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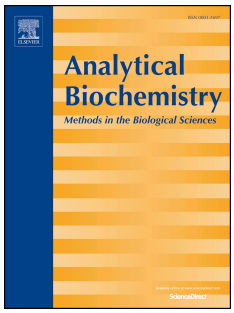

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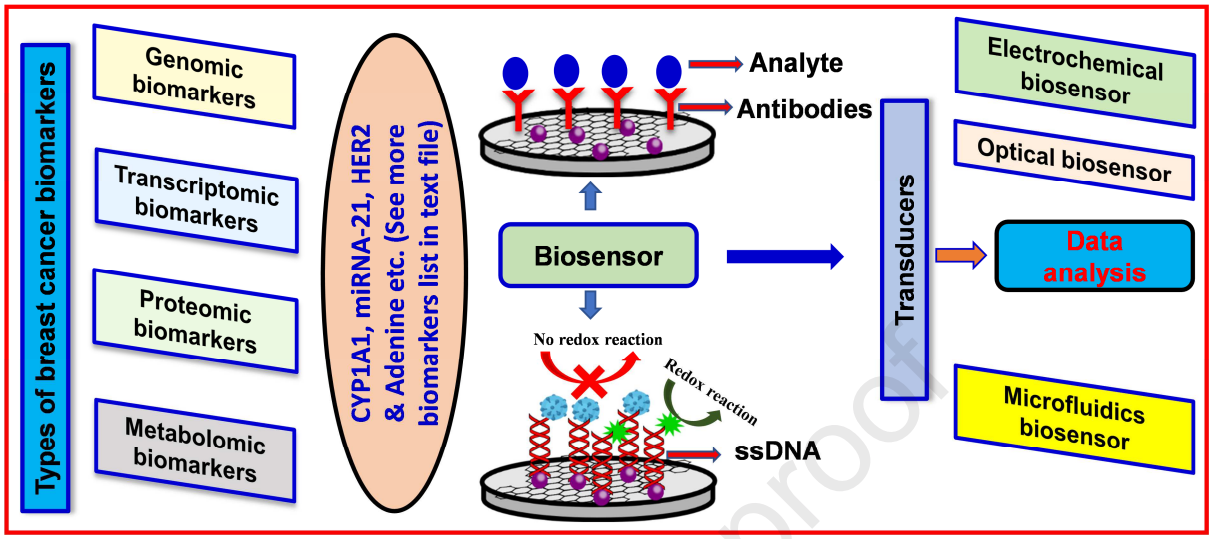

Biosensor-based diagnostic approaches for various cellular biomarkers of breast cancer: A comprehensive review

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Abstract:

Breast cancer is the most commonly occurring cancer among women which lead to thousands of deaths worldwide. The chances of survival are more if the breast cancer is diagnosed at early stage. At present, mammography, magnetic resonance imaging, ultrasound and tissue biopsies are the main diagnostic techniques available for the detection of breast cancer. However, despite of offering promising results, requirement of expensive setup, skilled supervision, expert analysis, invasive procedure (biopsy) and low capacity of multiplexing are the main limitations of these diagnostic techniques. Due to high cost, these screening tests are out of reach of people belonging to low socioeconomic groups and this poses serious health burden to the society. Recently, biosensor-based diagnostic technology for early detection of various types of cancer and other non-oncological disorders have gained considerable attention this is because of their several advantageous features over existing diagnostic technologies such as high throughput, noninvasive nature, cost effectiveness, easy interpretable results and capacity for multiplexing**.** Further, biosensors can be designed for biomarkers which are confined to particular type of cancer. In this review we have discussed about various genomic, transcriptomic, proteomic and metabolomic biomarkers associated with breast cancer, various biosensors based diagnostic approaches designed for detection of specific biomarkers associated with breast cancer are also discribed. Further, this review throws insight on various biomarkers linked with breast cancer which can be effectively exploited to develop new diagnostic technology. The assessment of these biomarkers associated with BC using biosensors in large population are cost-effective, non-invasive and high throughput. They help in risk assessment of disease at very initial stage even in backward areas and also help to lower the disease burden of society and economic cost of treatment for a common man. This review would provide new avenues for the development of biosensor based diagnostic technology for the detection of biomarkers associated with breast cancer.

Keywords:

Breast Cancer, Biosensor, Breast Cancer Biomarkers, Electrochemical, Optical, Microfluidics.

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1. Introduction:

Cancer is a disease in which cells divide uncontrollably, resulting in the formation of tumors. Further, the cells from tumors can metastasize to other organs via blood vessels, spreading the diseases to other body parts [1]. Cancer is the second leading cause of deaths worldwide, after cardiovascular diseases and also it is reported that the percentage of improvement in the chances of human survival for different cancer types with early diagnosis is more as compared to that of later stage. Globally, breast cancer (BC) is the most prevalent type of cancer in women which caused (626,679) 6.6% of cancer deaths in 2018, being the fourth leading cause of cancer deaths in the world [2]. Recently, Dhillon et al. have reported that breast cancer contributed to 8.2 % deaths among Indian women which are significantly higher as compared to world statistics [3]. The reasons behind higher mortality and morbidity in developing countries could be the poor clinical facilities, lack of awareness and poverty. Early diagnosis is the key to better prognosis of BC and it can be achieved by assessment of biomolecules which are known as biomarkers. The plethora of potential biomarkers associated with BC is being exploited to detect BC at very early stage, even before appearance of symptoms. So far, we found no report which provides discrimination between early and later stage biomarkers in case of breast cancer is found. However, genomics-based biomarkers using mutation screening methods have potential to predict the risk of occurrence of BC before the symptoms arise [4, 5]. Nevertheless, the experimental screening of biomarkers associated with BC in larger cohort is necessary to find out the difference between early and late phase breast cancer biomarkers. The assessment of biomarkers at early stage can predict the cancer occurrence probability at preclinical phase and helps to manage in delaying of disease/prognosis along with future therapy prediction [6]. According to study, the percentage of survival rate of patients enhanced significantly when the cancer is diagnosed at early stage as compared to later stage (Figure 1). It strengthens the fact that survival rates of patients significantly improved when cancer is diagnosed at early stage [Web Ref. 1].

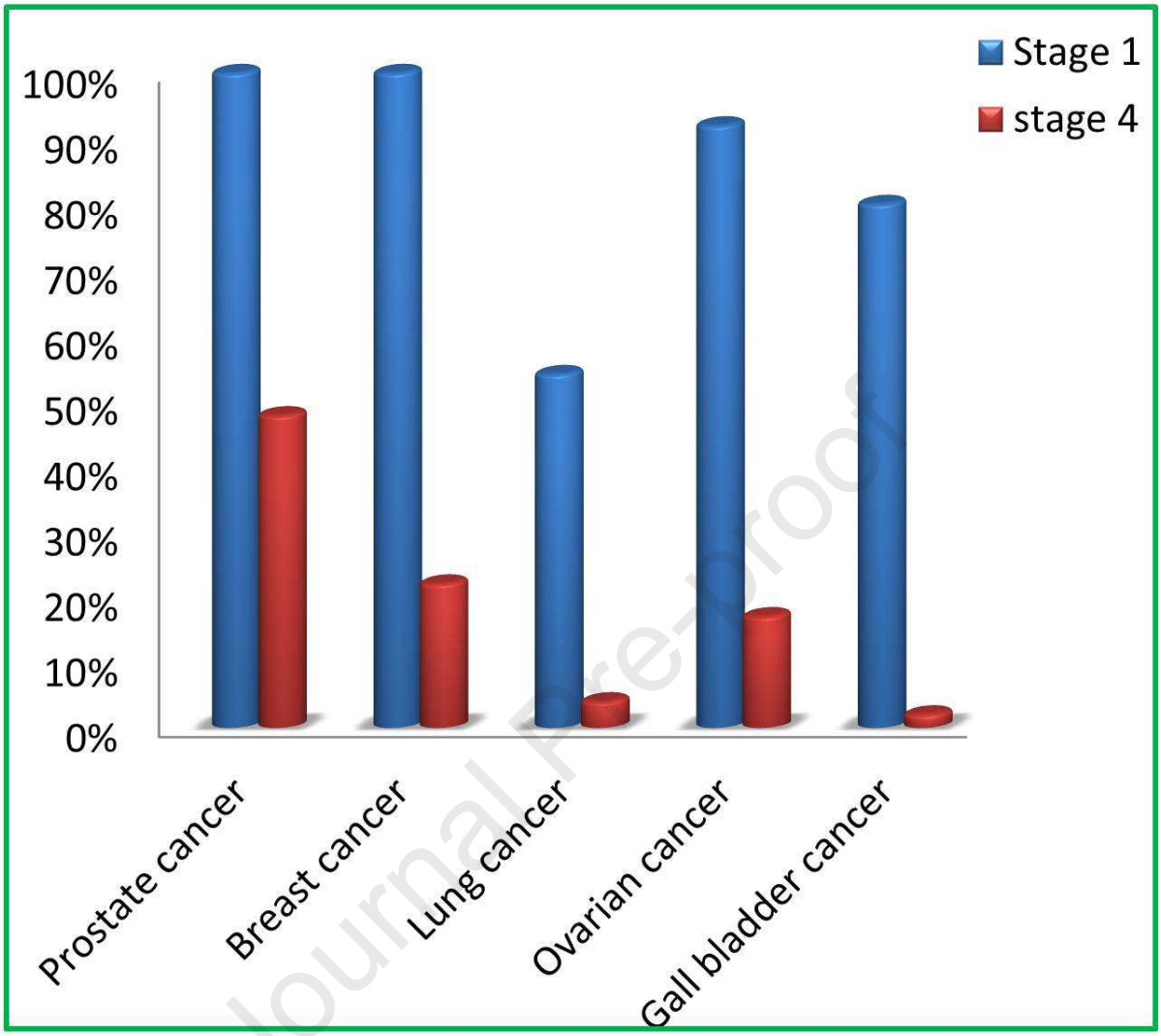

Figure 1: Five years survival rates of different types of cancers diagnosed at early and later stages.

Currently, mammography, magnetic resonance imaging (MRI), X-ray imaging, ultrasound, CT scan and tissue biopsies are the mainly approved diagnostic techniques for BC detection [7]. However, certain limitations like high cost, requirement of skilled supervision, expert data analysis and need of invasive procedure (in biopsy) hamper their usage despite of promising results [7]. Women after the age of 40 years are recommended for yearly checkup for BC. Unfortunately, due to high cost involved these diagnostic screening tests, the non-affordability of women belonging to low socio-economic groups is leading to delayed diagnosis of BC and associated high morbidities and mortality. Therefore, the diagnostic techniques that are

affordable by majority of the people, which simultaneously provide high sensitivity and specificity is the prime need of the hour. Recently, plethora of studies revealed that analysis of cancer associated biomarkers in patient's bodily fluid holds promise for early diagnosis of cancer [8-10]. Further, due to advancement of biosensor technologies one can easily detect tumor associated specific biomarkers, and design a biosensor-based platform for early detection of BC. Biosensor devices consist of a biorecognition element and a transducer, which can be used to detect the presence of biomarker for diagnosis of certain disease conditions [11]. In this context, points-of-care (POC) biosensors are fast, sensitive, portable, easy to use, cost-effective and accurate devices which can detect cancer cells even before the symptoms arise [12, 13]. Biosensors can be classified on the basis of types of biorecognition element (antibody, enzyme, aptamer) and types of transducers used (electrochemical, optical) [11, 12]. Various electrochemical, optical and microfluidics-based biosensors available so far for the detection of various biomarkers associated with different type of cancers are enlisted in Table 1, in which, biorecognition elements, amplification method, assay type, limit of detection along with appropriate citation are also included. The classification of biorecognition elements and transducers is shown in Figure 2. Several reviews have reflected biosensor-based diagnosis of BC. Most of these reviews are technique specific, e.g., the reviews by Campuzanu et al., Hasanzadeh et al. and Nemeir et al. have specifically described the electrochemical and affinity biosensors for the detection of BC [14-16]. Further, they lack information about advanced optical and microfluidics-based biosensors developed for early detection of BC using various biomarkers. Another review has described advances and applications of biosensors in various fields including glucose monitoring, cancer cell detection and food analysis, but lacks technical details on sensor application [17]. Mittal et al. discussed about various biomarkers and bio-transducer techniques for the detection of BC, but the scope of study is limited and lacks information about microfluidic and other recently developed biosensors for the detection of BC [18]. Recent literature of reviews, are more focused on electrochemical biosensing approaches for the detection of cancer [19-22]. In this review, a comprehensive description is made on various biomarkers which are classified on the basis of their molecular omics viz., nucleic acids, proteins and metabolites but also discussed various biosensors (electrochemical, optical, microfluidic) along with respective biorecognition techniques, transducers and amplification methods. Further, present review focuses on technical details regarding fabrication of biosensors.

This review also sheds light on recent advances (graphene, nanomaterials and Raman-based sensors) in the field of biosensing technology.

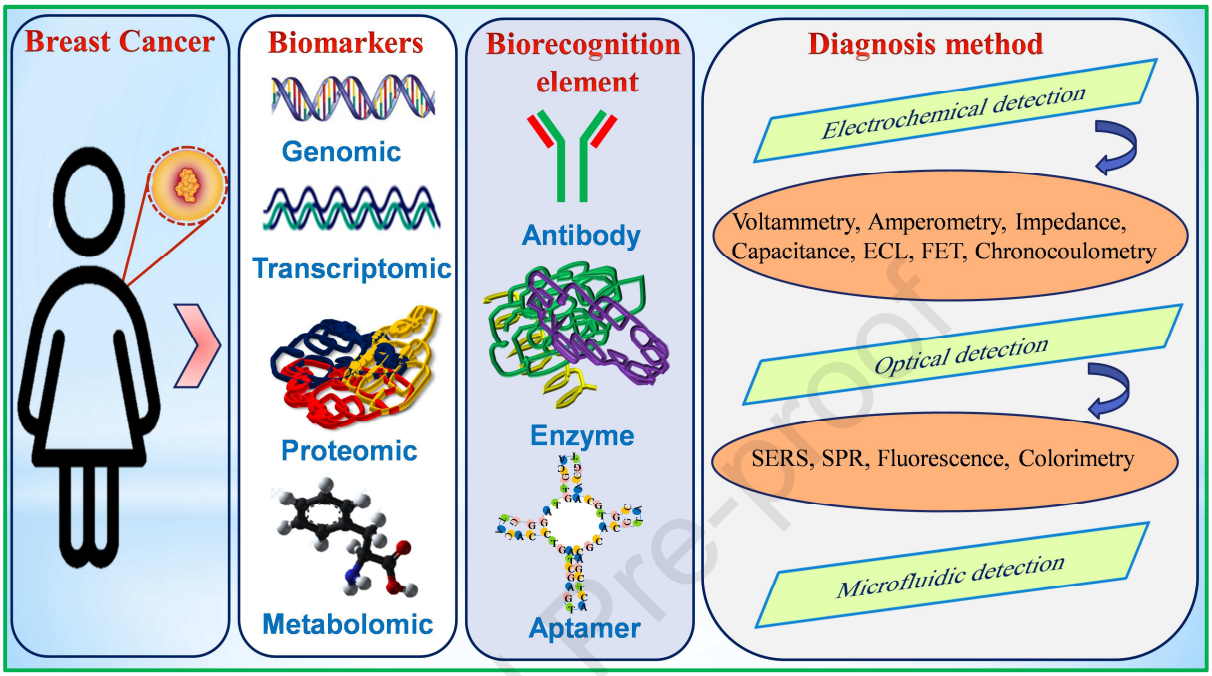

Figure 2: Types of biosensors on the basis of cancer biomarkers, biorecognition and transducers.

There are several molecular biomarkers approved/recognized and/or are under investigation by health organizations which are specifically associated with early stage BC [9, 10]. The biomarkers are the biomolecules which involve in various cellular and physiological processes and can be categorized as genomics, transcriptomics, proteomics and metabolomics. Various types of biomolecules-based biomarkers and their relation with each other and also illustrates the integration of these biomarkers which is useful tool for disease indication are shown in Figure 3.

2. Biomarkers associated with breast cancer

Biomolecules are the basic constituents of living organism. They are involved in various cellular processes which are vital for life, and any alteration in their concentration level indicating particular disease condition is termed as biomarkers. The cells in our body consist of different types of biomolecules viz., DNA, RNA, proteins and metabolites. The proper interaction between these biomolecules is important for cell division, proliferation, differentiation and apoptosis. Changes in structure, function, expressions, or abundance of these biomolecules may

result in abnormal cellular proliferation which could be exploited as biomarkers to distinguish between normal and diseased conditions. Further, the difference in the structure, function and expression of these biomarkers in the body fluids can provide significant diagnostic and prognostic insight of cancerous situation. In this context, a 70-gene microarray study called as MammaPrint from Agendia, Amsterdam, The Netherlands and a 21-gene expression profile using RTPCR method known as OncotypeDx from Genome Health, Redwood city, CA, USA [4, 5]. These methods got FDA approval for commercial use to predict the recurrence of tamoxifen treated node-negative BC and for gene expression profiling to assess clinical outcome of BC in 2002 and 2004 respectively. The FDA approved omics-based biomarkers are presented in Table 2 [23-30] [Web Ref. 2-6].

Different biomolecules exist in our body and bodily fluids have been enlisted in literature as potential biomarkers to assess prognostic and diagnostic conditions of various pathophysiological disorders. These biomarkers are categorized and named by appending the suffix "-omics" (*omics refers to the methods used to explore the roles and relationships of various types of biomolecules involved in cellular process in an organism*). Therefore, based on their origin, structure and function biomarkers can be classified as Genomic (DNA molecules), Transcriptomic (RNA molecules), Proteomic (protein molecules) and Metabolomic (small metabolites) [31-33]. The changes in any type of biomolecules are reflected in another type of biomolecules and the relationship between these omics-based biomarkers are shown in Figure 3. The study of biomarkers on the basis of omics technology also allows us to understand better the communication among the biomolecules, risk and progression of cancer and drug resistance besides diagnosis, prognosis and monitoring. Cancer biomarkers are the molecules produced by the tumor cells or other cells in response to the cancerous cells. These cancer markers can be detected in blood, serum, saliva, urine, stool etc. Biomarkers can be used in risk assessment, diagnosis, prognosis, pharmacokinetics and pharmacodynamics of cancer [8, 9]. Omics-based biomarkers are classified mainly in to following types:

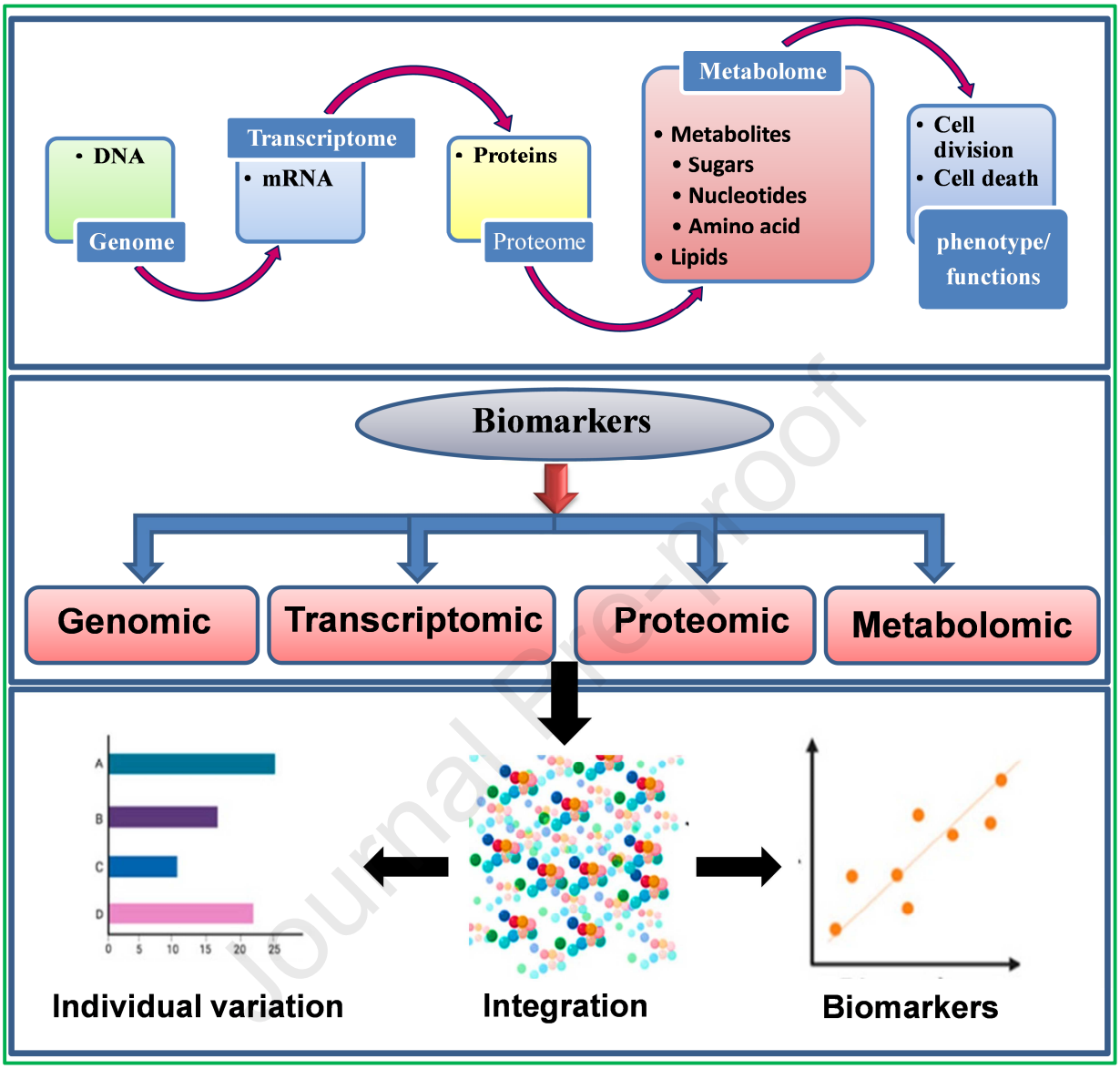

Figure 3: Relationship between biomolecules exploited as biomarkers for designing of biosensors.

