reasons for breast cancer. The chemical absorption phenomena used by the authors based on self-assembled ferrocene-cored poly(amidoamine) dendrimers pairing with ssDNA of interest on the gold electrode surface. The developed system could be able to detect non-corresponding fragments of DNA and a single mismatch base with high sensitivity. The sensor highlighted the superior sensitivity of 0.13 μA/(ng/ml) with a linear response of 1.3–20 nM and a LOD of 0.38 nM.

Recently, Cardoso et al. [128] showed the rapid detection of miRNA-155 with the aid of electrochemical biosensing mode. A gold-coated screen-printed electrode was thiolated for the attachment of anti-miRNA-155 following further capturing the regions showing the non-specific sites using mercaptosuccinic acid. The ultimate design delivered a tactful determination of miRNA-155 from 10 aM to 1.0 nM with a low detection limit of 5.7 aM in the case of actual human samples. Cui et al. [129] used zwitterionic peptide SAM matrix for the detection of breast cancer marker, BRCA1 associated fragments. The electrochemical biosensor displayed antifouling properties of the peptide SAM which could be confirmed by EIS. The proposed sensing system attained a linear range of detection from 1.0 fM to 10.0 pM with a lower detection limit of 0.3 fM. Freitas et al. [130] reported the development of throwaway electrochemical immunosensor based on CdSe@ZnS Quantum dots for the salient *in-situ* determination of breast cancer biomarker, HER-2-ECD (the extracellular domain of human epidermal growth factor 2). This immunosensor has a transducer in the form of screen-printed carbon electrodes for the development of immunocomplexes. An electroactive tag was chosen as quantum dots which were utilised for the attachment of antibodies and other relevant proteins of interest. The

developed electrochemical immunosensor acted in a linear fashion against HER-2-ECD with a broad range from 10–150 $ng \cdot mL^{-1}$ and a limit of detection of 2.1 $ng \cdot mL^{-1}$ of the proposed immunosensor was found promising for the analysis of different human serum samples and biomarkers of breast cancer. Guo et al. [131] proposed a photoelectrochemical (PEC) biosensor based on a two-fold signal multiplication assay for the identification of HER-2 biomarker. Ti mesh-based electrode formed of nanowire array of tungsten sulfide (WS2 NW/TM) was hand-picked to produce photoelectrical sign by the stimulation of visible light. An aptamer of HER-2 was enveloped around the whole nanowire for binding very specifically to HER-2 biomolecules. The authors altered Au NPs by attaching glucose oxidase enzyme molecules with HER-2 protein was simultaneously used for signal amplification in double mode. The photoelectric current signal was improved with HER-2 identification in the range of 0.5–10 $ng \cdot mL^{-1}$ with a limit of detection of 0.36 $ng \cdot mL^{-1}$. A similar PEC sensor was applicable for the detection of the biomarker in breast serum samples in accordance with commercial ELISA results. Malecka et al. [132] demonstrated an electrochemical sandwich assay based on magnetic beads for the fast and selective detection of the femtomolar concentration of HER-2/*neu* biomarker protein generally showed hyper-secretion in variety of breasts cancers. An assay was developed either by taking primary antibody or by using magnetic beads modified by aptamers between which HER-2/neu antigens were captured followed by a sandwich formation by employing secondary antibody or cellulase linked aptamers. Further, a film of nitrocellulose membrane was used for the fabrication of graphite electrode on which, a sandwich layer was assembled for recording the electrical performance of the electrodes being modified. The signal produced by the sandwich assay was linear between 10^{15} to 10^{10} M with a low detection limit of 1 fM in detected human serum. Majd et al. [133] proposed a direct hybridization method for the detection of miRNA-155 in real human samples. This field-effect transistor biosensor was developed by utilising a newer 2D material molybdenum disulfide (MoS_2), as a coating material for the fabrication of the FET device. The sensitive limit of detection displayed by the sensor was 0.03 fM and concentration was in the range of 0.1 fM to 10 nM. This biosensor showed better performance and ability to detect fully and mis-match bases of miRNAs in human cell line samples. Ribeiro et al. [134] used poly(toludine blue) as artificial imprinted polymer receptor membrane due to its conducting nature for the construction of electrochemical biosensor for the ultrasensitive detection of CA 15-3, a breast cancer biomarker protein. The MIP based biosensing system was very simple, economical, and robust for the detection of CA 15-3 antigens in the concentration range of 0.10 U mL^{-1} to 100 U mL^{-1} with an LOD of 0.10 $U \cdot mL^{-1}$. Salahandish et al. [135] proposed an electrochemical geno-biosensor for miRNA-21 detection based on the nanocomposites of nitrogen-doped functionalized graphene (NFG), polyaniline (PANI) and silver nanoparticles (AgNPs) nanomaterials. Incorporating the attributes of this sensitive, reproducible and nanocomposite have driven nano-genobiosensor, the miRNA-21 detection in a dynamic range of 10 fM–10 μM and a lower detection limit of 0.2 fM was successfully achieved. Shahrokhian and Salimian [136] developed an electrochemical DNA biosensor based on the composite of reduced graphene oxide nanolayer sheets and Pyrrole-3-carboxylic acid as conducting polymer for the label-free, sensitive detection of the BRCA1 biomarker gene. The DNA sensor showed a limit of detection of 3 fM with a wide linear range of 10 fM–0.1 μM with outstanding reproducibility and reliability for detection of short fragments of targeted DNA in human blood plasma.

