Biosensors and Bioelectronics 88 (2017) 217–231

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Biosensors for breast cancer diagnosis: A review of bioreceptors, biotransducers and signal amplification strategies

Sunil Mittal [a], Hardeep Kaur [a,*], Nandini Gautam [a], Anil K. Mantha [b]

[a] Centre for Environmental Science and Technology, Central University of Punjab, Bathinda, 151001 India
[b] Centre for Animal Sciences, Central University of Punjab, Bathinda, 151001 India

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
10 August 2016
Accepted 11 August 2016
Available online 12 August 2016

Keywords:
Breast cancer
Biomarkers
Nanomaterials
Redox mediators
Redox cycling

ABSTRACT

Breast cancer is highly prevalent in females and accounts for second highest number of deaths, worldwide. Cumbersome, expensive and time consuming detection techniques presently available for detection of breast cancer potentiates the need for development of novel, specific and ultrasensitive devices. Biosensors are the promising and selective detection devices which hold immense potential as point of care (POC) tools. Present review comprehensively scrutinizes various breast cancer biosensors developed so far and their technical evaluation with respect to efficiency and potency of selected bioreceptors and biotransducers. Use of glycoproteins, DNA biomarkers, micro-RNA, circulatory tumor cells (CTC) and some potential biomarkers are introduced briefly. The review also discusses various strategies used in signal amplification such as nanomaterials, redox mediators, p19 protein, duplex specific nucleases (DSN) and redox cycling.

1. Introduction

Breast cancer is a serious health concern for women. It contributes to about 23% of the cancer cases, worldwide, (Mahfoud et al., 2014) and accounts for the second largest number of deaths among all cancers (DeSantis et al., 2014; Misek and Kim, 2011). Resource limited countries are more severely affected due to their poor financial status, deprived clinical facilities and lack of awareness. Since, numerous factors are involved in onset of cancer and these factors may lead to diverse symptoms depending upon the type and site of tumor formation, hence, the treatment warrants an early stage diagnosis, efficient treatment procedures and post treatment care to avoid its reoccurrence. The breast cancer diagnostic techniques include mammography, biopsy, MRI, sonography, molecular breast imaging, thermography etc. These are quite efficient methods and are able to detect 80–90% of breast cancers in women (Michaelson et al., 2002). Apart from these, biomarker based expression techniques such as enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry (IHC) also cater the present diagnostic needs. Although all these techniques are efficient, still, these are associated with some limitations as discussed in Table 1. False positive or negative results complicate the situation by wrong interpretations and unnecessary biopsies. So, researchers have emphasized on development of detection methods that are highly sensitive, non-invasive and needed for the point of care (POC) diagnosis. For this, biosensors are sensitive, specific and cost effective devices. These also exhibit the benefit of quick response due to direct assessment in physiological fluids (blood, serum, urine, saliva, milk etc.) in a non-invasive way.

A biosensor for cancer diagnosis usually consists of biomarker (target molecule), bioreceptor (recognition element) and compatible biotransducer. These components play an imperative role and decide the technical specifications of the biosensor device. The biomarkers present on the cell surface or their shed off extra cellular domains (ECD) in the serum are the common analytes used as biomarkers (Al-Khafaji et al., 2012; Marques et al., 2014). These biomarkers are not very specific and represent varied levels of expression at different cancer stages. Therefore, multiplexing of the biomarkers at single diagnostic platform may lead to better interpretation. Accurate measurements of these biomarkers individually or in combination have been proposed to facilitate the classification of tumor cells that ultimately decipher the prognosis information. A correct understanding of the cancer type is necessary for prognosis and responsible for selection of better treatment therapies that maximizes the efficacy and minimizes toxicity risk and mortality. In order to recognize a biomarker, some recognition elements called bioreceptors are used which interact with biomarkers and produce relative dose dependent response. Further, the selection of particular type of biotransducer is required. It depends on the kind of biochemical signal produced by the

* Corresponding author.
E-mail addresses: sunil.cevs@gmail.com (S. Mittal), hardeep_kaur007@rediffmail.com (H. Kaur), ngautam86@gmail.com (N. Gautam), anilmantha@gmail.com (A.K. Mantha).

http://dx.doi.org/10.1016/j.bios.2016.08.028
0956-5663/

Table 1
Techniques in use for breast cancer diagnosis and their limitations.