2.1 Genomic biomarkers

Several researchers revealed that a person's genome profile, including interactions of genes with each other and with the person's environment gives indications about the present health and the future vulnerabity**.** Changes in genes such as deletion, amplification, or mutation of tumor suppressor genes, proto-oncogenes, cell-cycle regulator genes etc., may lead to cancer and hence

play an important role as cancer biomarkers [34]. The single nucleotide polymorphism (SNP) is a variation in single base-pair in a particular gene at a specific position predispose a person to higher chances of cancer. For instance, SNP in many genes such as CYP1A1, RAD1, BRCA1 and BRCA2 led to breast cancer. The BC risk can also be estimated in patients by genome screening for BRCA1 and BRCA2 genes on chromosome number 13 and 17 respectively. Mutations in these tumor suppressor genes indicate risk of hereditary breast and ovarian cancer. These genes were first discovered by Mary-Claire King, at University of Washington [35, 36]. Other genetic mutations which are used to predict in BC diagnosis are PTEN, TP53, CHEK2, ATM, PALB and BRIP1 2, FGFR2, MAP3K1, and TGFB1 respectively [37-39]. Recently, monitoring of circulatory tumor DNA (ctDNA) and cell free DNA which shed by apoptotic or necrotic cells into the bloodstream have gained considerable attention as cancer specific biomarkers [8]. Here, PCR based technique along with computational algorithm has been used for detection of ctDNA as a biomarker in patient's samples. The Phosphoinositide 3 kinase (PIK3CA) gene mutations have been found in subjects with breast cancers which led to amino acid sequence change and enhanced protein activity [40]. An allele-specific PCR and scorpion probe for monitoring of mutation of PIK3CA gene have been exploited for detection of BC [41]. The most commonly explored genome-based biomarker for designing of biosensor for early detection of BC is mutation in the genes BCRA1 and BRCA2. Although, detection of mutated BCRA1 and BRCA2 genes for early diagnosis of BC using various biosensor-based techniques such as voltammetry, amperometry, impedimetric, and colorimetry have shown promising results. The biosensor-based detection of other genomic biomarkers are need to be explored further.

2.2 Transcriptomic biomarkers

The complete set of messenger RNA (mRNA) transcribed from DNA in the cell plays an important role as biomarker as it reveals the functional profile of proteins. The mRNA from cancer cells gives insights into the diagnosis, type and subtype of cancer, prognosis and effect of given therapy. The panel of RNA consisting of mRNA, miRNA, exRNA, incRNA, cirRNA, etc., isolated from patient's samples such as blood, serum, plasma and urine through liquid biopsy, which is examined for early detection of cancer [42]. A study using RT-qPCR based quantification of microRNAs, miR-126-5p and miR-34a in blood of triple negative patient, have

shown 84% sensitivity for detection of BC [43]. Furthermore, exosomal miR-101 and miR-372 are found considerably higher in the serum exosomes of patients with BC than in healthy controls [44]. The miR-373 is found to be significantly high in triple-negative patients as compared to luminal cancers or healthy controls. Several biosensors involving different detection techniques have been explored for detection of specific BC-associated miRNA-21 in human serum samples such as fluorescence, electrochemical, surface enhanced Raman scattering (SERS), colorimetry, surface plasmon resonance enhanced light scattering and field effect transistor. The results of these studies showed miRNA-21 as a promising biomarker for early detection of BC. Similarly, biosensor-based detection of miRNA-34a, miRNA-155, miRNA-1246 and dual detection of miRNA-21 and miRNA-155 revealed that miRNA-based biomarker detection offers good selectivity and sensitivity.

2.3 Proteomic biomarkers

The proteomic biomarkers are the most studied molecules found to be associated with progression and development of BC. After the transcription and translation of DNA and RNA, proteins are expressed which ultimately perform the intended functions. Protein biomarkers are relatively abundant than RNAs or DNAs, and therefore play an important role in clinical disease diagnosis. There are several protein biomarkers found to be associated with development of BC such as Epidermal growth factor receptors (EGFR)/human epidermal growth factor receptors (HER) family, CA15-3, CA15-9, CA27.29, CA242, CA125, CA19-9, p53, CEA VEGF, EGF, Cathepsin D, Cyclin E, Human epidydimal protein 4, which serve as potent biomarkers to detect BC [45-53]. Inflammatory proteins such as fibrinogen and soluble intracellular adhesion molecule 1 are associated with BC risk directly and inversely respectively [54]. Another study, using reverse phase protein arrays (RPPA) discovered two metabolism related proteins viz., serine hydroxymethyltransferase 2 (SHMT2) and amino acid transporter ASCT2, to be poor prognostic biomarkers of BC [55]. Among various proteomic biomarkers, only two biomarkers viz., HER2 and CA125 are FDA approved biomarkers for clinical use. Various proteomic biomarkers for BC and biosensor techniques have been summarized in Table 1. [Figure 3 reveals that all biomarkers are related with each other and play an important role in various cellular and physiological processes which are vital for the living organisms].

The EGFR and HER are transmembrane protein receptors belongs to ErbB family encoded by ErbB gene. The receptor family mainly consists of four receptors viz., EGFR/HER1, HER2/neu, HER3, and HER4. These receptors are mainly involved in autophosphorylation of tyrosine residues and downstream activation of tyrosine kinase pathway. It subsequently activates other signal transduction pathways which regulate cellular motility, proliferation, differentiation and survival. The elevated HER2 protein can be detected in serum and saliva of HER2 positive BC patients, which serve as a diagnostic tool [45-47]. Further, Trastuzumab, a humanized monoclonal antibody which targets HER2 positive BC cells is an efficient treatment [48]. However, some of the HER2 positive BC patients show resistance to Trastuzumab treatment. Certain metagenes were also identified which mirror the level of pathway activation according to the expression of proteins and/or phosphorylated-proteins pertinent in cancer. Identification of such metagenes is done by integrating RNA and protein expression data from RPPA (reverse phase protein assay analysis) of HER2 positive BC patients from TCGA (Tumor Cancer Genomic Atlas). Among the identified metagenes, AnnexinA1 metagene is found responsible for Trastuzumab resistance in HER2 positive BC patients [49]. Various biosensors with different transducer mechanisms have been developed for detection of variants of EGFR/HER receptor, such as electrochemical impedance spectroscopy, differential pulse voltammetry (DPV) and Field Effect Transistor (FET). However, for HER2 protein receptor biosensor-based techniques such as Cyclic Voltammetry (CV), DPV, amperometry, conductometry, EIS, etc., have been used successfully [56, 57]. The EIS and voltammetry techniques were further employed for detection of HER3 and HER4 biomarkers in BC patient's sample in serum and saliva respectively.

The CA15-3 and CA27.29 are epitopes of polymorphic epithelial Mucin (PEM) and epithelial membrane antigen (EMA) Mucin. It is an extensive O-linked glycosylated protein encoded by gene MUC1. Mucin mainly plays an important role in protection of cells from infection by binding its extracellular domain with pathogens. Besides protection from infection, Mucin also participates in signal transduction, apoptosis, transcriptional, and acetylation of histone proteins. The elevated Mucin has been observed in different types of malignancies and hence exploited in immunohistological and liquid biopsy-based diagnosis of cancer [46]. The epitopes of Mucin, CA15-3 and CA27.29 are detected in blood samples of BC, ovarian cancer, endometrial cancer, bladder cancer etc. It is also observed that CA27.29 is more sensitive and specific in cancer

diagnosis than CA15-3. Despite extant conventional approaches, different biosensor types such as amperometry, impedimetry, conductometry, voltammetry, electrochemiluminescence (ECL) and plasmonic fluorescence-based have shown CA15-3 and CA27.29 as a promising biomarkers for BC.

The CA242 is a carbohydrate antigen present on cell surface, or in serum attached to core proteins and sialic acid. It is observed that on cancer progression, the concentration of CA242 increases in cancer tissues as well as in serum. Different methods like monoclonal antibodies, enzyme linked immune sorbent assay (ELISA), radioimmunoassay (RIA), and chemiluminescence immunoassay (CLIA) are used to detect CA242 in serum. The CA242 is not a very specific marker for cancer diagnosis as its concentration is found to be increased in a number of malignancies such as oesophageal, colon, ovarian, rectal, breast and pancreatic cancers. The CA242 is also observed to be elevated in non-cancerous diseases like type-II diabetes mellitus, coronary heart disease and myocardial infarction. The CA242 when used in combination with other biomarkers gives more sensitive results in cancer diagnosis. For instance, elevated levels of CA50, CA19-9 and CA242 in serum support in very early diagnosis of cancer. However, in this regards square wave voltammetry, amperometry, and other electrochemical platform-based biosensors have been used to detect the levels of CA242 [58].

Carbohydrate antigen, cancer antigen, carcinoma antigen-125 are epitopes of a cell surface associated glycoprotein mucin-16/MUC16 which is encoded by gene MUC16 present on chromosome 19 in humans. Detection of CA125 in serum is a clinically approved method for screening of ovarian cancer. The MUC16 proteins are known to be involved in tumor formation and invasion [50]. Therefore, it is a potent biomarker for diagnosis and prognosis in different types of cancers like ovarian, pancreatic, and gastric cancers. Over expression of CA125/MUC16 protein on tumor cells causes chemotherapy resistance (cisplatin- a platinum based genotoxic drug). Thus, detection of CA125 also helps to decide better therapy and predict therapy response in cancer patients. Recently, square wave voltammetry, chemiluminescence, SPR imaging, EIS, DPV, chronoamperometry, cyclic voltammetry, FET, amperometry based biosensor systems have been used to detect CA125 in BC patients' samples.

The CA19-9, another carbohydrate antigen has also been reported as potential biomarker for BC, lung, ovarian and pancreatic cancers. For the screening of CA19-9, capacitive, EIS, ECL and SPR-based biosensors have been employed.

Carcinoembryonic antigen (CEA) is a group of glycoproteins secreted by gastrointestinal cells during fetal development before birth. Elevated serum levels of CEA are seen in heavy smokers, Ulcerative colitis, Pancreatitis, Crohn's disease, Adenocarcinomas and other cancers as well. Clinically, CEA is used in monitoring of colorectal carcinoma and its recurrence. Increase in CEA also indicates the type of tumor such as aneuploid/diploid, benign/metastatic, and whether it is well differentiated or not, since CEA is also seen to be elevated in non-cancerous conditions. CEA as a diagnostic biomarker which gives sensitive and specific results in combination with other biomarkers. Several biosensor systems such as chronoamperometry, DPV, SWV, conductometry, EIS, fluorescence, resonance energy transfer, magnetic DNA nanoprobes, gold interdigitated capacitor transducer, SPR etc., have shown that CEA may act as a promising biomarker for detection of BC [59-71].

The P53 is well-known tumor suppressor genes encode p53 protein which involved in cell cycle regulation during cell division. Mutation in gene led to alteration in expression of p53, which acts as important biomarker for various cancers including BC. For measurement of p53 in patient's serum biosensors based on ECL, optical, fluorescence, EIS, colorimetric, DPV SWV, amperometry techniques have shown promising results.

Recently, cytochrome P450 (CYP1A1) which is involved in xenobiotic detoxification reactions for removal of toxic compounds from the body has been linked with BC progression and development. A combined PCR-RFLP and gel electrophoresis technique was used for monitoring of CYP1A1 protein [72]. Efforts have also been made to detect of CYP1A1 protein using two–photon fluorescence-based platform for selective detection of CYP1A1 (cytochrome P450 1A1) protein. Researchers constructed an imaging probe by modification of isopropyl functional group on BN, (4-isopropyl-7H-benzo[de]benzo [4,5]-imidazo[2,1-α] isoquinolin-7-one (iPrBN), a fluorescence material. These probes were selectively bound to CYP1A1 to exhibit imaging of protein and detected the concentration of 0.036 nM of CYP1A1 [73]. Similarly, a ratiometric fluorescence platform-based sensor, monitored through naked eye was utilized for the diagnosis of CYP1A1. They also constructed a fluorescence device using fluorophore active 1,

8-nephthalimide with the advantage of visual detection. This NEiPN sensor showed good limit of detection of 0.04874 nM in biological specimen [74]. Recently, a sensor of near-IR fluorescence material containing O-chloroethyl group and dicyanoisophorone was developed for the detection of CYP1A with low limit of detection of 0.026 nM [75].

2.4 Metabolomic biomarkers

Metabolome is a collection of low molecular weight molecules present in biofluids, cells, tissues, organs in the body. These metabolites are the ultimate downstream yields of genomic, transcriptomic, and/or proteomic processes. Changes occur in the relative concentration of these metabolites in the metabolome pool of these biofluids during diseased state of the body. Such changes can be detected and exploited as potential biomarkers for disease diagnosis and prognosis [76-79]. There are different factors which effect the concentrations of metabolites in biofluids such as age, gender, population, lifestyle, diet and presence of other diseases like diabetes, some infections etc. [76]. In BC, lower levels of histidine and higher levels of glucose and lipids are associated with its development and progression [79, 80]. Recently, affinity sensors have been used to detect metabolomic biomarkers present in saliva. Studies have proved a direct relationship between estrogen levels, gut microbiota and risk of BC in post-menopausal women. Microbiome of postmenopausal cancer patients was found to be more diverse and abundant at species level [81-83]. Recently, shotgun metagenomics analysis also identified several bacterial species which are related to postmenopausal BC in women. These species and their abundance in feces samples could be used to diagnose BC early [83]. Another similar clinical trial is going on at University of Granada, Spain, to evaluate the relationship between bacteria, archaea, viruses and fungi in the gut and the risk of BC [84].

3. Biosensor designed for specific biomarkers associated with breast cancer

The alteration or perturbation in the level of these biomarkers not only affect the vital cellular process but also predispose individuals at the risk of various types of cancer and other non-oncological disorders. Therefore, the detection of these biomarkers in individual's body fluids can shed light on the person's health and give contraindication relevant with the disorders. Most promising genomic, transcriptomic, proteomic and metabolomic-based biomarkers associated with breast cancer for designing various types of biosensors for early diagnosis of BC. The irony

of biomolecules is that gene (DNA) transcribes into RNA (Transcriptomics biomarker), and RNA translated into proteins (Proteomics). The proteins involve in various metabolic processes and result in formation of metabolomic biomarkers. Therefore, biosensors could be designed to detect genes or their RNA or protein/ metabolic byproducts. In this review, biosensors which are designed to detect these biomarkers at various cellular levels are discussed in detail.

4. Biosensors for breast cancer diagnosis based on detection technique

Specific and sensitive diagnosis of biomarkers in early stage of BC is of great importance. Biosensors are analytical devices which detect the signals captured by transducer that indicates the disease state of biological molecules. Various types of biosensors have been used for the early diagnosis of biomarkers (biological components) in patients. They are categorized on the basis of their biorecognition elements such as enzyme, antibodies, aptamers and cell-based biosensors. On the basis of their transducer nature they are electrochemical, optical, microfluidic and mass-based biosensors. Several advantages of biosensors such as high specificity, high sensitivity, cost-effective, quick response time, small sample volume requirements, easy to detect, and portability serve as point-of-care diagnosis for cancer biomarkers. Various biosensors based on their transducer nature for the diagnosis of BC biomarkers are discussed in this section [85-87].

4.1 Electrochemical biosensors

An electrochemical biosensor is a device that studies the electrochemical behavior of the electroactive surface to generate the quantitative or semi-quantitative information using electrochemical transducer. Most commonly used electrochemical biosensors are voltammetry, amperometry, impedimetry, potentiometry, capacitive and field effect transistor for the detection of BC biomarkers. Electrochemical biosensors have been highly specific, highly sensitive, portable, no need to pre-treatment of biological samples. They have potential of miniaturization and detection up to ultra-low concentration of biomarkers within few seconds. In addition, electrochemical biosensor has potential to convert into electronic wearable biosensor and could be used to obtain results through portable device such as mobile (smartphone). It has potential to easily integrate with other techniques such as microfluidic-based biosensor to enhance the sensitivity and detect trace level of analyte. In this electrochemical approach, the working

principle and fabrication strategies with their advantages as well as limitation of electrochemical biosensors utilizing for the diagnosis of breast cancer biomarkers are discussed [88-90].

4.1.1 Voltammetric-based biosensors

The voltammetric technique measures the current as the function of applied potential on to working electrode versus reference electrode. In this technique, potential applied on the working electrode in different forms such as potential sweep, step potential, potential waveform, potential ramp etc. Voltammetric techniques are categorized as cyclic voltammetry, differential pulse voltammetry, linear sweep voltammetry, and square wave voltammetry. In the voltammetry-based biosensor fabrication, the electrode surfaces by conductive material are used. The immobilization of the biomarkers specific antibodies on sensor surface was done. Moreover, the sensitivity of biosensors are associated with the current response of material and observed that sensitivity increases with the increase of current response. However, the disease specific antibodies immobilized on sensor surface gain the selectivity against target biological analyte/antigen. When the specific antigen binds to antibodies, they generate electrical signals which were captured by transducer and intern estimate the diseases stage. Moreover, voltammetry-based biosensors are highly sensitive among other technique-based biosensors. Hence, it is so it was widely employed for the diagnosis of BC biomarkers [90, 91].

4.1.1.1 Cyclic Voltammetry-based biosensors

The CV investigates the electrochemical behaviors such as redox properties and reaction intermediate of electroactive material. This technique, measures the current with ramped of potential [92]. Various sensitive biosensor-based on cyclic voltammetry techniques were reported for early diagnosis of BC biomarkers. For instance, Vasudev et al. reported a label-free electrochemical biosensor for the detection of EGFR. Herein, they modified gold electrode using self-assemble monolayer (SAM) of dithiobissuccinimidyl propionate (DTSP) and then after immobilized the anti-EGFR antibodies to selectively detect EGFR. The CV technique was employed for the monitoring of EGFR detection with 1 pg/mL as LOD with 2.02 µA/M of sensitivity. Here, NH_2 group of the DTSP-SAM facilitates to bind with carboxylic group of anti-EGFR antibodies to enhance the charge transfer onto the modified electrode. This results in the increase in movement of electron transfer to the electrode and improve their sensitivity.