5. COMPARATIVE RESULTS

As illustrated in Table I, the individual sensors are being reviewed for the detection of a large number of cancer-based biomarkers, working on the necessary parameters being kept in consideration such as their sensing method, mode of detection, the limit of detection along with better sensitivity and ease of operation. The label-less detection of CD 59 potential biomarker for lung cancer makes use of the amperometric biosensor based on graphene oxide nanoparticles adhered antibodies into a graphite electrode to present a low detection limit ideally in the specific femtomolar range. The overall framework proved to be highly sensitive with low cost integrating attractive features of GO nanoparticles so as to enhance the conductivity phenomena of the sensor in a better way. One of the best miniaturised compact devices for use on the spot diagnosis. Similarly, prostate cancer biomarker PSA was successfully detected by amperometric biosensor using the framework of GO-PDDA, which consists a very low detection limit in femtomolar concentration could be better than any other biomarkers used. This method used BSA to elevate the sensitivity of the assay for the very first time thereby increased signal amplification. In the case of ovarian cancer biomarker CA-125, the best results were obtained with FRET fluorescence immunosensor based on a fluorescence detection mode. The carbon dots coated with aptamer are designed especially for producing amplified signals to indicate direct increment in the biomarker concentration in the samples. The lowest detection limit was noticed while detecting pancreatic marker microRNA-10b in the samples of blood, where the detection lain in attomolar concentration with a linear range of 3 aM to 100 pM. The Dual-SERS biosensor is strictly based on the Zetasizer nano method for the efficient and sensitive detection of microRNA-10b take advantage of constructed conjugates of Fe_3O_4@Ag-DNAAu@Ag@DTNB, where gold

Table I. Biosensors for detecting different types of cancer biomarkers.