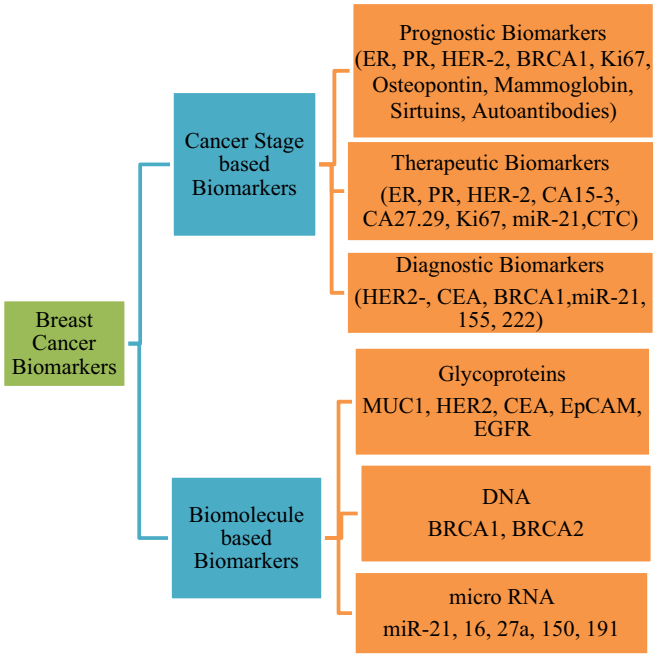
Technique	Limitations
Mammography	Detects only 70% of breast cancers Low sensitivity and specificity Sensitivity decreases as breast tissue density increases Fails to detect tumor in dense and small tissue at early stage Frequent false positive results Low energy X- rays may cause mutations in tissue Disintegration of tumor tissue during analysis may cause metastasis
Biopsy	May miss tumor cells Unnecessary surgery May lead to metastasis of tumor Need skilled expertize Performed for confirmation in later stages Expensive
MRI	Unable to detect all types of cancers such as ductal and lobular carcinoma Expensive
Sonography	Less sensitive and relatively expensive Need experienced expertize to analyse real time examination
FISH (Fluorescence in situ hybridization)	Provide semi-quantitative results Separate patients in biomarker positive or negative groups
ELISA	Time consuming Expensive Insensitive to low level markers Intrinsic color of analytes may lead to false results Require trained personal to carry out analysis
RIA	Radioactivity risk Complex procedure Time consuming Require trained personal to carry out analysis
IHC	Complicated technique Time consuming Require trained personal to carry out analysis

interaction of bioreceptor with biomarker. For example, change in proton concentration is measured by potentiometric biotransducer, discharge/uptake of electrons by amperometric biotransducer, light emission/absorption/fluorescence or reflectance by optical detection and change in mass by piezoelectric biosensors. Further, sometimes signal enhancers are (in the form of nanomaterials or high adsorptive properties harboring reagents) introduced in the system to achieve desired signal amplification.

The present review focuses on the major components and mechanisms entailed to develop POC devices for early breast cancer diagnosis. To simplify the understanding, current review has been divided into four major parts (1) breast cancer biomarkers, (2) associated bioreceptors, (3) biotransducers employed, and (4) the signal amplification strategies. These are further subdivided into their counter parts to discuss all the aspects associated with the biosensor development for breast cancer diagnosis.

2. Biomarkers for breast cancer diagnosis

There are certain biomolecules such as cell surface receptor proteins, mutated genes, micro RNAs, etc. that are variedly expressed in/on tumor cells and are indicative of cancer progression. These biologically relevant molecules are called biomarkers. Diagnostic assemblies demand that the biomarker should be an element easily extractable from the physiological fluids of the patients in a non-invasive procedure and should not exist in healthy person. The potential of biomarkers as target molecules for cancer diagnosis through biosensors have been vastly explored and elaboratively discussed already (Diaconu et al., 2013; Tothill, 2009; Vidi et al., 2013). But the studies are either not dedicated to breast cancer biosensors or does not include all the aspects of the same. The present review is therefore focused on all the technical points required to develop a specific breast cancer biosensor.