However, the self-assembly offers the high alignment of molecules on the sensor surface which is employed to enhance the sensitivity [93]. In another CV-based technique, Canbaz et al. reported highly sensitive electrochemical immunosensor for the detection of HER3. They fabricated an immunosensor by self-assembly monolayer of hexanedithiol and cysteamine on gold nanoparticle. Further with the help of cross linker like glutaraldehyde and anti-HER3 bioreceptor covalently immobilized on cysteamine. Gold nanoparticles are highly conductive materials and also provide more surface area for immobilization of bioreceptor to enhance the sensitivity of biosensor. In impedimetric measurement, which is used to observe the charge transfer resistant properties, they found that charge transfer resistance was increased after self-assembly of hexanedithione on gold nanoparticles. Consequently, hexanedithione acts as a blocking agent so that electron transfer between electrode and bulk solution is hindered. When cysteamine was immobilized on electrode surface, it facilitates the electron transfer and reduce charge transfer resistance. Moreover, glutaraldehyde also plays an important role during electrode fabrication, because the anti-HER3 binding increases with increase in glutaraldehyde concentration. Experimentally, the LOD of this biosensor is found to be between 0.2 to 1.4 pg/mL in serum sample [94]. A sandwich enzyme-linked immunosorbent assay (ELISA) was reported in which mouse anti-human ErbB2 antibody modified screen-printed electrode have been used for biosensor fabrication. This was further used for the detection of Human Epidermal Growth Factor Receptor-2 (HER2) with the detection limit of 4ng/mL [95]. The CV-based biosensor was widely applicable for detection of BC biomarkers, however due to non-Faradaic current interference, it lowers the sensitivity in comparison to other voltammetric techniques and makes it a limitation in biosensor application [96].

4.1.1.2 Differential Pulse Voltammetry-based biosensors

The DPV is a class of voltammetric technique which offers to detect a trace level of analyte with high sensitivity. In this technique, current is measured between two points of each pulse with respect to potential which is applied in the pulse form. The resulting peak height in the DPV curve is directly proportional to the analyte concentration [97]. For instance, Azimzadeh et al. reported a specific electrochemical immunosensor for early detection of miRNA-155 in unprocessed plasma sample. Herein, they constructed an electrode by graphene oxide with thiol containing gold nanorods on glassy carbon electrode. Since, graphene oxide is a poor conductor

but the oxygen functionality makes it favorable material in biosensing applications which facilitates the immobilization of antibodies to enhance the sensitivity. In addition, the thiol containing gold nanoparticles improve the conductivity which results in the increment of sensitivity of biosensor. They found that the electrode displays wide linear range of 2 fM to 8 pM with the LOD of 0.6 fM for miRNA-155 which was recorded via DPV [98]. In a similar approach, Omidfar et al. developed a DPV technique-based ultrasensitive biosensor of Fe_3O_4/N-trimethyl chitosan/Au nanoparticle composite and Platinum-modified electrode for the diagnosis of EGFR. The LOD of biosensor was 0.05 pg/mL. Moreover, the N-trimethyl chitosan was the potential to provided large surface area which helps to enhance the sensitivity of biosensor [99]. In another study, an electrochemical sandwich-assay aptasensor was reported for detection of EGFR biomarker. Herein, the anti-human EGFR aptamer immobilized on streptavidin-coated magnetic bead (MB) act as a capture probe while a polyclonal anti-human EGFR antibody binds with gold nanoparticles act as signaling probe. The diagnostic result was studied by DPV which resulted in LOD of 50 pg/mL for EGFR [100]. In another study, a BC biomarker, CA15-3 was detected by sensitive sandwich-assay immunosensor. This immunosensor was constructed by electrochemical bilayer of polypyrrole nanowire and poly (1, 5-diaminonaphthalene) (P (1,5 DAN) on screen printed electrode. Using CV in two-step process, they have done electro-polymerization of polypyrrole and P (1, 5 DAN) on screen printed electrode. Initially, polypyrrole is polymerized on GCE and afterwards P (1, 5 DAN) was deposited on polypyrrole surface. Moreover, the electrochemical polymerization has the advantage to control the deposition of layers of polymer on electrode surface by applying the number of cycles in CV technique, so that it can tune to their conductivity accordingly. In addition, it consumes less time with minimum wastage of chemicals and the process is quite easy. The bilayer of sensor provides high surface area and electro conductivity to enhance the sensitivity and selectivity by immobilization of anti-CA15-3 antibodies. To attain the amplification of signal, they used magnetic beads which were functionalized with horseradish peroxide. Due to high electrochemical activity of polypyrrole, it gives the advantage of enhancing their sensitivity. However, concentration of $LiClO_4$ also plays an important role during polymerization. Due to the increase of $LiClO_4$ concentration, the anodic current increases and also the polymerization process is enhanced. They reported that the 10 mM $LiClO_4$ was optimum for polymerization, but when then concentration of $LiClO_4$ further increases, the polymerization takes place at much

faster and forms a cauliflower like structure. So, they constructed sandwich like HRP-MBs-Ab2/CA15-3/Ab1-P15DAN/PPy NWs/SPEs immunosensor. The EIS was used for monitoring the successful immobilization of antibodies on electrode by measuring the increase in impedance of immunosensor. Detection of CA15-3 was done via DPV and the immunosensor showed linearity of 0.05 to 20 U/mL with the LOD of 0.02 U/mL [101]. In sandwich assay, the target analyte binds between two different anti-antibodies (Ab1 and Ab2) with similar epitopes, in which Ab2 anti-antibodies were conjugated with enzymes. This shows catalytic behavior which is used to obtained the detection of target analyte. For instance, a sandwich type electrochemical biosensor was developed for the detection of CA15-3 biomarker with high sensitivity and low LOD. They constructed biosensor of nano porous gold with graphene and thionine, and deposited on glassy carbon electrode. To achieve the amplification of signal, they used HRP-encapsulated liposomes. The reduction of H_2O_2 catalysis through HRP which is released from HRP-encapsulated liposomes are a promising tool to get results of biosensor. Here, thionine act as an electron arbiter to enhance the conductivity of sensor. Electrochemical studies of immunosensor has been done through DPV technique as illustrated in Figure 4. The LOD has been estimated as 5.0×10^{-6} U/mL with the limit of quantification (LOQ) of 1.7×10^{-5} U/mL at (S/N=3) along with the stability of immunosensor up to 30 days [102]. In another study, Akter et al. demonstrated graphene oxide nano composite-based sandwich assay electrochemical biosensor for selective detection of CA15-3 biomarker in serum. Herein, they synthesized the self-assembly of cysteamine onto the gold surface. Further, the electrode modification was done with the immobilization of functionalized graphene oxide (GO/Py-COOH) hybrid followed by anti-CA15-3 antibodies, for the specific detection of CA15-3 cancer cells. After synthesis, the electrodes were labeled with multi walled carbon nanotube containing ferritin immobilized with Ab2. The electrochemical behavior of sandwich electrode was monitored through DPV technique where it displayed detection of 0.009 U/mL in serum sample and the biosensor has good reproducibility and stable up to 45 days [103].

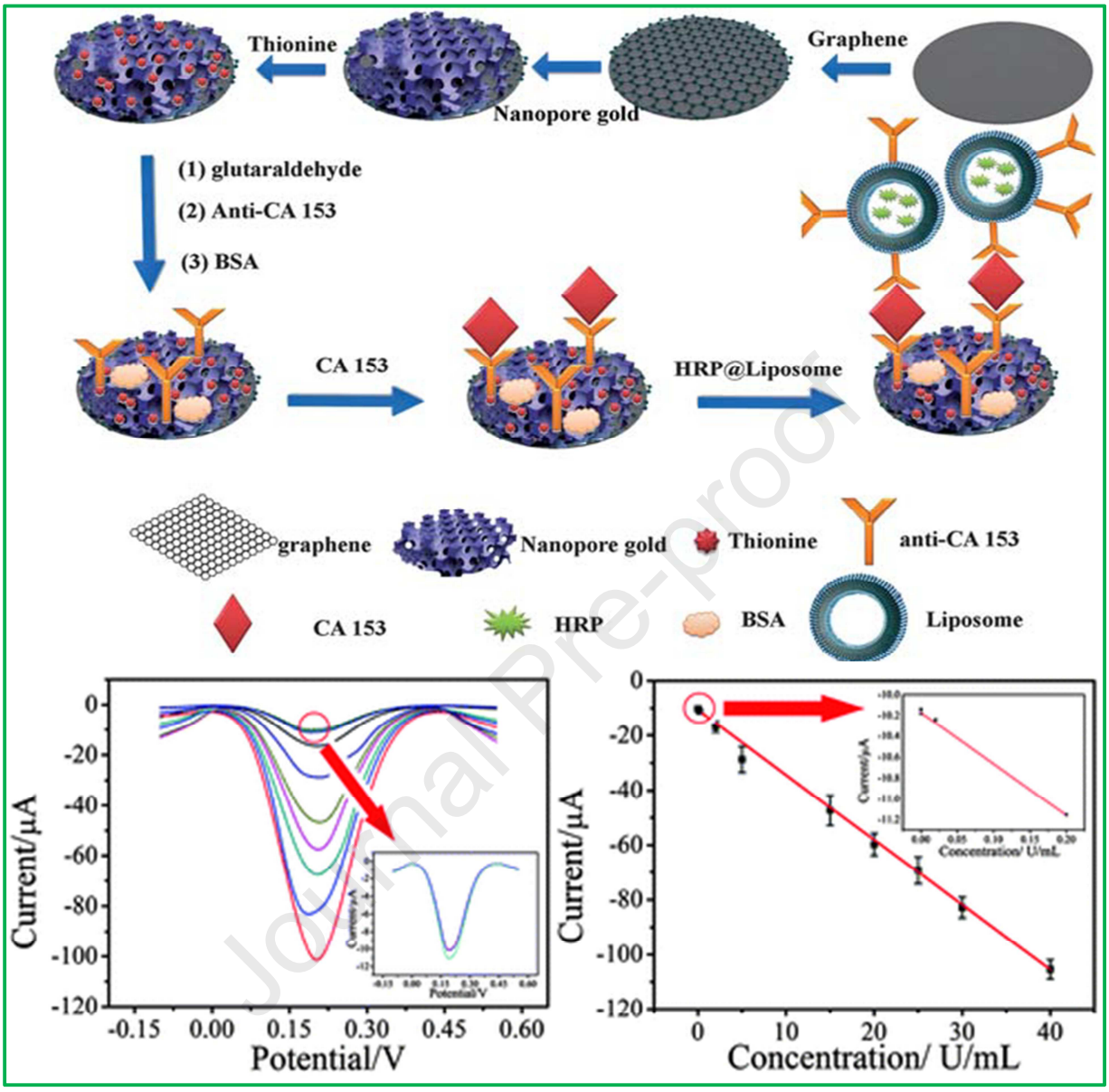

Figure 4: Representation of the assembly of the immunosensor, DPV response and calibration graph after incubation with different concentrations of CA15-3. Reprint with permission from Ref. [102]

Recently, SPE modified by graphene oxide and silica nanoparticles were demonstrated for the electrochemical sandwich-assay for the diagnosis of CA15-3 in serum samples. An electrode was synthesized by introducing copper catalyzed click reaction of acetylene group, which enables graphene oxide with azide group functionalized magnetic silica nanoparticles. This MSN/GO is

casted on screen printed electrode and further monoclonal anti-CA15-3 antibody is immobilized onto it. This hybrid MSN/GO composite exhibited great peroxide-like activity and through which detection of CA15-3 was done by DPV and resulted in excellent LOD of 2.8×10^{-4} U/mL in serum sample [104]. In another study, electrochemical aptasensor was utilized for the diagnosis of CA125. Further in the study, they synthesized silver nanoparticles doped polyacrylonitrile and deposited on ITO surface to make a thin film of AgNPs-PAN. It was further modified by oxime functional group using hydroxyl amine and ammonium hydroxide solution. To achieve selectivity against CA125, amine group containing aptamer was immobilized on electrode surface and it revealed that the aptamer had linearity of 0.01 to 350 U/mL with an excellent LOD of 0.0042 U/mL in serum sample [105]. Jafari el al. developed electrochemical biosensor platform for selective detection of CA125 cancer biomarker. For this purpose, they prepared conducting ink of silver nanoparticles and graphene quantum dots and immobilized on to a glassy carbon electrode via electrodeposition technique. Further diseases specific antibodies such as anti-CA125 were placed on modified GCE to selective detection of CA125 via DPV and SWV technique. It had a wide linear range of 0.01 to 400 U/mL and LOQ of 0.01 U/mL in plasma sample [106]. Recently, a non-enzymatic sandwich assay of electrochemical biosensor was demonstrated for the diagnosis of circulating miRNA-21 biomarker in serum sample. Initially, they synthesized electrode of gold nanoparticles and a reduced graphene oxide composite placed on screen printed carbon electrode. They further modified it with thiol containing compound which acts as capture surface. For the purpose of labeling, a detection probe was constructed by streptavidin immobilized on ferrocene capped gold nanoparticles. This sandwich assay showed wide linearity of 10 fM to 2 pM with LOD of 5 fM [107]. Liu el al. fabricated a label-free electrochemical biosensor for early detection of miRNA-155. Herein, the electrode was constructed by atomic layer deposition of gold nanoparticles on molybdenum disulphide to synthesize Au@MoS_2. Here they used conductive toluidine blue to further improve the sensitivity of probe and achieved lower LOD of 0.32 fM with wide linear range between 1 fM to 10 nM [108]. In other approach, Pacheco et al. reported an electrochemical molecularly imprinted polymer biosensor for the selective detection of HER2-ECD BC biomarker. The molecular imprint of HER2-ECD was done by electro polymerization of solution containing phenol to synthesize polyphenol and further HER2-ECD was attached to it. However, the advantage of molecular imprint with electro-polymerization to

controls the thickness of polymer by increasing or decreasing the number of cycles. This affects the conductivity of composite as well as high sensitivity and stability. The LOQ and LOD of biosensor was 5.2 ng/mL and 1.6 ng/L respectively in serum sample [109]. Another recently developed biosensor detects extracellular domain of protein biomarker human epidermal growth factor receptor-2 (HER2-ECD) in serum samples within 2 hours. For this purpose, they utilized the immunoassay of CdSe@ZnS core/shells quantum dots on the screen-printed carbon electrodes to detect the HER2-ECD. The biosensor was LOD of 2.1 ng/mL [110]. In another report, Yang et al. developed novel aptasensor for selective detection of HER2 biomarker through electrochemical techniques like CV, DPV and EIS. Herein, the biosensor was developed by ferrocene labeled DNA with gold nanospheres (FcNS). Aptasensors are made of sDNA, which is highly specific, sensitive and detect the ultra-low concentration of target analyte. In this aptasensor, it interacted with target HER2 via host-guest recognition to detect the analyte. The LOD of this aptasensor was found to be 4.9 ng/mL in serum [111]. In another study, N-doped graphene-based, label-free electrochemical biosensor was reported for detection of CA15-3 with the LOD of 0.012 U/mL and linear range of 0.1 to 20 U/mL. Graphene possesses excellent electrochemical conductivity because of their huge number of conjugated π-bond, however, the N-doping enhances their conductivity. The excellent conductivity of N-doped graphene has high surface to volume ratio, and hence, provides more surface to immobilization of anti-CA15-3 antibodies to improve sensitivity of biosensor [112]. In another approach, Ribeiro et al. demonstrated an electrochemical immunosensor for detection of CA15-3 antigen [113]. They constructed the electrode by electrochemical polymerization of toluidine blue through molecular imprinted polymerization on gold screen printed electrode. During this molecular imprinting polymerization, thiol group of toluidine blue conjugated with the gold surface. The free amine group activated by glutaraldehyde to form imine complex which further reacts with CA15-3 antigen for providing sensing platform. The MIP immunosensor exhibited linearity of 0.10 to 100 U/mL with LOD of 0.10 U/mL. Similarly, electrochemical biosensor was designed by molecular imprinting of 2-aminophenol on gold surface for the selective early detection of CA15-3 cancer biomarker. The LOD of this biosensor was 1.5 U/mL. In this MIP, tiny to macro molecule could be imprinted in any reaction condition; however, it is highly stable, and easy to fabricate [114]. Cui et al. reported the modified screen-printed electrode sandwich assay-based single electrochemical biosensor for the simultaneous detection of multiple analyte such as

CA125, CA15-3 and CEA BC biomarkers. They utilized graphene sheet since it was highly conductive and provided more surface area for immobilization of antibodies. Hence the sensitivity against analyte was increased which reduced the time slot for detection. So, they fabricated graphene sheet on screen printed electrode followed by immobilization of Ab1 antibodies. Further, the secondary antibodies (Ab2) were immobilized on mesoporous platinum electrode surface and finally constructed graphene sheet/Ab1/Ag/M-Pt-Ab2 on screen printed carbon electrode. In this process, they constructed three electrodes by immobilization of three different antibodies viz., CA125, CA15-3 and CEA in a single electrochemical biosensor and simultaneously detected the three-target analyte. These biosensors displayed linearity of 0.05-20, 0.008-24 and 0.020-20 U/mL with LOD of 0.002 U/mL, 0.001 U/mL and 0.007 ng/mL for CA125, CA15-3 and CEA, respectively [115]. Recently, a reduced graphene oxide, gold and palladium nanoparticles-based label-free electrochemical biosensor platform was developed for the early diagnosis of CA242 biomarker. This biosensor showed linearity of 0.001 to 10000 U/mL with a low LOD of 1.54×10^{-3} U/mL in blood sample**.** There are enormous advantages of bimetallic nanoparticle-based hybrid nanocomposites because they have high surface area, excellent electron mobility, better catalytic activity and excellent biocompatible properties. However, they need to control their aggregation of composites which make it a limitation in biosensor construction [116]. In another study, a label-free ultrasensitive electrochemical immunosensor was reported for the investigation of BRCA1, BC gene. For this purpose, dendrimer of ferrocene cored (polyamidoamine) was immobilized on cysteamine modified gold surface to construct electrode and followed by drop casting of single strand DNA onto it. The LOD of biosensor was 0.38 nM with high sensitivity of 0.13 ng/mL and 1.3 to 20 nM of linear range [117]. In this biosensor, the rate of electron transfer in the conducting polymer is found to be quite high, which play significant role in electrochemical sensing probe to give better sensitivity and reproducibility after binding with target analyte. In another context, Poly(3,4-ethylenedioxythiophene) (PEDOT) conducting polymer was utilized to fabricate electrochemical genosensor [118]. Herein, they electrochemically synthesized the citrate ion-doped PEDOT on GCE. Further, peptide moieties were immobilized onto functionalized PEDOT using Ni^{2+} ion, where free carboxylic group of citrate ion provided suitable surface and bonding ability for better binding of peptide. For selective detection of BRCA1, a capture DNA probe was also immobilized on to peptide surface. The developed genosensor showed low LOD of 0.03 fM. A

sandwich assay electrochemical biosensor was reported by Chen et al. for the detection of CEA and AFP simultaneously with LOD of 0.10 and 0.05 ng/mL respectively. Herein they prepared chitosan-functionalized gold electrode on glassy carbon surface and thereafter anti-CEA and anti-AFP were immobilized on functionalized gold surface [119]. In another approach, carboxylic group-functionalized graphene layer was modified separately of two different electroactive substances viz., toluidine blue (TB) and prussian blue (PB). Further, these CGS-TB and CGS-PB were immobilized with anti-CEA and anti-AFP antibodies respectively. When it combined with their target antigen, it displayed electrochemical activity which was measured by a DPV. A paper strip sensor based on signal-On and signal-Off strategy which was monitored by electrochemical technique was developed. Signal-On and signal-Off employed the enhancement and diminishing of signals during monitoring of target analyte. This paper-based sensor was utilized for the detection of H1047R, a missense mutation in exon 20 of the PIK3CA gene in breast cancer. Herein, they fabricated two different kinds of paper strips separately for both signal-On and signal-Off. For signal-On strategy, they modified filter paper via drop casting of gold nanoparticles further functionalized with DNA probe. When target DNA interacts with biosensor surface, a signal is generated. However, the intensity of signal was enhanced by binding of DNA probe with Ruthenium-based redox compound [$Ru(NH3)_6^{+3}$] and it achieved LOD of 5 nM with linearity of 10 to 100 nM. In case of signal-Off strategy, the gold surface of biosensors is modified with MB-functionalized DNA probe without use of Ru-redox compound and it showed LOD of 6 nM with linearity of 20 to 300 nM [120]. A sandwich type electrochemical immunosensor was reported for the detection of VEGFR2 protein. In this report, the electrode surfaces of GCE were coated with a thin layer of thionine followed by immobilization of reduced graphene oxide-chitosan composite and Ab1. After capturing the target analyte on the surface of Ab1, solution of streptavidin-HRP was placed on the antigen. In mechanistic study, thionine was oxidized by catalyzing of HRP in the presence of hydrogen peroxide. In DPV measurement, they found the LOD as 0.28 pM with linearity of 0.4 to 86 pM [121].