Cancer type	Biomarker	Sensing method	Detection mode	LOD	Linear range	Ref.
Lung cancer	NSE	Impedimetric immunosensor	EIS and CV	6.1 $fg \cdot mL^{-1}$	0.02–7.5 $pg \cdot mL^{-1}$	[37]
	NSE	Fluorescent biosensor	NSET (Nano surface energy transfer)	0.09 $pg \cdot mL^{-1}$	0.1 $pg \cdot mL^{-1}$ to 1000 $ng \cdot mL^{-1}$	[40]
	CYRFA 21-1, CEA and NSE	Multiplexed immuno fluorescence assay	Quantum dots and micro-magnetic beads	1.0 $ng \cdot mL^{-1}$ (364 $pg \cdot mL^{-1}$ for CYRFA21-1, 38 $pg \cdot mL^{-1}$ for CEA, 370 $pg \cdot mL^{-1}$ for NSE)	–	[41]
	CD59	Amperometric immunosensor	EIS and CV	1 $fg \cdot mL^{-1}$	1 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$	[47]
	Ethyl acetate (Volatile organic compounds)	Electrochemical sensor	CV, DPV and EIS	0.376 ppb for SnO_2 0.398 ppb for Ni–SnO_2 0.377 ppb for Cu–SnO_2	1 to 20 ppb	[48]
	Adenosine	Colorimetric aptasensor	Microplate reader & Bionic electronic-eye (E-eye)	0.17 μM or 0.48 μM	5.0 μM–60.0 μM or 5.0 μM–50.0 μM (in urine samples)	[49]
	Cytokeratin 19 fragment 21-1 (CYFRA21-1)	Electrochemical immunosensor	CV, EIS and DPV	43 $pg \cdot mL^{-1}$	0.1 to 150 $ng \cdot mL^{-1}$	[50]
	Carcinoembryonic antigen (CEA)	Graphene based biosensor	EIS	0.23 $ng \cdot mL^{-1}$	1.0–25.0 $ng \cdot mL^{-1}$	[51]
	Cytokeratin 19 (CK-19)	SPR immunosensor	CV	0.05 $pg \cdot mL^{-1}$	0.001 to 100 $pg \cdot mL^{-1}$	[52]
	EGFR	Electrochemical genobiosensor	CV and N_2 adsorption–desorption analysis	120 nM	0.1 μM to 3 μM	[53]
	EGFR	Paper-based electrochemical biosensor	DPV, EIS and CV	0.167 nM	0.5 nM to 500.0 nM	[54]
Prostate cancer	PCA3	Electrochemical and impedance-based biosensor	EIS	0.128 $nmol \cdot L^{-1}$	–	[56]
	PSA	Electrochemical aptasensor	DPV and EIS	1.0 $pg \cdot mL^{-1}$	0.005–20 $ng \cdot mL^{-1}$ (DPV) and 0.005–100 $ng \cdot mL^{-1}$ (EIS)	[1]
	PSA	Si NW FETs	–	23 $fg \cdot mL^{-1}$	23 $fg \cdot mL^{-1}$– 500 $ng \cdot mL^{-1}$	[61]
	PSA	Amperometric biosensor	EIS and SWV	0.01 $fg \cdot mL^{-1}$	100 $ng \cdot mL^{-1}$ to 1 $fg \cdot mL^{-1}$	[8]
	PSA and CEA	Capillary-based biosensor	Microplate reader	0.017 $ng \cdot mL^{-1}$ (PSA)/ 0.044 $ng \cdot mL^{-1}$ (CEA)	0.02 to 2.5 $ng \cdot mL^{-1}$ (PSA)/0.063 to 16 $ng \cdot mL^{-1}$ (CEA)	[73]
	PSA	Resonator biosensor	–	0.5 $ng \cdot mL^{-1}$	–	[59]
	PSA	Electrochemical immunosensor	EIS	100 $fg \cdot mL^{-1}$	100 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$	[62]
Ovarian cancer	CA125 antigen	NIR-emitting PL Ag_2S QDs	–	0.07 $ng \cdot mL^{-1}$	0.1 to 106 $ng \cdot mL^{-1}$	[68]
	CA125 antigen	FRET immunosensor	Fluorescence	0.5 $fg \cdot mL^{-1}$	1.0 $fg \cdot mL^{-1}$ to 1.0 $ng \cdot mL^{-1}$	[69]
	MUC1	Aptasensor	Fluorescence	0.13 $ng \cdot mL^{-1}$	0.2–100 $ng \cdot mL^{-1}$	[70]
	MUC1	Aptasensor	Fluorescence	5.3 $ng \cdot mL^{-1}$	5.3 to 200 $ng \cdot mL^{-1}$	[71]
	CA125 antigen	ECL immunosensor	EIS	0.4 $mU \cdot mL^{-1}$	0.001–5 $U \cdot mL^{-1}$	[27]
	CA125 antigen	ECL immunoassay	ECL and CV	5×10^{-5} $U \cdot mL^{-1}$	10^{-4} $U \cdot mL^{-1}$ to 1 $U \cdot mL^{-1}$	[73]

Table I. Continued.