Scheme 1. Classification of breast cancer biomarkers.

As other cancer biomarkers, breast cancer biomarkers also follow two modes of classification, as stage dependent biomarkers and over expressed biomolecules based biomarkers (Mishra and Verma, 2010) as summarized in Scheme 1. From diagnostic point of view, biomolecules based biomarkers have more significance than therapeutic or prognostic biomarkers. But there is always a correlation between the diagnostic and prognostic value of a biomarker. For example, human epidermal growth factor receptor 2 (HER-2), oestrogen and progesterone receptors are regarded as diagnostic as well as prognostic biomarkers (Misek and Kim, 2011). Biomolecule biomarkers identified as target molecules for bio recognition are discussed briefly in further sections.

2.1. Glycoproteins

Glycoproteins mainly consist of surface bound glycoprotein receptors like HER-2, Mucin1 (MUC1), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR) etc. These are usually deregulated and mediate cancer cell proliferation by production of growth factors which alter MAPK and PI3K/Akt pathways (Raina et al., 2004) and induce metastasis (Nath and Mukherjee, 2014). Among the various glycoproteins, HER-2 and MUC1 are the most widely accepted diagnostic and prognostic biomarkers used in the detection of breast cancer.

HER-2 is a proto-oncogene encoding trans-membrane glycoprotein (M. Wt-185 kDa). It's over expression is detected in nearly

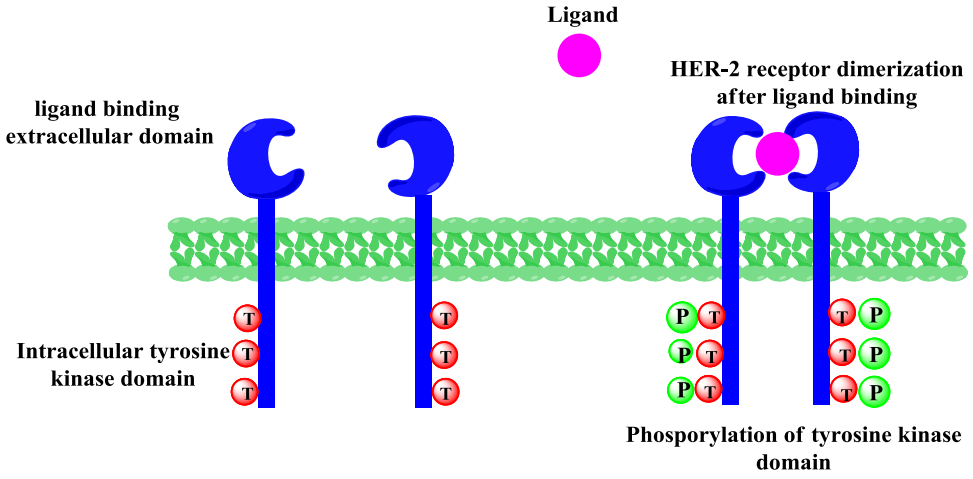


Fig. 1. HER-2 receptor response to ligand binding: HER-2 receptor has an extracellular domain that undergoes dimerization upon ligand binding. In response, the intracellular protein kinase domains get phosphorylated and activate other signalling cascades.