4.1.1.3 Square Wave Voltammetry and Linear Sweep Voltammetry-based biosensors

These electrochemical techniques, measure the resulting current with respect to applied potential. However, in SWV, the current is measured by the difference between forward and reverse

current, while in LSV, the current is measure by varied potential at constant rate through scanning. The rapid result and high sensitivity makes it more advantageous among other electrochemical techniques [122]. For instance, a pH responsive label-assisted click reaction based sandwich-assay electrochemical biosensor was reported for the diagnosis of CA242 antigen in serum sample by Zheng et al., Since sandwich assays are highly sensitive and could detect ultra-low concentration of analyte. They fabricated this sensing device using two anti-antibodies which can selectively bind with two different epitopes of an analyte. Herein, initially polyethyleneimine (PEI) mixed with graphene oxide was coated on the glassy carbon electrode followed by immobilization of Ab1. In the next step, they constructed Ab2 immobilized on copper containing polydopamine composite to fabricate a modified glassy carbon electrode by drop casting. Here, the antigen acts as a binder between these two anti-antibodies and make sandwich assay electrode. In this electrode, amine group of polyethyleneimine provided better surface to immobilization of antibodies to improve their sensitivity while catechol and quinone of polydopamine improved the immobilization of Ab2. The biosensor achieved the detection of CA242 antigen which was examined through SWV technique with the LOD of 20.74 μU/mL in serum sample [123]. An electrochemical biosensor of modified gold electrode was reported for early diagnosis of a cancer biomarker CA125 in serum. Herein, the electrochemical deposition of polydopamine was done on glassy carbon electrode which is, further modified with reduced graphene oxide. Cysteamine-functionalized gold nanoparticles were deposited onto rGO surface electrophoretically to make cys-Au-rGO/GCE electrode. For specific detection of CA125, monoclonal antibodies were immobilized on to electrode. The developed electrode displayed linearity of 0.1 to 400 U/mL with LOQ of 0.1 U/mL [124]. Recently, an electrode of polyaniline-polythionine gel was reported for the diagnosis of CA125 ovarian cancer biomarker based on electrochemical detection technique [125]. The proposed biosensor was fabricated by electropolymerization of aniline and thionine into 3D arrangement of polyaniline-polythionine on glassy carbon electrode followed by gold nanoparticles on it. The gold nanoparticles have better ability to catalyze hydrogen peroxide which is observed for diagnosis of CA125 antigen. This biosensor had great linearity from 0.001 U/mL to 1 kU/mL with LOD of 0.00125 U/mL. In another study, Hasanzadeh et al. developed an electrochemical biosensor platform for detection of p53 cancer biomarker [126]. They fabricated a composite of gold nanoparticles with graphene quantum dots and immobilized poly L-cysteine on to the electrode surface. For monitoring of

p53 antigen in unprocessed plasma sample, they employed DPV and SWV techniques and calculated the linearity of 0.195 to 50 pM and 0.0244 to 0.369 pM, respectively, with ultra-low LOD of 12.1 fM. They also reported a mediator-free biosensor platform for the diagnosis of p53 in unprocessed fluids. Herein, electrodes were fabricated by deposition of poly L-cysteine on gold nanoparticle followed by the immobilization of biotin-functionalized p53 antibodies. The linearity of electrode was found to be 0.0369 to 50 pM and 0.018 to 2.5 pM with LOD of 48 fM and 18 fM via SWV and DPV respectively [127]. In particular attention, a dual detection of miRNA-21 and miRNA-155 was reported through electrochemical immunosensor. The electrode was constructed using tetrahedron DNA structure. For multiple analysis of biomarker, ferrocene and methylene blue were immobilized with DNA probe, and observed that miRNA-21 selectively conjugates with ferrocene while miRNA-155 conjugates with methylene blue to produce two different signals [128]. These hybrid electrodes display dynamic linearity range from 0.1 fM to 10 nM with LOD of 18.9 aM for miRNA-21 and 39.6 aM for miRNA-155. An enzyme-free sensitive sandwich assay aptamer was reported by Shen et al. for the diagnosis of HER2 [129]. Herein, the aptamer was fabricated by self-assembly of DNA primer with HER2 to generate electric current which helps in the recognition of ligand as well as for generation of signal. They reported that the directional nature of DNA strand enhances the sensitivity of biosensor as well as selectivity against HER2 as compared to in absence of DNA aptamer. It generates electrochemical signal to interact with molybdate and detected 0.047 pg/mL concentration of HER2. Rebelo et al. have detected CA125 cancer biomarker based on molecularly imprinted polymer modified gold electrode [130]. Herein, the gold surface were modified with self-assembly of cysteamine where the thiols group are attached on gold surface. The amine groups were available for the reaction and in next step, electrochemically polymerization of pyrrole to polypyrrole was done in presence of CA125 template. The constructed MIP electrode displayed linearity of 0.01 to 500 U/mL with LOD of 0.01 U/mL in serum sample. Yang et al. reported a sandwich assay biosensor for the diagnosis of interleukin-6 biomarker having both electrochemical as well as fluorescent properties. Initially, they fabricated the biosensor by layer by layer deposition of gold nanocluster on thin layer of PDDA functionalized boron nitride followed by immobilization of Ab2. The PDDA serves as stabilizer for boron nitride and the fabricated biosensor have biolabels. Further, they fabricated another layer of graphene sheet which was labeled as Ab1. In this mechanistic study, the previously

fabricated Ab2 containing biolabels capture interleukin-6 antigens, and then conjugate with Ab1 to make sandwich assay. The significant change in fluorescence emission and electrochemical signal response helps to determine the detection response of biosensors. It was found that the SWV response displayed the linear range of 0.005 to 100 ng/mL with LOD of 1.3 pg/mL for IL-6 [131]. Similarly, Marques et al. developed a dual screen-printed electrochemical biosensor for the detection of two biomarkers CA15-3 and HER2-ECD in single approach in human serums. Herein, they constructed the electrode by immobilization of either anti-CA15-3 antibodies or anti-HER2-ECD antibodies on gold surface. Here, the anti-antibodies selectively react to antigen while gold surface offer excellent conductivity in sensing platform. The LOD of the biosensor was 5.0 U/mL and 2.9 ng/mL for CA15-3 and HER2-ECD respectively [132].

4.1.3 Amperometry-based biosensors

Amperometric technique depends upon electrochemical behavior of electrode surface. In this technique, current is determined as a function of constant applied potential [133]. With the advantage of amperometry, Hong et al. reported a novel electrochemical biosensor for the detection of CA15-3 biomarker. Herein, they fabricated the biosensor by amine functionalized ferrocene carboxylic acid (Fc-COOH)-doped silica nanoparticles as core-shell nanostructured morphology. In this experiment, shell-silica nanoparticles covalently bind with core-ferrocene carboxylic acid to form 3D structure using water in oil emulsion method. Further, APTEOS functionalize the amine group on Fc-COOH-doped silica NPs. The introduction of amine group which facilitate the immobilization of antibodies results in the enhancement the biosensor sensitivity. Moreover, hydrogen bonding between Fc-COOH and silica NPs helps to reduce the leaching of Fc-COOH from electrode. For the biomarker analysis, they employed CV technique for the detection of CA15-3 antigen and found that the stability of this biosensor was up to 30 days with linear range of 2.0 to 240 u/mL and LOD of 0.64 U/mL with signal to noise ratio of 3 [134]. In another study, Tang el al. developed a label-free highly sensitive amperometric biosensor based on hydrogel (SA-Pb^{2+}-GO) composite for selective detection of CA242 antigen [135]. Herein, sodium alginate (SA) was mixed with Pb^{2+} and graphene oxide and placed on glassy carbon electrode. Further, the composite surface was functionalized with chitosan and they bind to the surface through electrostatic force of attraction. Though chitosan is a poor conductor, it is oxygen and nitrogen-rich functional group that helps to immobilize the anti-

antibodies on sensor surface to improve their sensitivity. Moreover, to enhance the electrochemical activity of electrode, a layer of Pb^{2+} was immobilized on chitosan-containing composite which facilitated the electron movement in the electrode. Thereafter, with the help of glutaraldehyde, anti-CA242 antibodies were immobilized on electrode surface to selectively bind with CA242 antigen for the detection. This amperometric biosensor revealed improved sensing features with linearity of 0.005 to 500 U/mL and LOD of 0.067 U/mL. In a similar approach, a lactate oxidase labeled sandwich assay electrochemical biosensor was developed for the ultrasensitive detection of CA125 [136]. Herein, they synthesized the composite of chitosan-gold nanoparticles with multi-walled carbon nanotube and graphene oxide placed on glassy carbon electrode and functionalized with CA125 antibodies. Thereafter, another composite of gold nanoparticle with lactate oxidase and antibodies (Ab-AuNPs-LOx) were also synthesized. The CA125 antigen binds CS-AuNPs-MWCNT-GO-Ab and Ab-AuNPs-LOx surface. In mechanistic study, they found that lactate oxidase catalyzes the conversion of L-Lactic acid into pyruvate and hydrogen peroxide. Further monitoring of electrochemical oxidation of hydrogen peroxide was done by chronoamperometry and found that biosensor showed ultra-low LOD of 0.002 U/mL and exhibit dual linearity of 0.01 to 0.5 U/mL and 0.5 to 100 U/mL in serum sample. In another study, a selective biosensor was constructed with DNA-RNA antibodies immobilized on magnetic beads and coated on screen printed electrode. It resulted in electrochemically detection of miRNA within 120 min [137]. The biosensor had broad detection range of 8.2 to 250 pM with LOD of 2.4 pM in biological sample. An amperometric biosensor platform was reported for the diagnosis of TP53 gene by Avila et al [138]. Herein, the biosensor was constructed by immobilization of reduced graphene oxide and O-carboxymethylcellulose nanocomposite onto screen printed electrode. Further it was modified by two amino groups containing DNA probe. Herein, the reduced graphene oxide improved the conductivity while nitrogen functionality increased the immobilization of antibodies to enhance the sensitivity of the biosensor. Moura et al. reported magnetic particle conjugated biosensor for multiplex detection of exosomes (CD9, CD24, CD44, CD54, CD63, CD81, CD326 and CD340) which was isolated from three different BC cell lines (MCF-7, MDA-MB-231 and SKBR3) in serum sample. To explore the primary information of exosomes, they studied the imaging of exosomes through confocal microscopy [139]. Moreover, initially for electrochemical detection, exosomes were conjugated with magnetic particle and then functionalized with their disease-specific anti-monoclonal antibodies

for particular exosome detection. In another study, an amperometric immunosensor platform was reported for selective detection of p53 autoantibodies. Immunosensor was constructed with magnetic microcarrier and modified with Halo tag fusion 53 placed on screen printed carbon electrode to capture selective p53 autoantibodies for the generation of electrochemical signals [140]. For instance, researchers synthesized reduced graphene oxide wrapped microspheres ($ZnMn_2O_4$@rGO) through solvothermal technique which can detect H_2O_2 secreted by BC cells (MCF-7) with low LOD of 0.012 μM [141]. Moreover, amperometric biosensors suffer because of their low current response due to interference between enzymes and the other chemicals present in the sample.

4.1.3 Impedance-based biosensors

In EIS, the resistive and capacitive properties of the electroactive have been determined through the current response. The EIS has a significant role in fabrication process and determination of biomarkers in electrochemical biosensor. Moreover, when the biosensor surface was fabricated with biomolecules such as antibodies, antigen, DNA, enzyme, the movement of electrons was hindered or slowed down, which resulted in the increase of impedance of biosensor. In addition, it offers label-free detection of analyte with high sensitivity in rapid manner [142]. For instance, a label-free electrochemical immunosensor was developed made of peptide probe modified with ferrocene carboxylic acid on gold electrode surface. The EIS technique was used for monitoring EGFR detection with LOD of 3.7×10^{-11} g/L [143]. Similarly, an electrochemical impedance based label free assay was developed for the detection of EGFR glycoprotein [144]. They constructed electrode surface using gold nanoparticles followed by cysteamine, PDITC (1, 4-phenylene diisothiocyanate) and protein-G. However, proteins-G improved the orientation of antibodies which were immobilized on working electrode surface to enhance the binding capacity of EGFR with its antibody to enhance the sensitivity. The LOD of this electrode was 0.34 pg/mL in PBS while 0.88 pg/mL in human plasma with wide detection range of 1 pg/mL to 1μg/mL. They revealed that the variation of LOD in PBS and plasma samples was due to the interference created by proteins, ions or other molecules present in the plasma sample. An impedimetric biosensor constructed by graphene on SPE and its surface was modified by polyaniline followed by anti-CA125 antibodies displayed detection range of 0.92 to 15.20 pg/μL [145]. A single use cysteine modified label-free impedimetric affisensor was reported for the

detection of HER2 in which gold nanoparticles-screen printed electrode functioned as sensor probe while affibody as a bioreceptor. To investigate the potential of affibodies, surface plasmon resonance technique was utilized. The LOD of this affisensor was found to be 6 µg/L in serum sample [146]. Similarly, an electrochemical technique-based capacitive biosensor made of interdigitated gold micro electrode fabricated by self-assembly of thiol containing DNA aptamer was used for the early diagnosis of HER2. In an electrochemical technique, impedance was used for the capacitive measurement of aptasensor. For impedance measurement of HER2, there was no needs of redox couple, and only two middle IDµEs were provided platform as two-electrode systems. This biosensor displayed low LOD of up to 1 pM with wide detection range of 1 pM to 100 nM in undiluted sample [147]. In another study, Wang et al. reported a biosensor constructed by immobilization of gold nanoparticles on amine functionalized polyethylene glycol on GCE. It was further modified with thiol containing oligonucleotide to make label-free electrochemical biosensor (S1/PEG/AuNPs/GCE) for diagnosis of BRCA1 gene in human serum sample [148]. The sensor had a wide linear range of 50 fM to 1.0 nM with LOD of 1.72 fM. An electrochemical biosensor of PAMAM dendrimer-based platform was reported for the investigation of miRNA-34a, a cancer biomarker. The electrode showed better LOD of 0.95 µg/mL in PBS while 0.52 µg/mL in diluted FBS:PBS solution [149]. In another study, at low level concentration of miRNA-155, BC biomarker in serum was detected by an electrochemical immunosensor [150]. The sensors were constructed of screen-printed electrode which were coated by gold nanoparticles, and further modified with anti-miRNA-155 for selective monitoring of miRNA-155. The Thiol functionalized succinic acid was also placed to avoid non-specific binding. This modified electrode achieved wide detection range of 10 aM to 1.0 nM with LOD of 5.7 aM. Similarly, a novel electrochemical biosensor having excellent reproducibility was reported for the detection of HER3 [151]. Herein, they fabricated an electrode by self-assembly of 4-aminothiophenol on gold electrode surface. The CV and EIS technique were used to further monitor each fabrication step of working electrode. Herein, the sulphur atoms of 4-aminothiophenol were introduced via self-assembly on gold electrode through strong S-Au bond. Further, the interaction between negatively charged redox probe and positively charged amino acid facilitates the enhancement of the electron transfer. Further, the amino group of 4-ATP was activated by glutaraldehyde to increase the binding efficiency with anti-HER3 on electrode surface. The advantage of 4-ATP is that it facilitates the electron transfer, and thus by increasing

its concentration, the electron transfer properties also increase. They achieved the detection range of 0.4 to 2.4 pg/mL. Ayadin et al., a label-free electrochemical biosensor for p53 diagnosis [152]. They constructed poly-glycidylmethacrylate, a star-shaped polymer on ITO glass using spin coater to make a uniform coating of polymer on ITO. Further, anti-p53 antibodies were immobilized on polymer to facilitate the selective detection. The ESI technique monitors the detection of p53 antigen and found to have LOD of 7 fg/mL in serum sample. Since, EIS is a highly sensitive technique but in another way the non-specific interference makes it a limitation in biosensor application.

4.1.4 Capacitance-based biosensors

In capacitive technique, it measures the capacitance and thickness of the dielectric layer or/and change in dielectric properties of electroactive materials without interference of non-Faradaic current. For instance, an affimer-based gold interdigitated electrode, based on capacitive measurement in undiluted serum was reported by Zhurauski et al. for the selective diagnosis of HER4 protein [153]. Herein, the biosensors were fabricated by immobilization of self-assembly of anti-HER4 affimer conjugated with C-terminal cysteine on gold electrode surface. The advantage of affimer-based biosensor is that they gain good selectivity and sensitivity against biomolecules as compared to antibodies-based biosensor. The surface plasmon resonance (SPR) technique was used to examine the interaction between HER4 biomarker and affimer. They measures capacitance of electrode before and after interaction with HER4 biomarker with the help of impedimetric measurement, and found that the capacitance decreased when HER4 is present in serum. This electrode exhibits better linearity of concentration range from 1pM to 100 nM against HER4 as well as high sensitivity of 0.049 μF/log C_{HER4} with linearity of 1pM to 100 nM and LOD of 1pM in undiluted serum sample. Similarly, an electrochemical immunosensor of modified screen-printed interdigitated platform was reported for investigation of p53 [154]. They utilized self-assembled monolayer of gold nanoparticles to design the electrode which has detection limit of 0.68 U/mL in serum. Recently, a graphene oxide and carbon nano-onion composite based screen-printed interdigitated platform was utilized for the diagnosis of CA19-9 antigen of pancreatic cancer [155]. For a targeted selectivity against diagnosis of CA19-9, the anti-CA19-9 antibodies were immobilized on electrode surface. Moreover, development of interdigitated electrode was completed within few minutes in a simple process, which have high

sensitivity, and make it promising for point-of-care application. The biosensor detected the 0.12 U/mL concentration of CA19-9 cancer biomarker.