Cancer type	Biomarker	Sensing method	Detection mode	LOD	Linear range	Ref.
Ovarian cancer	CA125 and CA15-3	ECL immunoassay	EIS	0.1 $\mu U \cdot mL^{-1}$ and 10 $\mu U \cdot mL^{-1}$	1 $\mu U \cdot mL^{-1}$– 1 $U \cdot mL^{-1}$ and 0.1 $mU \cdot mL^{-1}$– 100 $U \cdot mL^{-1}$	[74]
	Human epididymis protein 4	Electrochemical immunosensor	CV and EIS	1.21 $\mu g \cdot mL^{-1}$	10 to 65 $\mu g \cdot mL^{-1}$	[75]
	CA125 antigen	Aptasensor	CV and EIS	58 $\mu U \cdot mL^{-1}$	100 $\mu U \cdot mL^{-1}$ to 200 $U \cdot mL^{-1}$	[78]
	CA125 antigen	Electrochemical immunosensor	CV, EIS and DPV	5.5 $U \cdot mL^{-1}$	10–100 $U \cdot mL^{-1}$	[79]
	CA125 antigen	Electrochemical biosensor	SWV	4.4 $mU \cdot mL^{-1}$	0.01 to 5000 $U \cdot mL^{-1}$	[80]
	CA125/MUC126	Electrochemical immunosensor	CV	2.5 $ng \cdot \mu L^{-1}$	2.5 $ng \cdot \mu L^{-1}$ to 1 $\mu g \cdot \mu L^{-1}$	[82]
	CA 125	Graphene biosensor	Potentiostat/ Galvanostat (Autolab)	0.923 $ng \cdot \mu L^{-1}$	0.92 $pg \cdot \mu L^{-1}$ to 15.20 $ng \cdot \mu L^{-1}$	[83]
	CA 125	Electrochemical immunosensor	DPV and SWV	0.01 $U \cdot mL^{-1}$	0.01–400 $U \cdot mL^{-1}$	[84]
	CA 125	Electrochemical immunosensor	CV, EIS and CHA (Chronoamperometry)	0.002 $U \cdot mL^{-1}$	0.01–0.5 $U \cdot mL^{-1}$ and 0.5–100 $U \cdot mL^{-1}$	[85]
	CA 125	Amperometric immunosensor	SWV (Square wave voltammetry), CV and EIS	0.336 $\mu U \cdot mL^{-1}$	0.0001 to 100 $U \cdot mL^{-1}$	[86]
	CA 125	Electrochemical immunosensor	DPV and CV	0.01 $U \cdot mL^{-1}$	0.1 $U \cdot mL^{-1}$ to 200 $U \cdot mL^{-1}$	[87]
	HE4	ECL biosensor	EIS	3.3 $fg \cdot mL^{-1}$	10 $fg \cdot mL^{-1}$ to 10 $ng \cdot mL^{-1}$	[88]
Pancreatic cancer	CEA	Microelectrode array (MEA)	CV and DPV	0.5 $pg \cdot mL^{-1}$	1 $pg \cdot mL^{-1}$ to 100 $ng \cdot mL^{-1}$	[108]
	PEAK1	Electrochemical biosensor	DPV	10 $pg \cdot mL^{-1}$	10 $pg \cdot mL^{-1}$ to 10^6 $pg \cdot mL^{-1}$	[109]
	miRNA-492	Paper-based electrochemical biosensor	DPV	6 nM	10 to 1000 nM	[110]
	CA 19-9	Immuno-chromatographic assay	–	5 $U \cdot mL^{-1}$	5 U mL^{-1} to 100 $U \cdot mL^{-1}$	[111]
	CA 19-9	Capacitive biosensors	EIS	0.12 $U \cdot mL^{-1}$	0.3–100 $U \cdot mL^{-1}$	[102]
	ULBP2	Immunosensor	EIS/Impedance analyzer	1 $pg \cdot mL^{-1}$	–	[113]
	MicroRNA-10b	Dual-SERS biosensor	Zetasizer nano	1.8 aM	3 aM to 100 pM	[114]
Breast cancer	HER-2	Surface plasmon resonance	Optical fibers	0.6 $\mu g \cdot mL^{-1}$ (5.16 nM)	–	[116]
	HER-2	Capacitive aptasensor	CV and EIS	1 pM	1 pM to 100 nM	[117]
	HER-2	Fiber-optic biosensor	Interferometry	0.1 $nm/(ng \cdot mL^{-1})$	2 to 25 $ng \cdot mL^{-1}$	[118]
	CA 15-3	Electrochemical immunosensor	CV, DPV and EIS	38.63 $ng \cdot dL^{-1}$	50 $ng \cdot dL^{-1}$ to 700 $ng \cdot dL^{-1}$ (8 $U \cdot mL^{-1}$ to 120 $U \cdot mL^{-1}$)	[121]
	ErbB2	Flow-through biosensor/Optofluidic silicon metasurface	Guided mode resonance	0.7 $ng \cdot mL^{-1}$	0.01–10 nM	[123]
	MUC1	Aptasensor	CV and EIS	2.7 nM	5 to 115 nM	[126]
	BRAC1 gene	Electrochemical biosensor	CV and DPV	0.38 nM	1.3–20 nM	[127]
	miRNA-155	Electrochemical biosensor	CV, EIS and SWV	5.7 aM	10 aM to 1.0 nM	[128]
	BRAC1	Electrochemical biosensor	EIS	0.3 fM	1.0 fM to 10.0 pM	[129]

Table I. Continued.