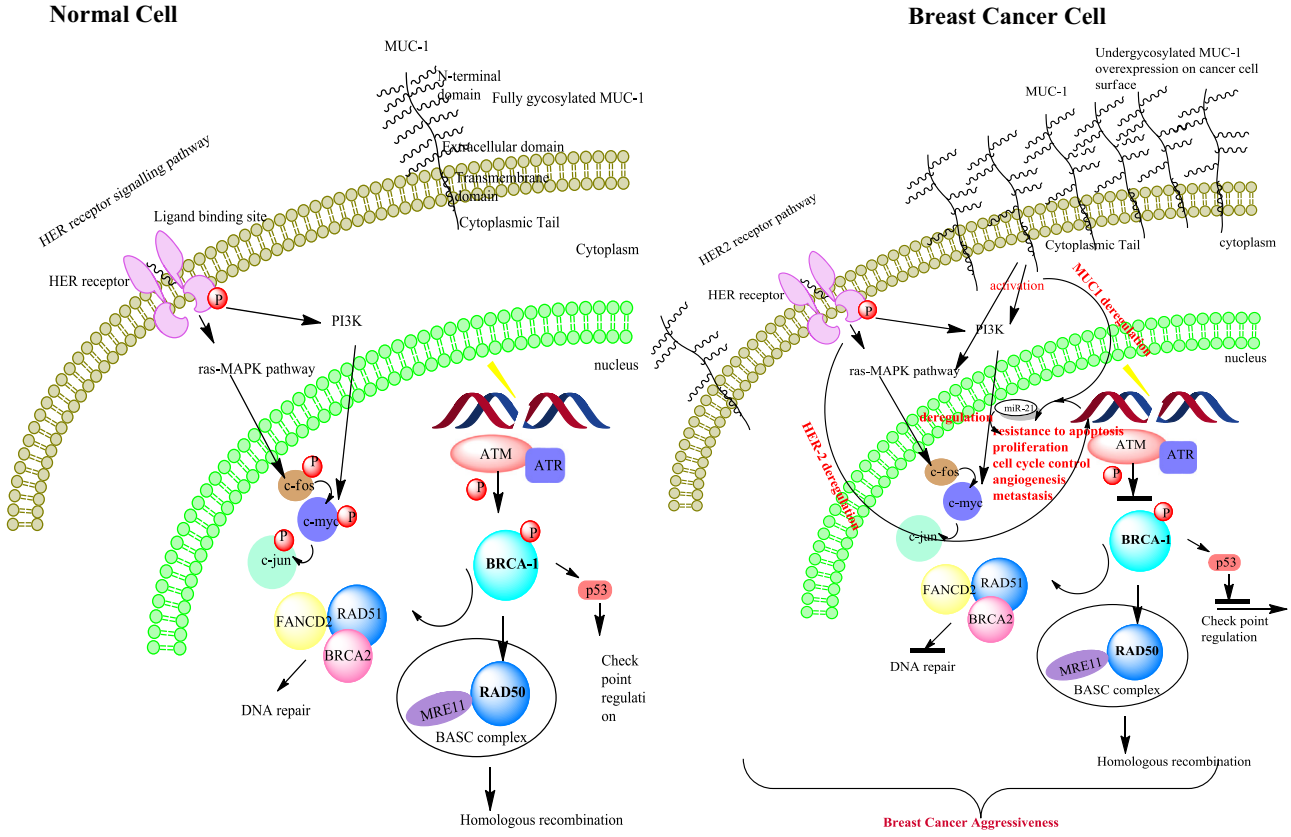


Fig. 2. Representation of HER-2, BRCA-1 and MUC-1 biomarker deregulation in the breast cancer vs normal cellular functions: Deregulated HER-2 receptor pathway lead to metastasis, cell cycle deregulation, angiogenesis, increased proliferation and reduction of apoptosis in the breast cancer cells. MUC-1 protein is under gycosylated and overexpressed throughout the membrane of cells leading to deregulation of PI3K pathway. MiR-21 overexpression leads to deregulated *bcl-2* expression leading to metastasis and activation of MAPK pathway on HER-2 stimulation. BRCA-1 down regulation in cancer cells leads to prevention of control over DNA repair and cell cycle regulation.

20–30% breast cancer cases which makes it a suitable biomarker for breast cancer diagnosis (Al-Khafaji et al., 2012). HER-2 positive breast cancers are very much aggressive than any other type of breast cancer. HER-2 receptors consist of extracellular ligand binding domain that undergo dimerization after ligand binding and an intracellular tyrosine kinase domain which gets phosphorylated in response to dimerization as illustrated in Fig. 1 (Burgess et al., 2003). This phosphorylation event in turn activates various receptors and results in downstream signalling cascade pathways like MAPK, P13/Akt etc. as exemplified in Fig. 2 (Menard et al., 2000).

MUC1 is a glycoprotein that helps in protection from pathogenic infections and is encoded by *MUC-1* gene. It is normally expressed at apical surface of epithelial cells in several organs including breast. But in cancerous cells, MUC1 is over expressed, leading to the presence of these glycoprotein over whole cell surface (Hollingsworth and Swanson, 2004).