4.1.5 Electrochemiluminescence-based biosensors

Electrochemiluminescence or electrogenerated chemiluminescence is the luminescent activity where the measuring of emitted light due to electron transport reaction when a potential is applied on electrode. The light intensity of this assay is directly proportional to the concentration of total number of analytes participated in chemical reaction. Their high sensitivity and selectivity make them promising choice in biosensor applications [156]. For instance, Wang et al. reported a highly sensitive sandwich-assay luminescent electrochemical biosensor for the detection of CA15-3. Initially, they fabricated electrode by modification of palladium nanocage (PDNCs) surface with poly(ethylenimine) and Ruthenium (II) luminophores conjugated with polystyrene polymer. The immobilization of selective anti-antibodies to further increases the specificity and sensitivity of biosensor was done. The luminophores are mostly semiconductors or transition metal complexes which generate luminescent signals when potential applied on electrode via electron transfer mechanism. Secondly, gold nanoparticles with their surface modified with graphene was introduced as electrochemical luminescent platform due to its high electron transfer properties for capturing of antibodies. They detected the antigen after binding between both electrode and estimates that the linearity of luminescent biosensor was 0.01 to 120 U/mL with the LOD of 0.003 U/mL for target biomarker CA15-3 as shown in Figure 5 (I) [157]. In another approach, a luminescent biosensor of protein-aptamer containing 3D-DNA provided a specific electroluminescent platform for the selective detection of Mucin1 [158]. Initially, they synthesized 3D-DNA nano-machine in which (oxides of cobalt and iron) $CoFe_2O_4$ were mixed with gold nanoparticles. Further, S2 and HP1 DNA were immobilized onto it to synthesize $CoFe_2O_4$@AuNPs-S2-HP1 magnetic nanocomposite. To fabricate the 3D immunosensor, the $CoFe_2O_4$@AuNPs-S2-HP1 nanocomposites were mixed with ABEI-HP2 which forms $CoFe_2O_4$@AuNPs-S2-HP1-HP2-ABEI hybrid magnetic nanocomposite. Because of high stability, large surface area and great separation characteristics, this was found to be promising material to facilitate the immobilization of DNA on biosensor surface to enhance the LOD and sensitivity against targeted analyte. This sandwich electrochemiluminescence biosensor achieved high sensitivity with detection range of 1 fg/mL to 1 ng/mL and LOD of 0.62 fg/mL. An

electrochemical luminescent biosensor was proposed for the detection of CA19-9 [159]. The composite of luminol and multi-walled carbon nanotube was coated with chitosan on glassy carbon electrode surface in which chitosan facilitated the uniform coating of thin film, and further it was modified by anti-CA19-9 antibodies. When antigen was coupled with antibodies, the intensity of signal was lowered. This advanced luminescent sensor exhibited linearity of 0.0001 to 10 U/mL with low LOD of 0.000046 U/mL in serum. Similarly, Zhang et al. developed a sandwich assay based electrochemiluminescence biosensor for the diagnosis of carbohydrate antigen 19-9 [160]. Herein, they constructed biosensor of magnetic carbon nanotube and luminol with Ag@BSA. Initially, the Ab1 was immobilized on magnetic carbon nanotube through glutaraldehyde cross linker. Then after, Ag@BSA was functionalized with luminol which has unique property to display chemiluminescence after interaction with selective target antibodies. In the detection process, Ab1 interacted with Ab2 which were conjugated with luminol functionalized Ag@BSA to generate luminescent properties and to detect the CA19-9. This biosensor had low LOD of 0.0002 U/mL (S/N= 3) with linearity of 0.0005 to 150 U/mL in serum samples. In a similar approach of electrochemiluminescence-based biosensor of magnetic graphene oxide was demonstrated by Sha et al. for the detection of CA19-9 [161]. Herein, the nano Fe_2O_3@GO composite was functionalized with an electrochemically active chemiluminophore N-(4-aminobutyl)-N-ethylisoluminol which exhibited luminescenct property while anti-CA19-9 antibodies were immobilized on sensor surface to selectively interact with CA19-9 antigen for detection. This sensor displayed wide linear detection range of 0.001 to 5 U/mL with LOD as 0.0005 U/mL. In another study, an electrochemiluminescence biosensor was developed for the detection of c-MycmRNA gene in MCF7 cells. Herein, the antisense DNA functionalized CdSe@ZnS QDs were modified with reporter DNA. When c-Myc mRNA reacts with modified QDs, reporter DNA could detach and sensed by the luminescent device. Moreover, quantum dots of less than 10 nm possess excellent fluorescent property and hence commonly utilized for fluorescent detection of analyte [162]. Application of metallic-organic framework (MOF) for designing of electrochemiluminescence biosensor was proposed by Xiong et al. for the diagnosis of CA15-3 BC biomarker. Herein, they synthesized a solution of $Ru(bpy)_6^{+2}$@UiO-66-NH_2 (MOF) by mixing of $Ru(bpy)_6Cl_2.6H_2O$, $ZrCl_4$ and H_2NH_2BDC. After the preparation of MOF solution, it was mixed with Nafion solution. Further, the prepared composite was drop casted on glassy carbon electrode to develop sensor surface. Further, the

sensor surface was modified via anti-CA15-3 antibodies for selective detection. In this experiment, they observed that $Ru(bpy)_6^{2+}$ acts as highly luminescent material while UiO-66-NH_2 helps to increase the electrochemiluminescent signal by providing large surface area. When CA15-3 antigen covalently binds with antibodies, the signal intensity of sensor was reduced due to the quenching effect. Moreover, they utilized tri-n-propylamine to enhance the signal intensity of biosensor. Proposed biosensor displayed linearity of 5×10^{-4} to 5×10^{2} U/mL with lower limit of detection of 1.7705×10^{-5} U/mL in serum [163].

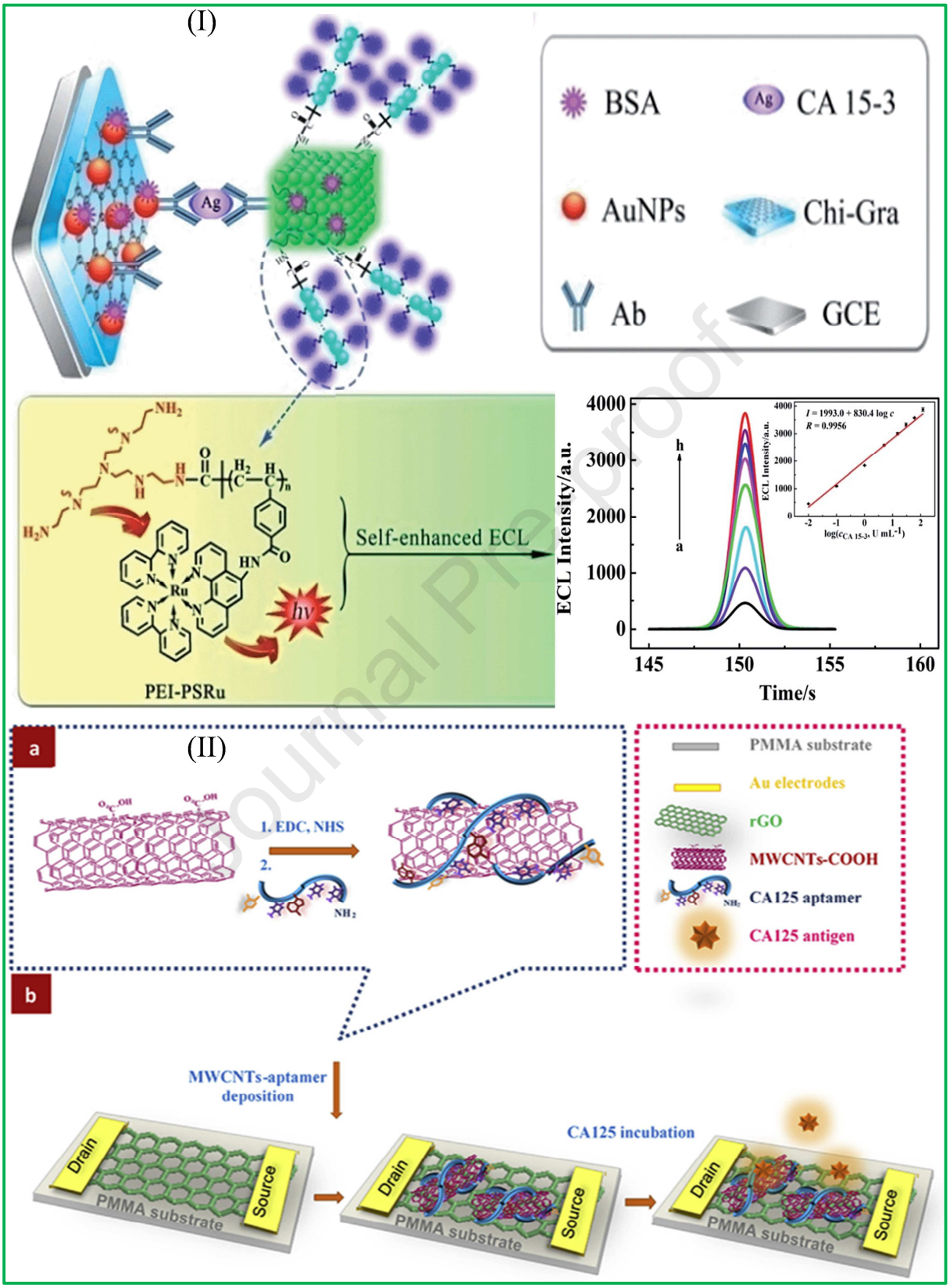

Figure 5: Schematic of (I) process and reaction mechanism of the ECL immunosensor and ECL profiles of the immunosensor for CA15-3 detection. Reprint with permission from Ref. [157]. (II) Fabrication of rGO FET aptasensor: (a) Modification of carboxylated-MWCNTs with CA125 ssDNA. (b) RF sputtered gold source and drain electrodes on the rGO polished onto the surface of the flexible PMMA substrate. Reprint with permission from Ref. [168].

4.1.6 Field Effect Transistor-based biosensors

The FET-based biosensor enables to estimate the variation of conductivity generated through the electric field in source-drain channel. Hence, the target analyte was detected on the estimation of change in current-voltage of source drain channel. For the detection, the disease-specific biological element which has capability to interact with target analyte was immobilized on sensing surface and connected to source and drain electrode [164]. The FET-based biosensors are rapid, highly sensitive, specific, potential for miniaturization and make them promising in biosensor application. For instance, field effect transistor-based sensor probe was employed for the diagnosis of miRNA-155 biomarker in serum and cell line clinical samples [165]. Sensor probe consisted of MoS_2 flacks, coated on field effect transistor surface to monitor the capturing of target analyte miRNA-155 which generated the signal after interaction, which estimates the detection of miRNA-155. The developed biosensor exhibited dynamic detection range of 0.1 fM to 10 nM with LOD of 0.03 fM in both serum and cell line samples. Similarly, it was reported that the FET-based highly sensitive immunosensor which was made of ZnO based thin film transistor functionalized with EGFR monoclonal antibodies which displayed high sensitivity, selectivity and low LOD of 10 fM [166]. The role of the nano structure of ZnO-bio TFT immunosensor was to control the super hydrophilicity, which reduced the requirements of liquid sample and improved the sensitivity of biosensor. Similarly, the FET based biosensor constituted of reduced graphene oxide functionalized nanoparticle was reported for the simultaneous diagnosis of HER2 and EGFR BC biomarkers [167]. Herein, they constructed a biosensor by assembling the binding of oppositely charged surfaces of graphene oxide and 3-aminopropyltriethoxysilane (APTES) immobilized on gold surface conjugated with silicon oxide nanoparticles. They further reduced the graphene oxide to enhance the conductivity of the sensor. For the selective diagnosis of HER2 and EGFR, they immobilized the disease specific monoclonal antibodies (HER2-antibodies or EGFR-antibodies) on sensor surface. Biosensor had

good LOD of 1.0 and 100 pM for HER2 and EGFR respectively. A COOH functionalized multi-walled carbon nanotube with reduced graphene oxide-based field effect transistor was used to design flexible aptasensor as shown in Figure 5 (II) [168]. The electrode was made of poly methyl methacrylate (PMMA) which provided flexibility to the electrode. The developed FET sensor can effectively detect CA125 with good linearity of 1×10^{-9} to 1 U/mL with LOD of 5×10^{-10} U/mL in serum sample.

4.1.7 Chronocoulometry-based biosensor

The chronocoulometry is used to study the kinetics of chemical reactions, diffusion processes, and adsorption. In this technique, a potential step is applied to the electrode and the resulting cumulative charge versus time is observed. For instance, Ganguly et al. demonstrated chronocoulometry biosensor for diagnosis of miRNA-21 cancer biomarker. Herein, biosensors were constructed by electrochemical deposition of gold nanoparticles on MoS_2 surface placed on screen printed electrode. The electrode surface was modified by anti-miRNA-21 for selective capturing of miRNA-21 biomarker for detection. The monitoring of interaction of electrode and biomarker was observed by integration of $[Ru(NH_3)]^{+3}$ attached to oligonucleotides. In biosensor, thickness of MoS_2 played significant role by enhancing or diminishing the sensitivity of sensor. Hence, it was observed that sensitivity of sensor was inversely proportional to the thickness of MoS_2. It had the LOD of 100 aM [169].

4.2. Optical biosensors

Optical biosensors offer distinct advantage over traditional analytical technique in providing real-time and level-free detection of biological and chemical substances in a highly sensitive, specific and cost-effective manner. Optical-based biosensors have been developed for the diagnosis of various types of biomarkers. In principle, these biosensors work on the basis of emission of optical signal by absorption of light source. They are categorized as SERS, SPR, fluorescence, colorimetry and chemiluminescence. Their advantages such as real-time analysis, high sensitivity and selectivity, easy to use, result observed through naked eye in rapid manner make it promising in biosensor applications [85, 170]. For instance, Chen et al. reported a plasmonic biosensor made of gold nanorods for the rapid, sensitive and simultaneous dual detection of CA15-3 and bio-copper concentration in human serum [171]. The sensor showed the

LOD of 1.0×10^{-10} M for bio-copper in BC serum sample. In another report, Park et al. explored the detection of tumor assisted O-linked glycoprotein CA15-3 cancer biomarker based on lactin based optical biosensor platform [172]. This biosensor exhibited LOD of 1.2 and 0.4 U/mL for PNA and SNA respectively. Liang et al. specifically proposed ultrasensitive detection of p53 marker using fiber light coupled optofluidic waveguide (FLOW) biosensor. The proposed biosensors are based on interaction of flowed liquid sample and light emitted from device made of optical fibers. Moreover, interaction might have caused shifting of wavelength of transmission spectra. The biosensor recorded wide dynamic range of 10 fg/mL to 10 ng/mL (Figure 6 (I and II)) [173]. Similarly, Gohring et al. reported a label-free optofluidic ring resonator (OFRR) biosensor for the detection of HER-2 biomarker within 30 minutes with detection range of 13-100 ng/mL [174]. A label-free optical aptasensor was reported for the rapid diagnosis of mammaglobin protein (a BC biomarker) at very low concentration of 49 cells/mL. Herein, the optical biosensor was fabricated of gold nanoparticles functionalized with thiolated single strand DNA or RNA to make gold modified tilted fiber Bragg grafting (TFBGs) which displayed optical activity for the diagnosis of Mammaglobin protein with very low LOD with high specificity [175]. In a similar approach, the detection of HER2 BC biomarker in serum specimen as well as PBS was achieved through optical biosensor [176]. Herein, the biosensor was constructed through flame brushing technique in which tapering of a commercial optical fiber to interferometry sensor having length and diameter of 1.2 cm and 8 µm respectively. This constructed sensor was further hydroxyl functionalized using piranha solution followed by functionalization of amine group through APTES. The glutaraldehyde was used as cross linker for anti-HER2 antibodies attachment for specific detection of HER2 antigen. The biosensors detected the change of surface refractive index during the binding of selective analyte, which showed low sensitivity of 0.1 ng/mL in serum sample. Optical biosensors are rapid and have great potential for the diagnosis of target analyte. However it suffers with the low spectral resolution and required bulky and costly instrumentation.

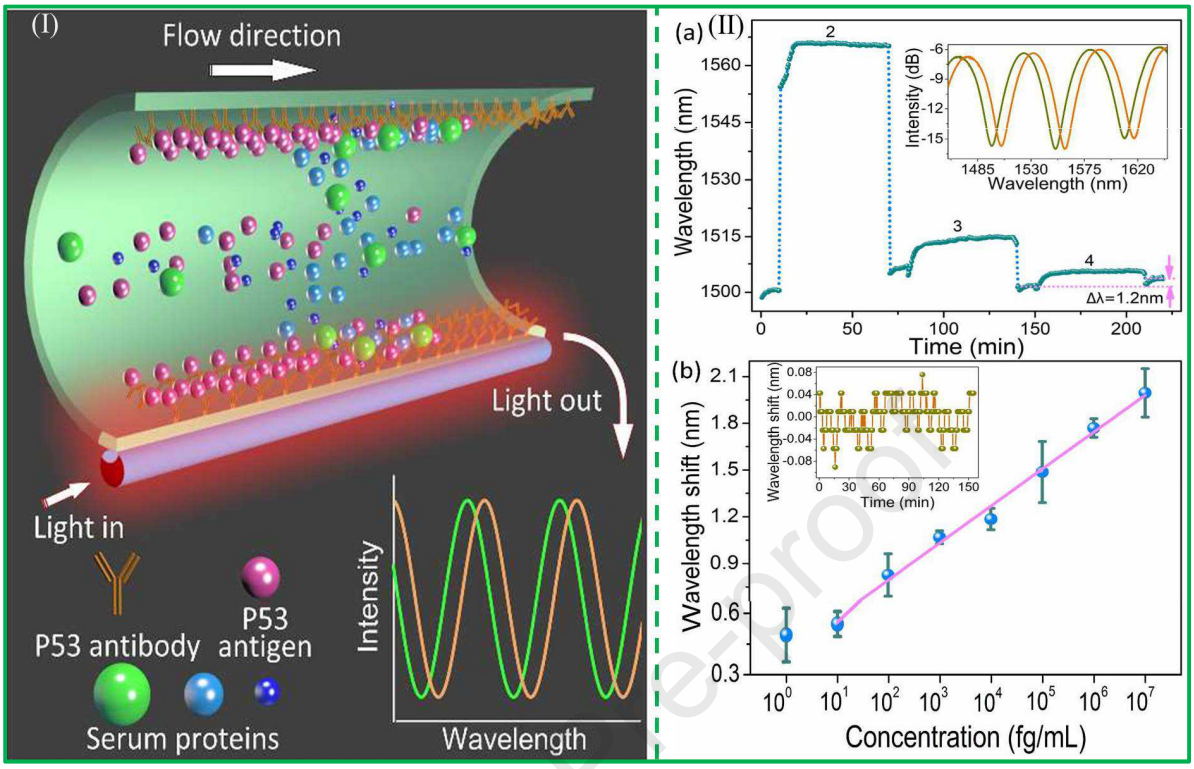

Figure 6: (I) Schematic of Fiber light-coupled optofluidic waveguide (FLOW) immunosensor. Reprint with permission from Ref. [173] (II) Sensorgram for surface activation and p53 detection. Inset: Recorded spectral shift induced by p53 bimolecular binding. (b) Optical response as a function of p53 concentration. Inset: Measured dip wavelength drift over 150 min. Reprint with permission from Ref. [173]

Colorimetric biosensor offers highly sensitive point-of-care detection of biomarker through color changes which could be visually observed by naked eye [177]. This technique can be used for detection of analyte in solution as well as on strips. Recently, a colorimetric biosensor was developed for selective detection of miRNA-21. Herein, the sensor surface was fabricated of silver and platinum nanoparticles with single strand DNA template. When target analyte interacted with sensing probe, blue colour was emitted to catalyzing of TMB and hydrogen peroxide through DNA-functionalized Ag/Pt nano sensor. Sensor showed linearity of 1 to 700 pM and LOD of 0.6 pM [178]. A molecular beacon incorporated with palindromic tail (PMB) was reported for quantitative colorimetric diagnosis of p53 cell marker. Here, PMB act as recognition electrode chip and polymerization mediator and contained C-rich probe. These C-

rich probes attached to hemin and converted to DNA enzyme. In addition, it has ability to oxidise the ABTS to hydrogen peroxide as a result of emission of colour. It exhibited wide linearity of 100 pM to 200 nM with low LOD of 10 pM (Figure 7) [179]. The resulting products, DNAzyme units, generated from the combination of polymerase/dNTPs with nikase implement the colorimetric signal amplification via catalyzing the oxidation of ABTS by H_2O_2 in the presence of hemin. The capability of PMB-based system to quantify target DNA. UV-Vis absorption spectra in the presence of various concentration of target DNA ranging from 0 to 300nM are taken. Yang et al. reported a polymerase chain reaction free sandwich biosensor for the detection of BRCA1. Herein, biosensor were constructed as tetrahedron structured reporter probe (TSRP), where three sites are modified with digoxin, while another one is by detection probe. The amplification of signal and enzyme loading was done by TSRP and further enhanced the monitoring of cancer cell. The LOD of the biosensor was calculated the 10 fM [180]. Lu et al. demonstrated a label-free two-photon scattering-based colorimetric biosensor of multifunctional oval shaped gold nanoparticle for the diagnosis of SK-BR-3 BC cell line. Herein, sensors were constructed by oval shaped gold nanoparticle functionalized with anti-HER2 antibodies and S6 RNA aptamer. When sensor binds to SK-BR-3 cell line, it was observed that the color changes from pink to bluish due to agglomeration of nanoparticles after interaction with target cell line. It has efficiency to detect 100 cells/mL. The presence of SK-BR-3 also indicates the presence of low concentration of HER2 which helps for early diagnosis of cancer. It was also found that when sensor interacts with MDA-MB-231 cancer cell line or HaCaT, which is a non-cancer cell line, there was no such property exhibited as a colour change, which was observed with respect to SK-BR-3. The sensor displayed two-time higher sensitivity than normal colorimetric assay as well as almost 13 times higher intensity is observed [181]. Xu et al. developed a biosensor which uses nano particles modified with antibodies against common BC biomarkers. This colorimetric biosensor utilizes BSA as stabilizer and thymophthelin, phenolphthalein and curcumin for ER, PR and HER2 biomarkers, respectively. The biosensor works on the principle of self-assembled monolayers formed by nanoparticles in solvent. Sodium hydroxide is used as indicator which gives different colors in presence of different biomarkers [182]. Colorimetric biosensors have some challenges like reproducibility, shelf-life, as well as false positive and negative result may causes limitation in biosensor application.