Cancer type	Biomarker	Sensing method	Detection mode	LOD	Linear range	Ref.
Breast cancer	HER-2-ECD	Electrochemical immunosensor	DPV	2.1 $ng \cdot mL^{-1}$	10–150 $ng \cdot mL^{-1}$	[130]
	HER-2	Photoelectrochemical biosensor (PEC)	EIS	0.36 $ng \cdot mL^{-1}$	0.5–10 $ng \cdot mL^{-1}$	[131]
	HER-2/neu	Electrochemical immunoassay	CV and CC	1 fM	10^{-15} to 10^{-10} M	[132]
	miRNA-155	MoS_2 FET biosensor	Direct hybridization assay	0.03 fM	0.1 fM to 10 nM	[133]
	CA 15-3	MIP biosensor	DPV	0.10 U mL^{-1}	0.10 $U \cdot mL^{-1}$ to 100 $U \cdot mL^{-1}$	[134]
	miRNA-21	Electrochemical nano genosensor	DPV	0.2 fM	10 fM–10 μM	[135]
	BRCA1 gene	Electrochemical biosensor	EIS and DPV	3 fM	10 fM–0.1 μM	[136]

nanoparticles were used for signal generation and enhancement. Excellent results were seemed to be obtained with an electrochemical biosensor for breast cancer biomarker examination with a good detection limit upto 5.7 aM. Furthermore, it is evident from the above-reviewed data that mostly electrochemical immunosensors based on the nanomaterial platform mainly carbon and gold could be highly and efficiently employing on a very large scale due to the major benefits coming out from their use, but before using these carbon entities in an experimental platform, one should be highly recommended to evaluate and go through the significant progress, advantages and limitations offered by these persistent nanomaterials while working on the on-spot real human samples.

6. CONCLUSION AND FUTURE OUTLOOK

Traditional methods of premature cancer detection, its location and progression exactly on time are usually accomplished by taking the patient's blood and serum fluids, just before the tumor spread to other sensitive parts of the body and might convert into a dangerous malignant form. But these already existing techniques have a couple of disadvantages as for instance, they are time-consuming, high cost, follow lengthy experiments, highly inconvenient, complex nature, the requirement for costly reagents, invasive etc. which often limits their effective and regular usage at the clinical level, in fact, they are still in practice due to the severity of the disease and less availability of other established alternatives methods. This current survey gives an outline of the ongoing endeavours in the field of early cancer detection by jumping into the discovery of cancer-associated potential protein biomarker detection by incorporating varied synthetic biosensing strategies for sensitive, specific, robust, accurate and fast detection. By providing myriad illustrations of current research being carried out for exploring new cancer biomarkers proved that multidisciplinary technology-based cancer diagnostics are increasingly relevant alternatives to traditional techniques. Several different biosensor approaches have been investigated and discussed in this review. It is desirable to quote here that since the emergence of nanotechnology and nanoscience came into existence, various avenues have been disclosed that could work in the manufacture and fabrication of hybrid bio nanostructures and customized purpose of-care diagnostics utilizing the outstanding attributes of nanoscale materials which include, CNTs, Graphene, rGO, QDs, gold, silver nanoparticles and so many. These sensing platforms too, however, are not alone sufficient so, be that as it may, future enhancements of these advancements are as yet required before commercialization will get plausible. Hence, by keeping the future points of view under consideration for malignancy-related biomarker discovery lie in building up a productive recognition stage with high affectability and selectivity, scaling down, adaptability, high-throughput, and the distinguishing proof of new biomarkers explicitly early finding. New advancements and upgrades are relied upon to be accomplished by the participation and efforts from wide networks of scientific communities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Compliance

Research experiments conducted in this article with animals or humans were approved by the Ethical Committee and responsible authorities of our research organization(s) following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflicts of Interest

There are no conflicts to declare.

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