2.2. DNA biomarkers

Mutations, in oncogenes lead to gene amplification while in tumor suppressor genes, it causes loss of their functions which are

generally related with cancer incidence and have been employed as diagnostic biomarkers. Epigenetic alterations in nuclear and mitochondrial DNA are also used as indicator of breast cancer progression (Yadav and Chandra, 2013). In case of breast cancer diagnosis, genes *BRCA1 and BRCA2* are the most widely accepted DNA biomarkers. They are tumor suppressor genes expressed in normal cells and help in repair of double strand DNA breaks or induce cell death, if unable to repair (Friedenson, 2007; O'Donovan and Livingston, 2010). They are also responsible for control and regulation of cell cycle checkpoints and cell division. Hence, they play key role in maintaining genome integrity (Wu et al., 2010). Mutations in genes *BRCA1* and *BRCA2* are associated with the increased risk of breast cancer and responsible for approximately 21–40% of the inherited breast cancer cases (Friedenson, 2007; King et al., 2003). Expression of BRCA1 protein has been reported to be reduced in 30% of the sporadic breast cancer cases (Yang et al., 2001). Extent of down regulation of BRCA1 protein is dependent on the severity of the breast cancer and is inversely related to the expression of BRCA2 protein which has been employed as a tool for the diagnosis of sporadic breast cancer (Hedau et al., 2015). Therefore, *BRCA2* can be used as prognostic as well as diagnostic biomarker for breast cancer.

2.3. Micro RNAs

Micro RNAs (miRNAs or miRs) are small non coding RNA molecules involved in regulation of gene expression. They are usually up-regulated or downregulated in tumor cells, hence can be used as diagnostic biomarkers. The miRs up-regulated in cancer cells are miR-16, miR-21, miR- 222, miR-155, miR- 213, miR-27a, miR-29b, miR- 29c, miR- 93, miR-150, miR-191, miR-200c, miR-210, and miR- 451. Similarly, miR-145, miR-125b, miR- 100, miR- 10b, Let-7a-2, miR-205, miR- 497, and miR-193 are the miRs which are down-regulated in cancer cells (Andorfer et al., 2011; dos Anjos Pultz et al., 2014; Heneghan et al., 2010; Lv et al., 2013). Among all miRs, miR-21 is the most targeted one in breast cancer diagnosis because of its higher sensitivity (87.6%) and specificity (87.3%) at early stages than other biomarkers such as CEA and CA15–3 (Gao et al., 2013).

MiR-21 is over-expressed in blood plasma and cancerous tissue in comparison to normal breast tissue and hence can be used as diagnostic and therapeutic biomarker. It's upregulation has been linked to metastasis in breast cancer by activation of MAPK pathway on HER-2 stimulation (Huang et al., 2009). Although, miR-21 has provided non-invasive and stable biomarker identification platform in breast cancer diagnosis and prognosis events, yet, these have limitations such as sequence homology with related RNAs, occurrence in other cancers too, and low abundance in serum. Such challenges need to be addressed when working with miRs as the target molecules. Generally, northern blotting and *in situ* hybridization methods are employed for their analysis, but they offer low sensitivity and restrict economical use of time and money. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is a widely used technique which delivers high sensitivity and broad spectrum miR analysis but has same limitations as of other laboratory bound techniques. In this regard, electrochemical biosensors have been proved as valuable and succeeded as POC devices (Keshavarz et al., 2015). Preliminary studies of miR detection were mostly based on hybridization concept and guanine oxidation (Kilic et al., 2012; Lusi et al., 2009). Although, guanine oxidation study was the preferred method, still, it lacked sensitivity and reproducibility in cell lysates. So, owing to the need of another transduction principle, hybridization of miR–21 with its complementary probe has been investigated (Campuzano et al., 2014; Kilic et al., 2012, 2013; Labib et al., 2013b; Yin et al., 2012). Recently, it has been concluded that the detection levels of miR-21 can be lowered down to fentomols using iridium (III) complex with miR-21 recognizing G- quadruplex (Miao et al., 2016).