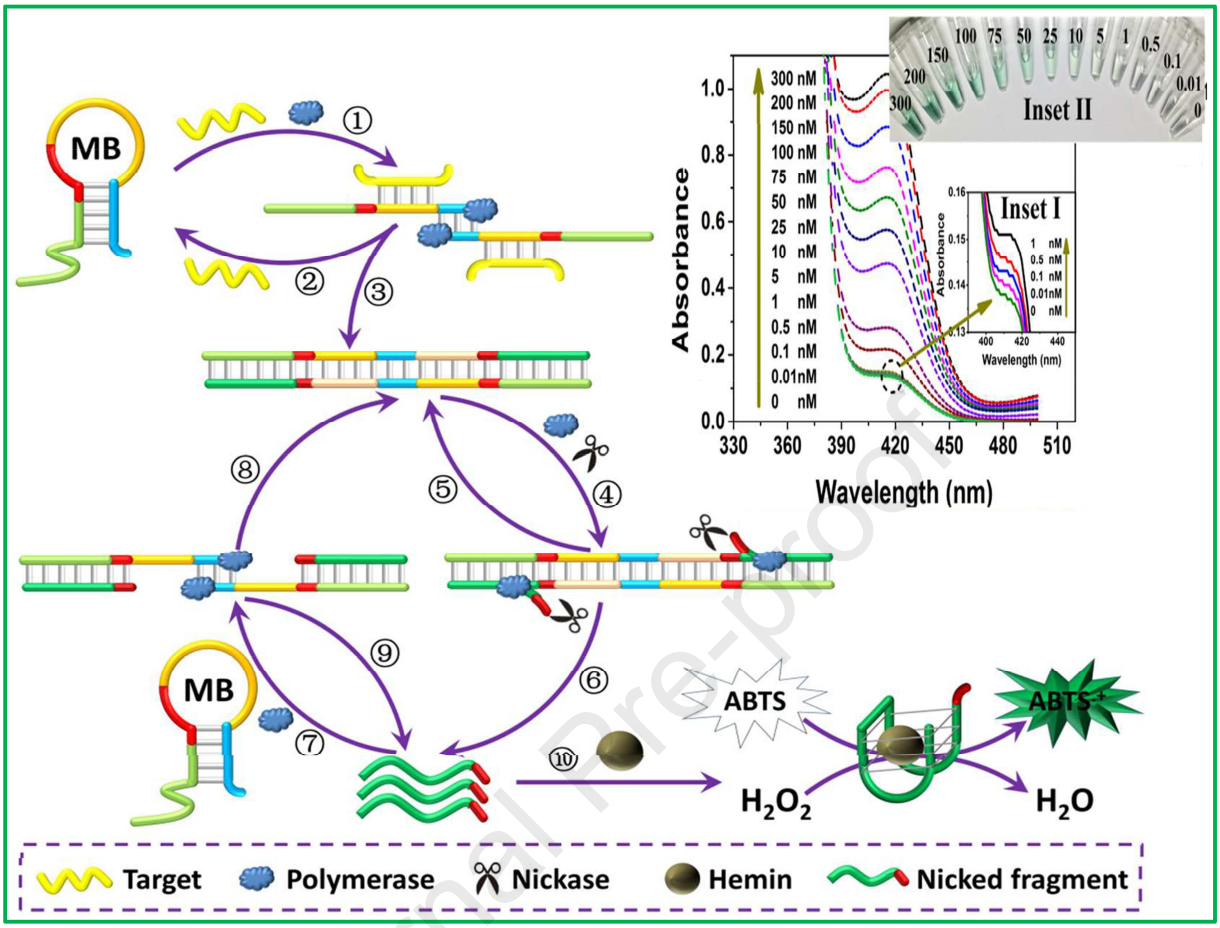

Figure 7: Scheme for the colorimetric detection of target p53 gene using a versatile PMB. The resulting products, DNAzyme units, generated from the combination of polymerase/dNTPs with nickase implement the colorimetric signal amplification via catalyzing the oxidation of ABTS by H_2O_2 in the presence of hemin. Reprint with permission from Ref. [179]

4.2.1 Surface Enhanced Raman Scattering-based biosensors

The SERS-based biosensors are a class of optical biosensor which works on the principle based on Raman spectroscopy. When a beam of laser light is focused on sample causes vibration in the molecules which recorded as spectral line. Even if the same type of molecule has distinct vibration level, it could be easily differentiated from each other. Moreover, the SERS has the ability to detect trace level of biomarkers through enhancing the signal intensity by using of Raman tag/Raman reporter. This could detect even single molecules with high sensitivity and selectivity [170, 183]. For instance, with the advantage of plasmonic material, a sensitive

biosensor was explored for the diagnosis of miRNA, which is based on monitoring by plasmonic coupling interference (PCI) also known as SERS-based technique [184]. Herein, the sensor probes were assembled on plasmonic silver nanoparticles labeled by Raman tag material to enhance the sensitivity of the probe. However, the coupling with the target miRNA to probe surface, resulted in the diminishing of signal intensity. In another contest, a SERS-based label-free biosensor was reported for the diagnosis of BC biomarker in tear specimen. They have fabricated a portable Raman spectrometer which can detect asymptomatic tumors and tumor recurrence with sensitivity of 92% and a specificity of 100%. The biosensor uses a multivariate statistics-based identification algorithm. The developed biosensor can detect metabolites in a range of fM using monolayers of gold nanospheres conjugated hexagonal-close-packed polystyrene (Au/HCP-PS) and equipped with a plasmonic SERS substrate [185]. A SERS chip was fabricated by immobilization of a single hexagonal close packed layer of polystyrene on silicon wafer via spin coating and with the help of e-beam evaporation. The gold layer was deposited on polystyrene surface to construct Au/HCP-PS plasmonic chip. Thickness of gold surface significantly contributes in enhancing the Raman intensity. They observed that the Raman intensity is maximum when the gold surface is around 150 nm thick. Through SERS chip, they analyze the BC biomarker in tear specimen. Similarly, SERS-based biosensor was reported which was fabricated by gold nanoparticles surface modified with ssDNA for the detection of CD24 and CD44 cell biomarker isolated from breast cancer cell line [186]. Recently, Xu et al. reported a label-free detection of two cancer cell (MCF-7 and MDA-MB-231) and one non-cancerous cell (MCF-10A) via dynamic liquid surface enhanced Raman scattering-based microfluidic biosensor [187]. Herein, microfluidics assay was assembled by Microsoft tube embedded 3D printing. However, silver nanostar mixed with the sample containing target analyte and dropped on microfluidic sample chamber which passed through channel whereas, silver nanostar improves the label-free SERS intensity Raman spectra. Zeng et al. reported a SERS imaging and NIR-triggered photo thermal therapy of BC by using modified nanoparticles [188]. Herein, the synthesized silver nanoparticle was coated with gold nanostar to construct Ag@Au core-shell structures. Further modified with DTTC, a Raman reporter, whereas gold nanostar reduced the cytotoxicity of silver nanoparticles. The Ag@Au nanostar-DTTC was further functionalized with mPEG-SH, to they enhance the SERS imaging and near infra-red triggered photo thermal therapy of BC. A SERS platform based biosensor was demonstrated for the

diagnosis of BRCA1, BC gene and their alternative splice variant exon 9, 10 and exon 5 [189]. A SERS-based DNA-Au-RTag fluorescent device was demonstrated for the diagnosis of alternating multiplexed splice variant of BRCA1 gene (different exons) with detection up to 1 fM [190].

4.2.2 Surface Plasmon Resonance-based biosensors

The SPR is widely used in biosensing application for the detection of cancer and other biomarkers. In principle, the analyte has been detected by determination of shift in refractive index (RI) of the material, when incident light interacts on material surface causes surface plasmon wave. It provided the binding and kinetic details of biosensor [170, 191]. In this category, surface plasmon resonance enhanced light scattering technique-based nano probes was reported for detection of miRNA-122 at ultra-low detection within 30 minutes [192]. Herein, the probes were fabricated by gold nanoparticles immobilized with monoclonal antibodies on gold surface to enhance selectivity. Biosensor achieved low LOD of 2 aM. The excellent plasmonic behavior of gold nanoparticles introduce as promising material in plasmon-based biosensor application. Similarly, an ovarian cancer (OC) biomarker CA125/Mucin16 was investigated by SPR imaging-based biosensor [193]. Herein, biosensor was fabricated by gold electrode followed by the immobilization of cysteamine and rabbit polyclonal antibodies for selective diagnosis with wide linear range of 2.2-150 U/mL in serum sample. Similarly, a SPR-based immunosensor was reported by Chung et al. for the early diagnosis of CA19-9 antigen [194]. Herein, they constructed a self-assembled monolayer of 11-mercaptoundecanoic acid on gold surface to detect CA19-9 with 410.9 U/mL. To further enhance the sensitivity of biosensor, there surface was modified by antibodies and consequently detection limit was improved to 66.7 U/mL.

4.2.3 Fluorescence-based biosensors

Quantitatively with high sensitivity as remarkable properties of fluorescent-based biosensor made them promising device for the detection of BC biomarker. For instance, several types of miRNA such as miRNA, miRNA-21, miRNA-155, miRNA-34a, and miRNA-1246 have been exploited as potential BC biomarker for early detection of BC. Various techniques have been explored for detection of miRNA in human serum sample or in other biological samples. In another contest, functionalized MoS_2 based fluorescent biosensor platform was utilized for the

diagnosis of miRNA-21 [195]. Herein, a dye containing ssDNA was immobilized on MoS_2 functionalized with folic acid–polyethylene glycol (ssDNA-MoS_2-PEG-FA). When the sensor interacted with the target analyte, the dye of fluorescent probe was detached and exhibited fluorescence activity. Similar, fluorescence-based strategy was reported by Li et al. for the detection of miRNA-21. They explored the detection based on induced nanocluster functionalized with terminal deoxynucleotidyl transferase (TdT) and duplex specific nuclease (DSN). When the target analyte interacts with hybrid material, they start releasing primer DNA and 3-hydroxy group by breaking of DNA with DSN to convert into long poly-T with the help of TdT to display fluorescent property of copper nanocluster. However, the analyte was not present in the sample, and hence it could not exhibit same properties due to lack of hybridization properties of functional materials. It could detect up to 18.7 pM of miRNA-21 biomarker in sample [196]. In similar approach, Ren et al. reported a FRET-based fluorescence probe for selective detection of miRNA-21 cancer biomarker. They synthesized single quantum dot modified with p19 protein, capture the specific miRNA-21 to improve sensitivity. The fabricated sensor displayed low LOD of 0.6 fM [197]. A fluorescence biosensor-based on FRET technique was developed by Nasirian et al. for the detection of miRNA-21 BC biomarker within 30 minutes in serum sample. Herein, the electrode was constructed by silver nanocluster modified with cytosine containing DNA moieties. To further improve the fluorescence intensity of electrode, it was modified with Cy-5.5. After capturing the selective miRNA on modified electrode, they display red fluorescence and quantitatively calculated that they had detection range of 0.02 to 100 nM with low LOD of 4×10^{-3} nM [198]. Furthermore, a fluorescence-based biosensor was demonstrated for detection of miRNA-1246, BC biomarker in plasma exosomes within few hours. Herein, they utilized a gold nanoflower modified with nucleic acid as a fluorescent probe which penetrated into plasma exosomes to exhibit fluorescence activity (Figure 8) [199]. Recently, fluorescence-based optical sensor was explored for the early detection of CA125. This biosensor was made of a fluorescence active phthalocyanine (Pc) with Ni compound to make NiPc complex doped in polystyrene to synthesize fluorescent film. To measure the change in intensity of fluorescent film with respect to different concentrations of CA125 biomarker and calculated the LOD of 1.0×10^{-4} U/mL in serum [200]. A sandwich assay-based fluorescent biosensor was proposed for the detection of p53 biomarker. Initially, cadmium sulphide was coated on GCE and then immobilized the antibodies on surface. On the other hand,

secondary antibodies were immobilized on graphene oxide/gold nanoparticles composite. Moreover, the secondary antibodies increase the selective binding with p53 marker and facilitated the luminescent properties. The biosensor displayed linearity of 20 to 1000 fg/mL with LOD of 4 fg/mL in serum sample [201]. Recently, p53 biomarker was screened with a DNA immobilized on magnetic nanoparticle-based fluorescent platform. They fabricated dye as fluorescent probe and DNA in place of enzymatic or antibodies-based strategy. The proposed biosensor had wide linearity range of 50 pM to 2 nM with low LOD of 8 pM [202]. Li et al. reported a biosensor probe-based on bio-photonic nanomaterial constructed of polydopamine nanospheres and DNAaseito were demonstrated for the detection of miRNA-21 with wide detection range of 1 pM to 10 nM with ultralow LOD of 55 fM [203]. In another study, a label-based oligonucleotide hybridized luminescent probe was reported for the detection of miRNA-21 in cell sample. Herein, the probe was labeled with free miRNA-21 and rluc (R. luciferase)-labeled miRNA-21. The probe exhibited the LOD of 1 fM in cell sample [204]. Rafiee et al. developed a biosensor which was based on a programmable DNA hybridization chain reaction (HCR) circuits. It utilizes an elicitors DNA coupled with anti-HER2 antibodies which on binding with HER2+ circulating cancer cells initiates the HCR cascade, and it gives an amplified fluorescent signal. Although the biosensor has some limitations such as low sensitivity and background noise [205]. Luo et al. developed a novel biosensor which detects cancer at double level to improve the accuracy in cancer diagnosis. The biosensor employs a DNA molecular beacon which stimulates the catalytic hairpin assembly-mediated Y-junction nicking enzyme assisted signal amplification (CHA-YNEASA) circuits to give dual signal amplification, when p53 gene is detected. The biosensor can be modified into a cytosensor to detect MCF-7 cells using the same circuits with MUC1 aptamer. The biosensor has LOD of 0.9 pM for p53 gene and 100 cells/mL for cancer cells [206]. Over the advantage of fluorescence biosensor, it suffer with the high background signal as well as the own fluorescence property of biological analyte.

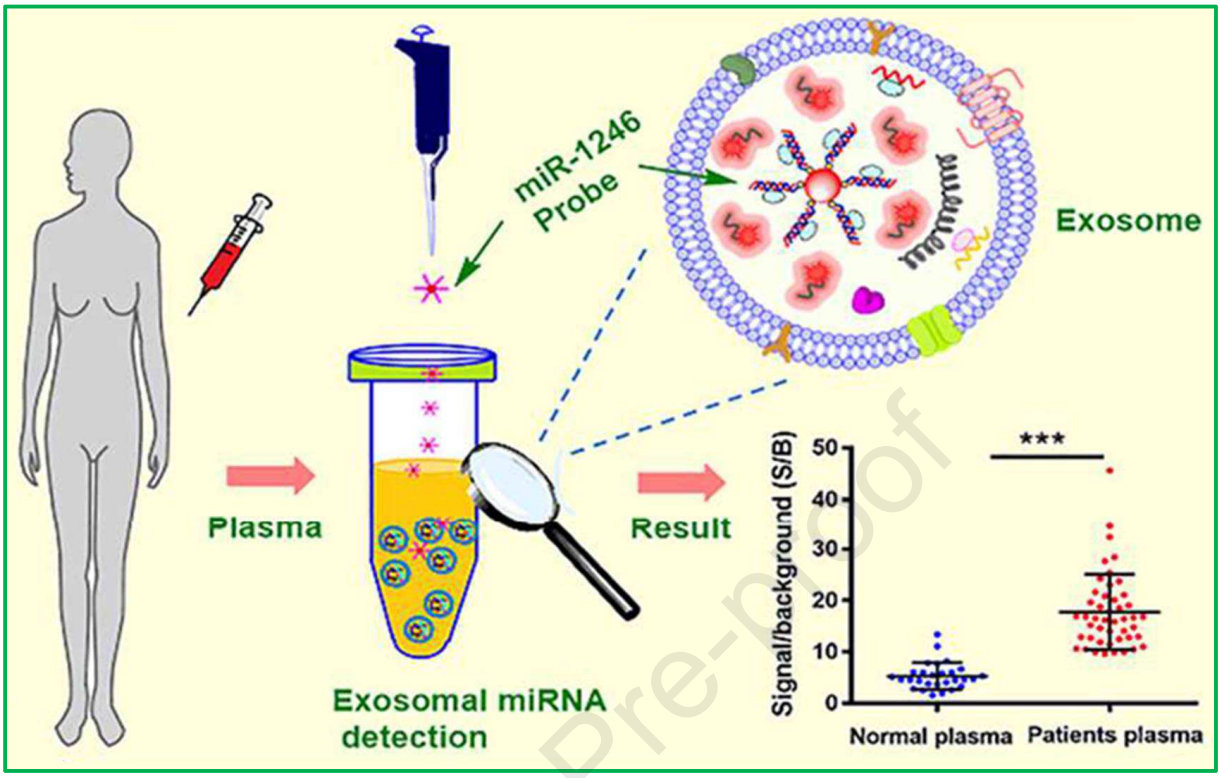

Figure 8: Schematic representation of diagnosis of plasma exosomal MicroRNA-1246 BC biomarker. Reprint with permission from Ref. [199]

5. Microfluidic-based Biosensors

In microfluidic device, the micro channels have been constructed on chip and modified by diseases specific antibodies through which target analyte has been detected. In mechanistic study, analyte containing small sample volume were placed on sample zone of chip, which flow through channel due to capillary action and separated, and detected at detection zone. In addition, microfluidics-based biosensors are high sensitive, specific and could be detected multiplex analyte in a single biosensor platform. The miniaturize nature, small sample volume with no need to pre-treatment of clinical sample as well as the analyte separation, mixing, and detection process have been done in a single chip make it promising to make in point-of-care application. Microfluidics chip are single use, portable, low cost and disposable device. Moreover, it can easily integrate with other techniques like electrochemical and optical to make it highly sensitive biosensors and could be detected up to a single cell in biological sample [207-211]. In this regard**,** an electrochemical technique-integrated microfluidic biosensor was utilized

for the early detection of EGFR2 or ErbB2, BC biomarker. The T-shaped microfluidic channel was made of porous graphene foam which on carbon doped $nTiO_2$, a semiconductor was immobilized on PDMS substrate. Since due to porous nature of graphene foam, it is easy to bind with $nTiO_2$, which enhance the impedimetric behavior of sensor. Further, electrode surface were functionalized with anti-ErbB2 antibodies for specificity of biosensor. The impedance and DPV measurement revealed that the linearity of biosensor to be 0.1 pM to 0.1 µM and 1.0 fM to 0.1 µM with sensitivity of 43.7 kΩ μM^{-1} cm^{-2} and 0.585 µA μM^{1} cm^{-2} respectively for ErbB2 protein marker [212]. In another report, microfluidic assay was demonstrated for the diagnosis of Glypican-1, an exosome protein which was extracted from MDA-MB-231 BC cell line in serum sample. For this purpose, the magnetic beads were functionalized with capture antibodies CD63. When functionalized magnetic bead is combined with exosome suspension, the magnetic beads serve as capture as well as separation of exosome, however, anti-GPC-1 antibodies serves to selectively bind to target analyte to form immunocomplex. Further β-Galactosidase, an enzymatic substrate which binds to detection of antibodies can catalyze the fluorescein-di-β-galacopyranoside to radiate fluorescent signal. These signals are count via microfluidic system to generate quantitative data and found to be detection of 10 exosomes µ/L in serum sample [213]. Recently, LSPR enable microfluidics chip was demonstrated for detection of multiple cancer biomarkers (CA153, CA125, CEA, ErbB2) in a single biosensor. Herein, the synthesized gold nanorods surface was modified with self-assembly monolayer of mercaptoundecanoic acid. Further, by the utilization of soft lithography, these modified electrodes were combined with microfluidic device. For the purpose of multiplex detection of biomarker, their different antibodies of biomarker are immobilized on modified electrode surface. The self-life of chip was up-to 3 months when stored in dark place [214]. A sandwich assay microfluidic device integrated with SERS technique was demonstrated for the diagnosis of multiplex biomarker (CEA, CA125 and CA153) of BC in serum samples. Initially, the inner surface of microfluidic channel was coated with silver nanoparticles, which serve as SERS material followed by immobilization of specific antibodies (anti-CEA, anti-CA125 and anti-CA153 antibodies) over it. Further, the target biomarkers are flowed through microfluidic channel which captured specific antigen. In next step, after capturing of antigens on antibodies, the SERS probe which was constructed of silver nanoparticles conjugated with Raman reporter and specific antibodies were injected through the channel. Moreover, the Raman reporter mixed with Ag nanoparticles and antibodies are specific

for each antigen such as 2NAT (2-naphthalenthiol) for CEA antigen, DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] for CA125 antigen and 4MBA (4-mercaptobenzoic acid) for CA153 antigen. The LOD was found to be 1 pg/mL, 0.01 U/mL and 0.01 U/mL for CEA, CA125 and CA153 respectively (Figure 9) [215]. An electrochemical integrated microfluidic channel-based aptasensor was developed for the diagnosis of interferon-γ (cytokine) secreted from the cell. The microfluidic channel was constructed by PDMS mould to make two layers on glass surface which was coated with gold nanoparticles**.** The SWV exhibited the detection of interferon-γ with LOD of 5 ng/mL [216]. Li et al. reported the aptasensor and enzyme containing microfluidic device for the diagnosis of cell membrane protein tyrosine kinases-7 (PTK7), a prognostic biomarker for BC. In the proposed device, a solution of PTK7, aptamer of hairpin probe and nicking endonuclease Nb.BbvCl enzyme were flowed through microfluidic channel. Fluorescence spectra determine the LOD of PTK7 as 0.4 nM and they found that the aptamer selectively binds with target analyte while enzyme enhances the fluorescent properties of the biosensor [217].

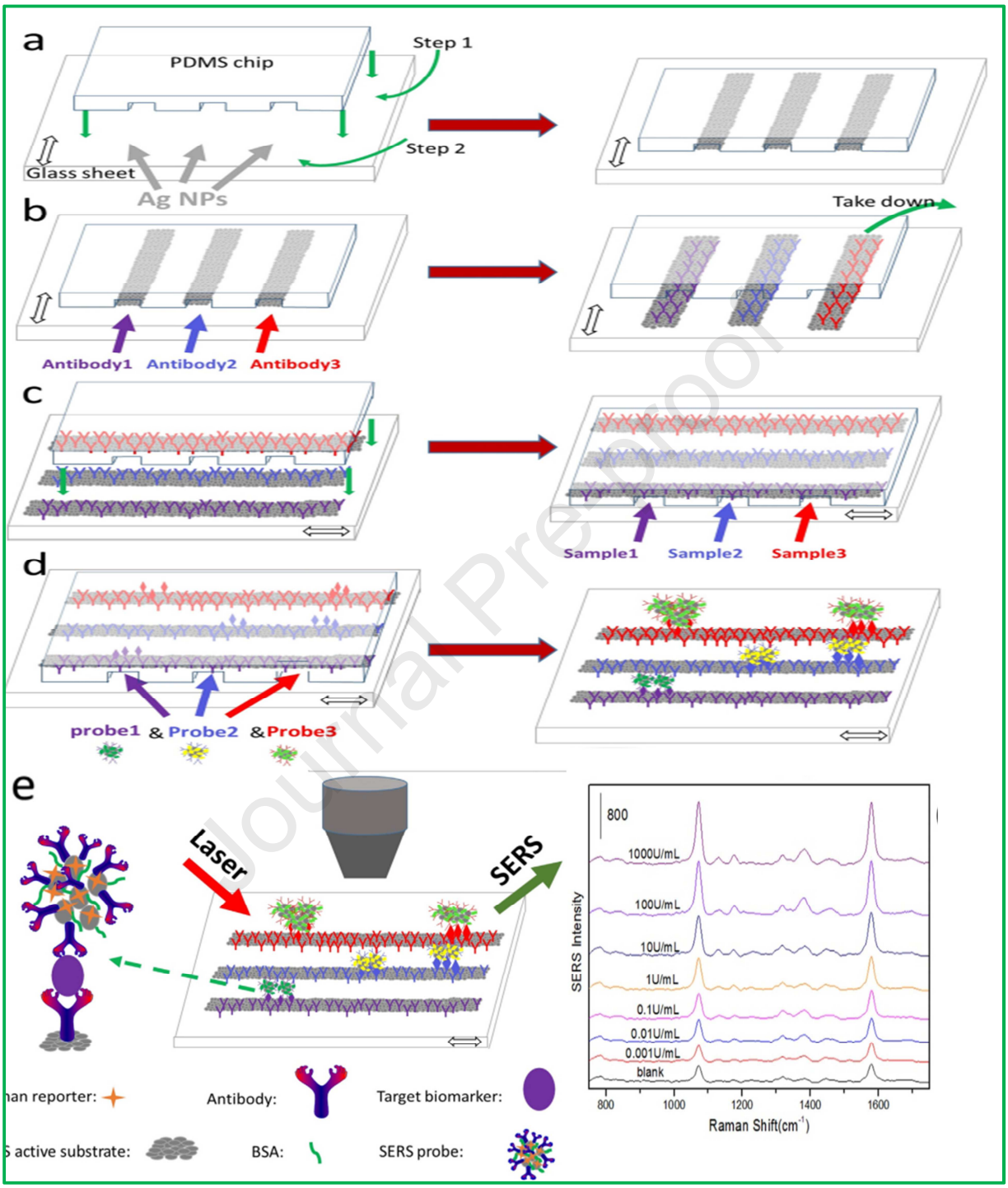

Figure 9: (a) Microfluidic system. (a) Polydimethylsiloxane (PDMS) chip bonded to the glass sheet. (b) Different antibodies were injected into the AgNPs modified microfluidic channels via syringe pumps. (c) PDMS chip removed and a new PDMS chip bonded to the glass sheet. (d)

SERS probes injected into the microfluidic channels. (e) Microfluidic channels and SERS signal of each crossing region. Reprint with permission from ref. [215]

In another study, A microfluidics device based on alternating current (ac) electrohydrodynamics (ac-EHD) induced surface shear forces technique was utilized for the colorimetric detection of multiple biomarkers like HER2, PSA and IgG in serum sample. This device contains gold electrode containing three channels, each is functionalized with their specific antibodies (anti-HER2, anti-PSA and anti-IgG) and the anti-fluorescent HRP was functionalized with antibodies which make it advantageous that it could be easily observed through naked eye. When TMB passed through the channel, the catalytic activity takes place which generates the fluorescent signal, and the as colour change in chip can be seen by naked eye (Figure 10 (I and II)) [218]. Similarly, a sandwich type fluorescent microfluidic device was demonstrated for the diagnosis of dual biomarker CEA and AFP in a single chip platform. Herein, they utilized quantum dots, since they have great fluorescent properties and biocompatibility. For this purpose, they synthesized quantum dots of CdTe/CdS and functionalized with goat anti-mouse IgG antibodies. However, microfluidic chip was modified with respective antibodies (anti-CEA and anti-AFP). The solution of functionalized QDTs was passed through channel. After capturing of antigens on their respective antibodies, colour change was observed. They found that the red colour displayed after CEA binding to anti-CEA while green colour due to AFP binding to anti-AFP, which quantitatively achieved LOD of 250 fM in serum sample [219]. The techniques such as, electrochemical and SPR integrated with microfluidic-based biosensor was reported for the diagnosis of ErbB2 biomarker. Herein, they fabricated the microfluidic chip by gold and graphene oxide based nanocomposite followed by the immobilization of anti-ErbB2 antibody. Gold NPs and graphene oxide nanocomposite are compatible with electrochemical and SPR techniques in biosensing applications. In amperometric detection of ErbB2, they estimated the linearity of 1 fM to 1 µM with LOD of 1 fM, while in SPR detection the linearity of biosensor was 0.1 to 1000 pM and 1×10^3 to 1×10^5 pM with LOD of 10 pM (Figure 10 (III)) [220]. Chen et al. also developed a biosensor which utilizes the microfluidic technology to detect cancer cells. The 3D printed biosensor uses anti-EpCAM (epithelial cell adhesion molecule) antibodies which capture EpCAM positive cancer cells. The high surface area and fluid flow manipulation, and

increasing capture efficiency was achieved using novel and specially designed interior structures through 3D printing technology [221].

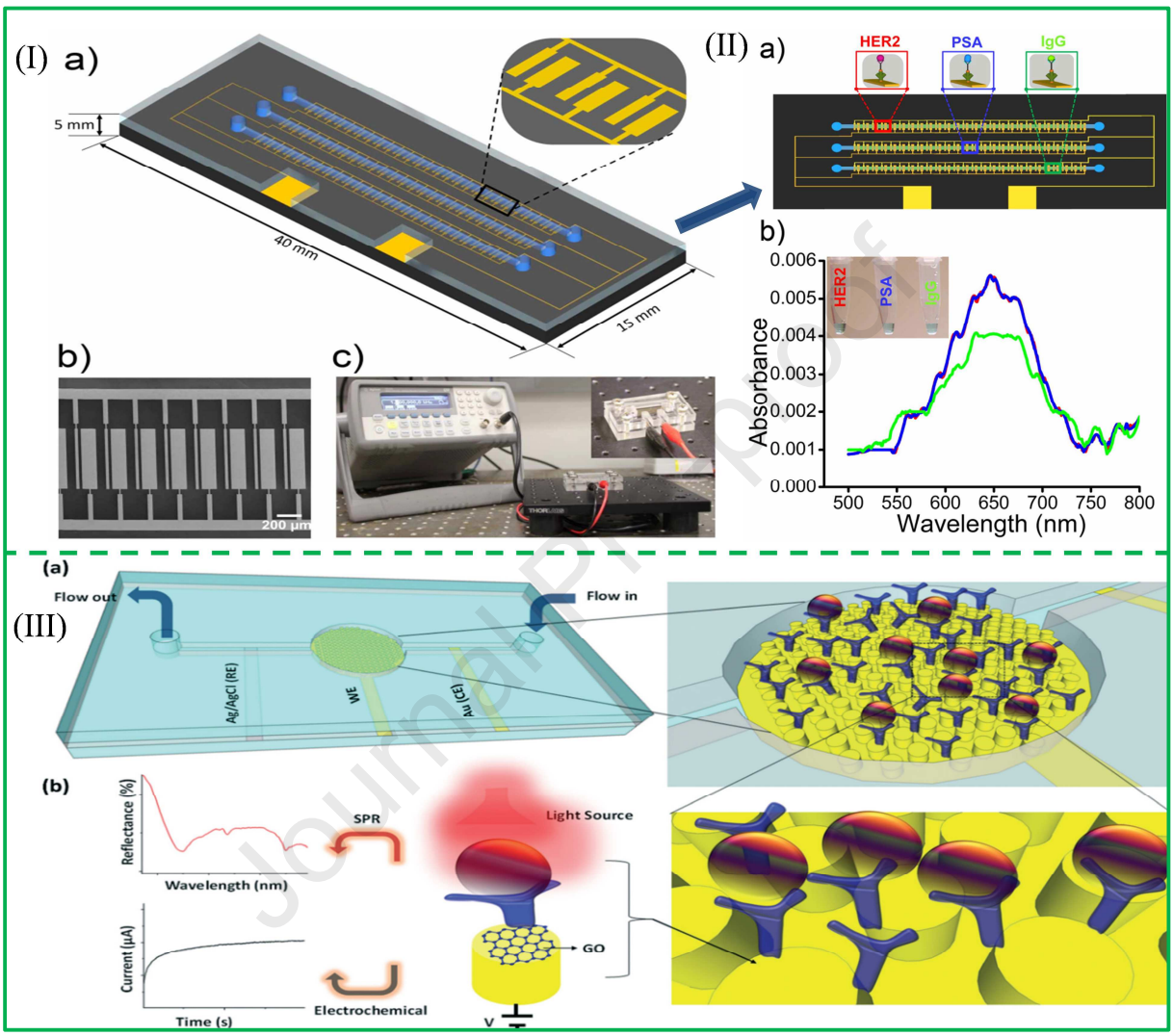

Figure 10. (I) Multiplexed nanoshearing device: (a) Multiplexed microfluidic device for protein biomarker detection. (b) SEM of enlarged segment of the device. (c) Protein biomarker detection using a multiplexed ac-EHD device. Reprint with permission from Ref. [218] (II) (a) Detection of multiple protein biomarkers. (b) UV-Vis absorption spectra of serum samples spiked with non-target proteins. Reprint with permission from Ref. [220] (III) (a) Integrated dual modality microfluidic sensor chip and antibody immobilization at plasmonic nanoposts (b) Integrated dual-modality sensor operation. Reprint with permission from Ref. [220]

6. Summary and Future Perspectives:

The detection of various cellular biomarkers associated with the development and progression of cancer holds promise for early detection of cancer. Several techniques such as polymerase chain reaction (PCR), RTPCR, ELISA etc., are available for the detection of biomarkers. Certain limitations of these techniques viz., expensive setups, critical data analysis and incapability of multiplexing hamper their applicability and adaptability. Considering early diagnosis of cancer as prime requirement to provide effective treatment on time, there is an urgent need for a technology which provides rapid, affordable, easy to use, reliable, highly sensitive platform. In this regard, biosensor-based platform for early detection of cancer provides a new ray of hope in the field of cancer screening as they offer several advantages over the existing molecular technologies viz., high throughput, non-invasive, cost-effective, easy to handle, and can be multiplexed for simultaneous detection of biomarkers. In this review, genomic, transcriptomic, proteomic and metabolomic biomarkers associated with BC, which can be explored to design biosensors for early detection of BC are discussed. Furthermore, extensive information about various biosensors viz., electrochemical, optical and microfluidic-based, which are available for the diagnosis of BC are evaluated. The optical biosensors are highly sensitive and rapid but they exhibit constraints of low resolution/intensity of spectral signals which significantly appear as false positive results. In this context, especially electrochemical and microfluidics-based biosensors provide the platform for the diagnosis of ultra-low concentration of cancer biomarkers. Among these, electrochemical-based biosensors are emerging, affordable, easy to use, rapid, real-time monitoring with high specificity, selectivity, devoid of sample pre-treatment with an efficacy to detect analyte concentration at femtomolar level. Moreover, microfluidics approaches have ability to integrate electrochemical and optical techniques which significantly enhances the sensitivity of device. Furthermore, it could simultaneously detect multiple biomarkers on a single chip device. These integrated multiplexed biosensors specifically designed for BC biomarkers can be used at very early stage through population screening via regular health checkup. These POC devices are cost-effective and does not require expensive infrastructure and expertise. With timely mass screening of disease one can assess its risk of occurrence in vulnerable populations; and also help in specific precision medicine/personalized treatment, therapy prediction and prognosis. Most of the biosensors presently available are designed to detect either one or two biomarkers simultaneously. However, multiplexing

enhances the amount of information gathered from single sample and provides information of sample profiling. The multiplexing enhances the sensitivity and specificity of disease detection. The combination of two or more markers of low predictive power individually can be exploited to get high predictive power in combination. In this context, recent advances in nanotechnology (carbon dots, carbon nanotubes, graphene, magnetic nanoparticles etc.), and microfluidics-based lab-on-chip devices open new avenues in the field of novel approaches for designing miniaturized, multiplexed and fully automated biosensors for disease diagnosis. These high throughput, portable, and cost-effective approaches provide enhanced sensitivity and low LOD along with potential for multiplexing of several biomarkers on a single platform, they can be explored for the optimization of preclinical, clinical and toxicological studies and also help in drug development strategy. Further, automation of the device with capacity to modify it for newly discovered cancer biomarkers along with its miniaturization for point of care testing of disease have lot of scope for future research. There is need of such devices for POCT in resource limited settings. The major challenge is to make these devices available at affordable prices in adequate numbers for POCT and continuous health monitoring in developing countries. Furthermore, this need scale up of potential devices along with networking for data transfer and storage to reduce global health burden. However, most of the microfluidic devices are PDMS-based due to their easy prototyping and amenable properties, which limits their mass production. This constraint can be overcome using, 3D printing technologies for mass manufacturing and fabrication of microfluidic devices. In this regard, high resolution and precision can be achieved using photopolymer jetting 3D printers. Nevertheless, the 3D printing technology offers great potential and several advantages in mass production of microfluidic devices, but the choice of resin material and printing resolution may limit their applicability. Biocompatibility and commercial availability of different types of printable materials which exhibit good mechanical, electrical, chemical and optical properties is another factor to restrict the device manufacturing. It is pertinent that, despite several advantages, the developments of 3D printed microfluidic devices are still not prevalent. However, advances in technology and availability of desired printable materials have potential to overcome the limitations for development of lab-on-chip device fabrication.

Another challenge while designing biosensor is to store and process biological samples (serum, plasma etc.) as they are highly perishable and therefore require proper storage facility before

testing. In addition, some biomarkers have low specificity and tendency to serve as biomarkers for more than one cancer type. This can be overcome using biosensor-based approach which can detect multiple biomarkers in a single device platform with high specificity and desirable sensitivity. This would also help to understand the role of biomarkers in disease development and progression and also shed light on the role of one altered parameter over other with respect to normal physiology of body. Recent technological advancements with respect to biosensor-based device fabrication for early cancer diagnosis have potential for easy and economically feasible treatment options for society. Therefore, it is anticipated that, this review will help researchers to design and develop potential biosensors as point-of-care devices for the detection of BC biomarker in early stage which would pave the way for enhanced cancer prognosis and therapeutics.

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Table 1: List of biosensors and potential Biomarkers associated with breast cancer.