2.4. Circulatory tumor cells (CTC)

The biochemical or morphological attributes of cancerous cells are distinct from normal cells that lead to their detection. Metastasis of cancer could be easily accessed by the analysis of CTC (Nadal et al., 2013). Sequestration and enumeration of such cells could assist in cancer diagnosis and prognosis. They also convey the invasive nature of cancer and could be used to understand anti-cancer drug response and mechanism of personalized anticancer therapy. The size, morphological attributes, magnetic properties etc. are taken as the key factors for their isolation from a population of cells. As their number is very low in blood stream (0–10 ml^{-1} of whole blood containing 10^9 erythrocytes and 10^6 leucocytes ml^{-1}), their enumeration is extremely difficult (Han et al., 2006). It is speculated that a high number could be directly associated with clinically imperative phase in breast cancer.

2.5. Miscellaneous biomarkers

There are few biomarkers that could be regarded as potential targets for breast cancer diagnosis. These include cell free DNA (cfDNA), autoantibodies, and antigens such as urokinase-dependent plasminogen activator system (uPA), the plasminogen activator inhibitor (PAI), and the Thomsen-Friedenreich (TF).

2.5.1. Cell free DNA (cfDNA)

Circulating, cell-free tumor DNA (cfDNA) holds promising potential as cancer biomarkers. Breast cancer is associated with excessive DNA damage which is released by apoptotic and necrotic cells, expressing 0- 2000 ng ml^{-1} DNA content in plasma, serum and urine (Kohler et al., 2011; Schwarzenbach and Pantel, 2015). Quantitative estimation of cfDNA could open up new route to non-invasive methods of breast cancer diagnosis and provide therapeutic information. Studies pertaining to use of cfDNA as breast cancer indicator reveal a direct relationship between breast cancer progression and cfDNA concentration (Schwarzenbach, 2013; Tangvarasittichai et al., 2015; Weigel and Dowsett, 2010). But, their application for biosensor construction is still in infancy state and needs more research.

2.5.2. Autoantibodies

Cancer incidence gained momentum since 1990s, but the role of autoantibodies in early breast cancer diagnosis have been implicated very late. It was observed that cancer malignancies result in circulation of antibodies against cancer antigens in serum that could be employed as biomarker to deduce cancer status (Lacombe et al., 2014). Autoantibodies against tumor associated antigens (TAA) can be detected in serum prior to any clinical manifestation. These are overexpressed in serum and saliva of the cancer patients and have been proposed to be effective biomarkers for early diagnosis (Arif et al., 2015; Azimzadeh et al., 2016; Laidi et al., 2015). Autoantibodies against cancer antigens such as p53, heat shock proteins (HSP – 27, 60, 90), GIPC-1, c – myc, c- myb, cyclin D1, cyclin B1, RS/DJ-1 etc. have been reported in breast cancer patients and a positive co-relation between their titre and cancer progression has been observed (Le Naour et al., 2001; Piura and Piura, 2010). Autoantibodies based microarray for 10 respective tumor antigens of breast cancer has been reported and successfully applied to investigate breast cancer samples (Yang et al., 2013). Simplified, non-invasive detection of autoantibodies in serum potentiates their application as diagnostic biomarker in near future, but, heterogeneous nature of breast cancer and our limited

understanding of humoral immune response resist their routine clinical use.

2.5.3. uPA, PAI and TF

Apart from above discussed biomarkers, combination of independent markers have been proposed to promote diagnostic efficacy. Urokinase-dependent plasminogen activator system (uPA), the plasminogen activator inhibitor (PAI) and the Thomsen-Friedenreich (TF) antigen have been recognized as effective disease predictors in pre and post-menopausal women (Deutscher et al., 2010; Qin et al., 2012). The application of these biomarkers for real sample analysis through biosensing is awaited and may open up sensitive and specific breast cancer diagnostic avenues.

3. Bioreceptors

Bioreceptors are the biological part of the biosensor that interact with the biomarker and undergo biological reaction to produce a signal. These bioreceptors could be any biological entity that has specificity towards a particular biomarker. Generally bioreceptors include antibodies, aptamers, DNA, enzymes, whole cells, etc. Bioreceptors extensively used for breast cancer biosensing are discussed below.