S.no.		Biomarkers	Type of Cancer	Biorecognition Element	Amplification Method	Assay Type	Limit of Detection	References
1.	Electrochemical a). Cyclic Voltammetry (CV)	Human Epidermal Receptor 1 (HER1)/c-erbB1 EGFR	Breast Cancer, Non-Small Cell Lung Carcinoma (NSCLC)	Antigen-antibody	Immobilized anti-EGFR antibodies on dithiobisuccinimidyl propionate (DTSE) self-assembled monolayer (SAM) on gold (Au) electrode.	Label free assay	1 pg/mL	[93]
		Human Epidermal Receptor 3 (HER3)	Breast Cancer Pancreatic Cancer, Gastric Cancer, (NSCLC), Colorectal Cancer	Antigen-antibody	Anti-HER-3 antibody attached on Cysteamine to gold nanoparticles to hexanedithiol on gold electrode surface.	-	0.2-1.4 pg/mL	[94]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer	Antigen-antibody	HRP linked Ab	Sandwich assay	4 ng/mL	[95]
	b). Differential Pulse Voltammetry (DPV)	miRNA-155	Breast cancer		Thiol functionalized-Au nanorods/graphene oxide		0.6 fM	[98]
		Human Epidermal Receptor 1 (HER1)/c-erbB1/ EGFR	Breast Cancer, Non-Small Cell Lung Carcinoma (NSCLC)	Antigen-antibody	Ferro oxide/ chitosan/ gold nanoparticle	Sandwich assay	0.05 pg/mL	[99]
		Human Epidermal Receptor (HER2)/neu/c-erbB2 EGFR	Breast Cancer, Gastric Cancer	Aptamer	Aptamer DNA primer	Sandwich assay	50 ng/mL	[100]

		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer	-	Poly (1, 5-diaminonaphthalene) and polypyrrole coated GCE	Sandwich assay	0.02 U/mL	[101]
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		HRP 2-encapsulated liposomes	Sandwich assay	5 µU/mL	[102]
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		Ab 3/ferritin/multiwall carbon nanotube	Sandwich assay	0.009 U/mL	[103]
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer,		Modified magnetic nanoparticles	Sandwich assay	2.8×10^{-4} U/mL	[104]

			bladder Cancer, Gastrointestinal Cancer					
		CA-125	Ovarian Cancer Breast cancer	Aptamer-cDNA	Amidoxime modified polyacrylonitrile nanofibers decorated with Ag nanoparticles (AgNPs-PAN-oxime Nfs)	-	0.0042 U/mL	[105]
		CA-125	Breast cancer, Ovarian Cancer	Antigen-Antibody	Silver nanoparticles modified graphene quantum dots ink (Ag NPs-GQDs)		0.01 U/mL (LOQ)	[106]
		miRNA-21	Breast cancer		Au/rGO hybrid nanocomposite	Sandwich	5 fM	[107]
		miRNA-155	Breast cancer		Au@MoS_2 nanocomposite		0.32 fM	[108]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer		Ferro ferricyanide	Label free assay	1.6 ng/mL	[109]
		HER2	Breast Cancer		CdSe@ZnS quantum dots	-	2.1 ng/mL	[110]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer		Horse Radish Peroxidase labeled DNA gold nanoparticles	Sandwich assay	4.9 ng/mL	[111]
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer,		-	Label free assay	0.012 U/mL	[112]

			bladder Cancer, Gastrointestinal Cancer					
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		Toluidine blue on gold electrode surface	Direct assay	0.10 U/mL	[113]
		CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		2-aminophenol on gold electrode surface	Direct assay	1.5 U/mL	[114]
		CA125, CA-153, CEA	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		Graphene sheet on Platinum nanoparticles	Sandwich assay	0.002 U/mL for CA-125, 0.001 U/mL for CA-153, 0.007 U/mL for CEA	[115]

		CA 242	Breast Cancer, Pancreatic Cancer, Gall Bladder Cancer, Digestive Tract Cancer		Reduced graphene oxide (rGO) gold-palladium nanocomposite.	Label free assay	1.54 X 10^{-3} U/mL	[116]
		BRCA1	Breast and Ovarian Cancer	Nucleic Acid	Self-assembled ferrocene-cored poly (amidoamine) dendrimers	Label free assay	0.38 nM	[117]
		BRCA1	Breast cancer	DNA	DNA immobilized on Zwitterionic peptide modified poly(3,4-ethylenedioxythiophene) PEDOT		0.03 fM	[118]
		CEA, AFP			Chitosan functionalized Au electrode		0.1 ng/mL for CEA, 0.05 ng/mL for AFP	[119]
		H1047R	Breast cancer		DNA modified Au nanoparticles on paper strip		5 nM (signal ON approach) 6 nM (signal OFF approach)	[120]
		VEGFR2	Breast cancer		Thionine-reduced graphene oxide-Chitosan composite	Sandwich	0.28 pM	[121]
	c). Square Wave Voltammetry (SWV) & Linear Sweep Voltammetry (LSV)	CA 242	Breast Cancer, Pancreatic Cancer, Gall Bladder Cancer, Digestive	Antigen-Antibody	Labeled antibodies attached to azide functionalized dsDNA as signal enhancer.	Sandwich assay	20.74 µU/mL	[123]

			Tract Cancer					
		CA-125	Breast cancer, Ovarian Cancer	Antigen-Antibody	Cysteamine capped gold nanoparticles-electrophoretic reduced graphene oxide (Cys-AuNPs/ERGO)	-	0.1 U/mL (LOQ)	[124]
			Breast cancer, Ovarian Cancer	Enzyme based	Redox polyaniline-polythionine hydrogel (PANI-PThi gel)	Label free assay	0.00125 U/mL	[125]
		p53	Breast Cancer, Colorectal Cancer	Antigen-Antibody	Poly I-cysteine, graphene quantum dots, gold nanoparticles (P-Cys/GQDs/AuNPs) multilayered film based	-	12.1 fM	[126]
		p53	Breast Cancer, Colorectal Cancer	Antigen-Antibody	Biotin conjugated p53 antibody on nanocomposite poly I-cysteine, 3D gold nanoparticles (P-Cys/AuNPs)	-	48 fM (SWV) 18 fM (DPV)	[127]
		miRNA-21, miRNA-155	Breast cancer		DNA probe	-	18.9 aM for miRNA-21 39.6 aM for miRNA-155	[128]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer	Aptamer	Aptamer DNA primer	Sandwich assay	0.047 pg/mL	[129]
		CA-125	Breast cancer, Ovarian	Gold Electrodes	Molecular Imprinting Polymers (MIP) on gold	Direct assay	0.01 U/mL	[130]

			Cancer		electrodes			
		IL6	Breast cancer		PDDA functionalized Boron Nitride-gold nanocluster		1.3 pg/mL	[131]
		CA15-3, HER-2 ECD	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		Gold nanoparticles	Label free assay	5.0 U/mL for CA 153 2.9 ng/mL for HER2-ECD	[132]
	d.) Amperometry	CA 242	Breast Cancer, Pancreatic Cancer, Gall Bladder Cancer, Digestive Tract Cancer	Antigen-Antibody	Redox sodium alginate-Pb2+-graphene oxide (SA-Pb^{2+}-GO) hydrogel.	Label free assay	0.067 U/mL	[135]
		CA-125	Breast cancer, Ovarian Cancer	Enzyme based	Chitosan-gold nanoparticle/multiwall carbon nanotube/graphene oxide (CS-AuNP/MWCNT/GO)	Sandwich assay	0.002 U/mL	[136]
		miRNA	Breast cancer		DNA-RNA modified Magnetic bead on SPCE	-	2.4 pM	[137]
		TP53	Breast cancer		Reduced graphene oxide –carboxymethylcellulose hybrid nanocomposite		3.4 nM (sccp53 probe) 2.9 nM(lcpp53	[138]

							probe)	
		CD9, CD24, CD44, CD54, CD63, CD81, CD326, CD340	Breast cancer		Magnetic particles	-		[139]
		p53	Breast Cancer, Colorectal Cancer	Antibody	Magnetic microcarriers (MBs) modified with immobilized Halotag fusion p53 protein.	-	-	[140]
	e). Impedimetric (EIS)	Human Epidermal Receptor 1 (HER1)/c-erbB1 EGFR	Breast Cancer, Non-Small Cell Lung Carcinoma (NSCLC)	Receptor-Ligand	Ferrocene bead coupled with peptides	Label free assay	0.37 ng/mL	[143]
		EGFR	Breast Cancer	Antigen-antibody	Cysteamine, PDITC (1, 4-phenylene diisothiocyanate) and protein-G modified gold nanoparticles	Label free assay	0.34 pg/mL in PBS 0.88 pg/mL in human plasma	[144]
		CA-125	Breast cancer, Ovarian Cancer		Polyaniline modified Graphene SPE system	Label free assay	0.92 pg/ml-15.20ng/ml	[145]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer	-	Cysteine modified Gold nanoparticles	Label free assay	6 µg/L	[146]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer	Aptamer	DNA aptamer	Label free assay	1 pM	[147]
		BRCA1	Breast Cancer, Ovarian Cancer	DNA	ss 19-mer oligonucleotide Complimentary DNA	Label free Assay	1.72 fM	[148]

					immobilized on Polyethylene glycol film-gold nanoparticles (PEG-AuNPs)			
		miRNA34a	Breast cancer		PAMAM dendrimer	-	0.95 µg/mL in PBS 0.52 µg/mL in FBS:PBS	[149]
		miRNA-155	Breast cancer		Au/thiol functionalized succinic acid on SPE	-	5.7 aM	[150]
		Human Epidermal Receptor 3 (HER3)	Breast Cancer, Pancreatic Cancer, Gastric Cancer, (NSCLC), Colorectal Cancer	Antigen-antibody	Self-assembled monolayers (SAMs) of 4-aminothiophenol on gold electrodes.	-	0.4-2.4 pg/mL	[151]
		p53	Breast Cancer, Colorectal Cancer	Antigen-Antibody	Star-shaped poly(glycidylmethacrylate) (starPGMA) with epoxy side group coupled with anti p53 antibodies	Label free assay	7 fg/mL	[152]
	f). Capacitive	Human Epidermal Receptor 4 (HER4)	Breast Cancer, Uterine Cervix, Prostate Cancer, Gastrointestinal Cancer	Protein-Affimer	Anti-HER-4 affimer with C-terminal cysteine self-assembled on gold interdigitated electrodes	-	1 pM	[153]

		CA 19-9	Breast cancer , Lung Cancer, Ovarian Cancer, Pancreatic Cancer	Antigen-Antibody	Interdigitated gold electrodes coated with self-assembled monolayer of anti-CA 19-9 antibodies.	-	0.68 U/mL	[154]
		CA 19-9	Breast cancer, Lung Cancer, Ovarian Cancer, Pancreatic Cancer	Antigen-Antibody	Screen printed interdigitated electrodes (SPIDEs) modified by carbon nano-onions (CNOs) and graphene oxide (GO) (SPIDEs/gold nanoparticles labelled detection antibody, anti-CA 19-9 capture antibody and CA 19-9CNOs/GO)	-	0.12 U/mL	[155]
	g). Electrochemiluminescence (ECL)	CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer	-	Palladium Nano cage captured Ru(II) luminophore	Sandwich assay	0.003 U/mL	[157]
		MUCIN-1	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder	-	3D DNA Nano machine probes using Protein-Aptamer binding complex, a mimic	Direct assay	0.62 fg/mL	[158]

			Cancer, Gastrointestinal Cancer		peroxidase			
		CA 19-9	Breast cancer, Lung Cancer, Ovarian Cancer, Pancreatic Cancer	Antigen-Antibody	Anti-CA 19-9 antibodies attached to Glutaraldehyde modified Multi-walled carbon nanotubes-platinum-luminol nanocomposites on the surface of glassy carbon electrodes	-	0.000046 U/mL	[159]
		CA 19-9	Breast cancer, Lung Cancer, Ovarian Cancer, Pancreatic Cancer	Antigen-Antibody	CA 19-9 antibodies attached via glutaraldehyde on Magnetic carbon nanotubes decorated with polyethylenimine, CA 19-9 detecting antibodies 2 immobilizing luminol on Ag@BSA core/shell microsphere.	Sandwich assay	0.0002 U/mL	[160]
		CA 19-9	Breast cancer, Lung Cancer, Ovarian Cancer, Pancreatic Cancer	Antigen-Antibody	Magnetic Graphene oxide (NanoFe3O4@GO) functionalized with N-(4-aminobutyl)-N-ethylisoluminol (ABEI) and CA 19-9 antibodies, attracted on the surface of glass carbon electrode.	Sandwich assay	0.0005 U/mL	[161]
		c-Myc mRNA	Breast cancer		CdSe@ZnS QDs based microfluidics channel	-	-	[162]

		CA153	Breast cancer		$Ru(bpy)_3^{+2}$.UiO-66- NH_2 MOF	-	1.7705 x 10^{-5} U/mL	[163]
	h). Field Effect Transistor (FET)	miRNA-155	Breast cancer		MoS_2 flack on FET	-	0.03 fM	[165]
		Human Epidermal Receptor 1 (HER1)/c-erbB1 EGFR	Breast Cancer, Non-Small Cell Lung Carcinoma (NSCLC)	Antigen-antibody	ZnO thin film transistor (ZnO-bioTFT) functionalized with monoclonal EGFR antibodies.	Direct assay	10 fM	[166]
		Human Epidermal Receptor 2 (HER2) EGFR	Breast Cancer, Non-Small Cell Lung Carcinoma (NSCLC)		Graphene oxide and APTES functionalized gold electrode	Direct assay	100 pM for EGFR 1 pM for HER2	[167]
		CA-125	Breast cancer, Ovarian Cancer	Aptamer based	Multi Walled Carbon nanotubes reduced Graphene Oxide poly Methyl Methacrylate (MWCNTs/rGO/PMMA)	Label free assay	5×10^{-5} U/mL	[168]
	i). Chronocoulometric	miRNA-21	Breast cancer		$AuNPs@MoS_2$	-	100 aM	[169]
2.	Optical	CA15-3	Breast Cancer, lung Cancer, Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer	-	Gold nanorods	Direct assay	1.0×10^{-10} M	[171]
		CA15-3	Breast Cancer, lung Cancer,	-	2 types of Lectin	Sandwic	1.2 U/mL for	[172]

			Ovarian Cancer, Endometrial Cancer, bladder Cancer, Gastrointestinal Cancer		modified fluoromicrobeads, Sambucus nigra agglutinin and peanut agglutinin lectins	h assay	PNA and 0.4 U/mL for SNA	
		p53	Breast Cancer, Colorectal Cancer	Antigen-Antibody	Fiber-light-coupled optofluidic waveguide (FLOW)	-	10 fg/mL-10 ng/mL	[173]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer	Antigen-affibody	Magnetic beads modified with enzymes, affibody were used instead of antibody	Sandwich assay	13-100 ng/mL	[174]
		SK-BR-3	Breast cancer		Au nanoparticles modified with thiolated terminated aptamer	-	49 cells/mL	[175]
		Human Epidermal Receptor (HER2)/neu/c-erbB2	Breast Cancer, Gastric Cancer		Silica nanofiber	-	0.1 ng/mL	[176]
		miRNA-21	Breast cancer		DNA templated Ag/Pt nanocluster	-	0.6 pM	[178]
		p53	Breast Cancer, Colorectal Cancer	Enzyme based	PMB probe-DNAzymes	Sandwich assay	10 pM	[179]
		BRCA1	Breast cancer, Ovarian Cancer	DNA	Enzyme based 3D DNA detection nanostructured reporter probe	Sandwich assay	10 fM	[180]
		SK-BR-3	Breast cancer		Oval shaped-Au nanoparticles modified with aptamer	-	100 cell/mL	[181]

	a). Surface Enhanced Raman Scattering (SERS)	miRNA	Breast cancer		Plasmonic silver nanoparticles		-	[184]
		Multiple Biomarker string 3404, 3654, 11676, 11742, 16504, 16910, 17503, and 7542-X	Breast cancer		Au/HCP-PS plasmonic chip		-	[185]
		CD44/CD24	Breast cancer		Au nanoparticles modified with ssDNA		-	[186]
		MCF-7, MDA-MB-231, MCF-10A	Breast cancer		Silver nanostar			[187]
		MCF-7	Breast cancer		Ag@Au nanostar-DTTC functionalized with mPEG-SH			[188]
		BRCA1 and its alternating splice variant Δ(11q)(thelast3309ntdeletedfromexon11), Δ(9,10)(exon9and10deleted),Δ(5)(exon5deleted), and Δ(5q,6)(thelast22ntofexon5andtheentireexon6 is deleted)	Breast cancer		DNA-Au-RTag		-	[189]
		BRCA1	Breast cancer		DNA-Au-RTag		1 fM	[190]
	b). Surface Plasmon Resonance (SPR)	miRNA-122	Breast cancer		Antibodies functionalized Au nanoparticles	-	2 attamoles	[192]

		CA-125	Ovarian Cancer Breast cancer	Antigen-Antibody	Gold chip	-	2.2-150 U/mL	[193]
		CA 19-9	Lung Cancer, Ovarian Cancer, Pancreatic Cancer Breast cancer	Antigen - Affibody	-	Sandwich assay	66.7 U/mL	[194]
	c). Fluorescence	CYP1A1	Breast cancer		Fluorescent probe of iPrBN	-	0.04874 nM	[73]
		CYP1A1	Breast cancer		Fluorescent probe NEiPN	-	0.026 nM	[74]
		CYP1A1	Breast cancer		Fluorescent probe of DCPI	Label free assay	1 pg/mL	[75]
		miRNA-21	Breast cancer		ssDNA-Folic acid – polyethylene functionalized MoS_2 based fluorescent probe		-	[195]
		miRNA-21	Breast cancer		Copper nanocluster based hybrid materials	-	18.7 pM	[196]
		miRNA-21	Breast cancer		Protein p19 conjugated DQs	-	0.6 fM	[197]
		miRNA-21	Breast cancer		DNA/Ag nanocluster/cytosine	-	4×10^{-3} nM	[198]
		miRNA-1246	Breast cancer		Au nanoflare modified with nucleic acid	-	-	[199]

		CA-125	Breast cancer, Ovarian Cancer	-	Phthalocyanine fluorophore in polystyrene matrix	-	1 X 10^{-4} U/mL	[200]
		p53	Breast Cancer, Colorectal Cancer	Antigen-Antibody	CdS nanocrystals immobilized on graphene oxide electrodes-gold nanoparticles (CdS NCs/ GO/AuNPs)	Sandwich Assay	4 fg/mL	[201]
		p53	Breast Cancer, Colorectal Cancer	Protein-consensus DNA	Fluorescent dye tagged Consensus DNA functionalized on magnetic nanoparticles	-	8 pM	[202]
		miRNA-21	Breast cancer		Bio photonic containing Dopamine nanosphare	-	55 fM	[203]
		miRNA-21	Breast cancer		Oligonucleotide hybridized luminescent probe	-	1 fM	[204]
3.	Microfluidic	EGFR2	Breast cancer		Hierarchical composite of porous Graphene foam/nTiO_2 nanofiber	-	1.0 fM – 0.1 µM (DPV) 0.1pM-0.1 µM (EIS)	[212]
		Glypican-1	Breast cancer		Ab CD63 functionalized Magnetic beads	-	10 exosome/µL (~10^{-17} M)	[213]
		CA153, CA125, CEA, ErbB2	Breast cancer		Modified Au nanorods	-	1.85 U/mL (CA153 in Human Serum) 1.021 kU/mL (CA125 in Human Serum)	[214]

							76.19 ng/ml (CEA in Human Serum) 31.9 ng/mL (ErbB2 in Human Serum)	
		CEA, CA125, CA153	Breast cancer		Ag nanoparticle conjugated with Raman reporter		1 pg/mL for CEA 0.01 U/mL for CA125 & CA153	[215]
		Interferon-γ	Breast cancer		Micropatterned Gold electrode		5 ng/mL	[216]
		PTK7	Breast cancer		Hairpin probe with nicking enzyme		0.4 nM	[217]
		HER2, PSA, IgG			Gold electrode conjugated with HRP and TMB		100 fg/mL	[218]
		CEA, AFP	Breast cancer		Antibodies functionalized CdTe/CdS QDs	Sandwich	250 fM	[219]
		ErbB2	Breast cancer		Au-GO nanoposts		0.001 pM for (Amperometric) 10 pM (SPR)	[220]

Table 2: List of omics-based FDA approved biomarkers for diagnosis of breast cancer:

S. no.	Omics Based Biomarkers		Biological Specimen	Cancer Type	Characteristic of Biomarker/ Clinical Use	Approval Year	Mode of Assessment/Concentration	References
1.	Genomics	BRCA1 and BRCA2	Tumor, Blood	Breast Cancer	Risk prediction, diagnosis	1998	Mutations detection	Web ref. [2]
2.		HER2/ NEU	Tumor,	Breast Cancer	Prognosis and selection of therapy	1997	Mutations detection	Web ref. [3]
3.		EGFR mutation	Tumor, Circulating free, Tumor DNA	NSCLC Breast cancer	Diagnosis	2016	Gene over expression	[23]
4.		KRAS	Tumor, Blood	Colorectal cancer Breast cancer	Selection of therapy	2009	Mutation	Web ref. [4]
5.	Transcriptomic	Mir-21	Blood	Breast cancer	Diagnosis	2009	Expression levels	Web ref. [5]
6.		Mir-155	Blood	Breast cancer		2014		Web ref. 6
7.	Proteomic	HER2	Serum	Breast Cancer	Screening, Monitoring	1998	15 ng/mL	[24]
8.		ER	Serum, urine	Breast Cancer	Monitoring, Prognosis, Selection of therapy	1999	4-10 fmol/mg of protein	[25]
9.		PR	Serum, urine	Breast Cancer	Monitoring, Prognosis, Selection of therapy	1999	-	[25]
10.		CA15-3	Serum	Breast Cancer	Screening, Monitoring	1997	25 U/mL	[26]
11.		CA27-	Serum	Breast	Screening,	2002	38 U/mL	[27]

		29		Cancer	Monitoring			
12.		CA 125	Serum	Ovarian cancer Breast Cancer	Screening, Monitoring	1997/ 2011	35 U/mL	[28]
13.		CEA	Serum and urine	Breast cancer, Colon Cancer	Screening, Monitoring		3.5 ng/mL	[29]
14.		VEGF	serum	Breast Cancer	Monitoring, selection of therapy	2011	62–707 pg/mL for serum and 0–115 pg/mL for plasma	[30]

- ❖ Recent biosensors-based techniques for early detection of breast cancer have been described in this review.
- ❖ Common breast cancer associated omics-based biomarkers such as genomic, transcriptomic, proteomic and metabolomic are discussed herein.
- ❖ This review provide platform to design specific biomarkers-based biosensor technique for early detection of breast cancer.