3.1. Antibodies

As convenient probes, antibodies have created explicit niche in breast cancer diagnosis by contributing real nano-sense to the system. The specificity, sensitivity, and homogeneous nature of antibodies have attracted researcher's interest. Immobilization of biomarker specific antibodies on electrode surface and alteration in electron transfer rate of electrode is the principle for the development of impedance based electrochemical sensors. This is a process of direct and unlabelled form of detection that depends on change in physical parameters upon antigen - antibody complex (Ag-Ab) formation (Arya et al., 2012; Arya et al., 2013; Eletxigerra et al., 2015; Elshafey et al., 2013; Fan et al., 2013; Li et al., 2013a; Li et al., 2013b; Myung et al., 2011; Norouzi et al., 2011; Seven et al., 2013; Sonuç and Sezgintürk, 2014; Sun et al., 2011; Vasudev et al., 2013; Zhu et al., 2012). Indirect method of detection involves labelling of antibodies with signal producing molecules such as enzymes (Al-Khafaji et al., 2012; Li et al., 2011c; Marques et al., 2014; Wei et al., 2011; Xiang et al., 2011; Yang et al., 2011), redox mediators (Prabhulkar and Li, 2009) or fluorophores (Chang et al., 2011) to capture voltametric or optical changes. Enzymes such as horseradish peroxidase (HRP) and alkaline phosphatase are usually employed to produce electro active products leading to redox reactions at electrode surface. Also, this approach makes use of sandwich immunoassay to make contact with electrode surface at one end and produce detectable signal at the other end (Al-Khafaji et al., 2012; Kellner et al., 2011; Marques et al., 2014; Wei et al., 2011; Xiang et al., 2011; Yang et al., 2011). The proteinaceous nature of antibodies enable easy preparation of surface assembled monolayer (SAM) with some cross linkers such as N-(3-dimethylamminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (NHS) (Arkan et al., 2015; Benvidi et al., 2016; Kilic et al., 2012; Xiang et al., 2011; Zhang et al., 2011), 3-aminopropyltrietoxy silane (APTES), glutaraldehyde (Wang et al., 2014), 11-mercaptoundecanoic acid (11-MUA), 3-mercaptopropionic acid (3-MPA) (Arya et al., 2012), Poly-(diallyldimethylammonium chloride) (PDDA), poly(sodium 4-styrenesulfonate (PSS) (Gao et al., 2011; Xiang et al., 2011), Succinimidyl 6-(3-[2-pyridyldithio]-propionamido) hexanoate (LC-SPDP) (Arya et al., 2013; Seven et al., 2013), dithiobissuccini- midyl propionate (DTSP) (Vasudev et al., 2013), and 1,4- phenylene diisothiocyanate (PDITC) (Elshafey et al., 2013). The limitations of antibodies are their thermal or physical instability and complex *in vitro* and *in vivo* models of their generation. Further, regeneration of antibody based systems and reproducibility of results are the major challenge in using antibodies as bioreceptors.

3.2. Aptamers

Aptamers, also known as chemical antibodies have come up as an alternative to native antibodies and are nowadays preferred bioreceptors in biosensors because of their specific binding efficiency, small size (one tenth of antibodies) and synthetic nature. They form tertiary structures of single stranded DNA (ssDNA) or RNA capable of making interaction with biomarkers such as cell surface glycoproteins and miRs (Won et al., 2013). Aptamers are also capable to interact with glycoproteins or miRs as activator or inhibitor of their biological functions under *in vivo* conditions. But, *in vitro* systems, they may be used as bait to pull down the target moiety from a pool of interfering biomolecules. An interesting fact about aptamers is that they can be selected through a process of molecular evolution called systemic evolution of ligands by exponential enrichment (SELEX) against unlimited range of targets extending from inorganic ions to proteins, pathogens, tumor cells etc. (Brody and Gold, 2000; Song et al., 2013). Compared to antibodies, aptamers are more thermo stable and can be easily modified with a minimal batch to batch variation (Sun and Zu, 2015).

Table 2
Aptamer sequences employed to capture breast cancer biomarkers.

Target	Aptamer Sequence	References
HER-2	5′CTTCTGCCCGCCTCCTTCC-(TGGGGCCTGGAT ACGGATTGGTAAGGATTAGTAGGGGGCATAGCT)-GGAGACGAGATAGGCGGACAC T3′	(Chun et al., 2013)
	5′GGGAGAGCGGAAGCGUGCUGGG–N40–AUAACCCA-GAGGUCGAUGGAUCCGGGG3′	(Won et al., 2013)
	5′GTAAAAGAACTGATCAGCACGGGATGGGATAGGGAGGGGAGTGTTGAAAA 3′	(Zhu et al., 2012)
	5′GGGCCGTCGAACACGAGCATGGTGCGTGGACCT AGGATGACCTGAGTACTGTCC3′	(Qureshi et al., 2015)
MUC1	5′GCAGTTGATCCTTTGGATACCCTGG3′	(Li et al., 2010a)
	5′GCAGTTGATCCTTTGGATACCCTGG3′	(He et al., 2012)
	5′GCAGTTGATCCTTTGGATACCCTGGTTTTTTTTTTTTTTT3′	(Florea et al., 2013)
	5′GCAGTTGATCCTTTGGATACCCTGG3′	(Yan et al., 2013)
	5′GCAGTTGATCCTTTGGATACCCTGG3	(Zhu et al., 2013)
	5′ACACGGCAGTTGATCCTTTGGATACCCTGGCGTGT-3′	(Hu et al., 2014)
	5′GCAGTTGATCCTTTGGATACCCTGG3′	(Cai et al., 2015b)
VEGF	5′TTCCCGTCTTCCAGACAAGAGTGCAGGG3′	(Zhao et al., 2011)
	5′GGGCCCGTCCGTATGGTGGGTGTGCTGGCTTTTAAAAAAAAAA3′	(Wang et al., 2015a)
EpCAM	5CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG-	(Song et al., 2013)
AS1411	5′GGTGGTGGTGGTTGTGGTGGTGGTGG3′	(Feng et al., 2011)

Table 2 enlists the sequences of aptamers designed to capture breast cancer biomarkers in various investigations. Aptamers have been proposed to work individually (Chun et al., 2013; Feng et al., 2011; Hu et al., 2014; Wang et al., 2015a), with some redox mediators like methylene blue, thionine, $[Ru(NH_3)_6]^{3+}$ etc. (Florea et al., 2013; Pérez et al., 2015; Yan et al., 2013; Zhao et al., 2011), or in association with antibodies (Won et al., 2013), or fluorophores (He et al., 2012) to produce sensitive dose dependent response. When used with redox mediators, their assembly in the presence or absence of target decides the proximity of mediator with electrode surface and hence influence the voltametric response. Sometimes, specific double stranded DNA (dsDNA) sequences could also bind to specific proteins such as beta protein 1 (BP1) to produce mediator assisted response (Pérez et al., 2015). Aptamer regeneration is postulated through addition of cDNA that releases target association with aptamer (Feng et al., 2011). Inspite of advantages over antibodies, aptamers also suffer few limitations. Their *in vivo* and *in vitro* interactions with proteins vary, but this is not a serious problem in case of biosensor application as cancer diagnosis is always done on physiological fluids under *in vitro* conditions. But, the low success rate and time consuming attributes of SELEX hinder the commercial application of aptamers in clinical diagnosis.

3.3. Complementary DNA (cDNA)

Apart from antibodies and aptamers, classical method of DNA detection with complementary DNA (cDNA) includes a huge number of breast cancer biosensors. The bioassay principle depends on simple hybridization of target gene with its cDNA immobilized on electrode surface or optical detection platform to produce target dependent response (Fig. 3A). In case of electrochemical biosensors, hybridization is confirmed by increase in charge transfer resistance (R_{ct}) at electrode surface through electrochemical impedance spectroscopy (EIS) or introduction of some electro active intercalating agents (Benvidi et al., 2015; Tiwari and Gong, 2009). SAM of cDNA with nanocomposits and cross-linkers on electrode surface enhances voltametric signals. Detection of target genes or miR through cDNA hybridization seems the most reliable method for their identification (Bartosik et al., 2013; Benvidi et al., 2015; Cardoso et al., 2016; Kilic et al., 2012, 2013; Labib et al., 2013b; Rafiee-Pour et al., 2016; Wang et al., 2015b; Zhang et al., 2016). Amplification of capture DNA probe after hybridization with target gene (e.g. BRCA1 mutant) on the electrode surface through DNA polymerase enzyme has enabled surface resistance based evaluation of gene mutation (Benvidi et al., 2015). Some modified sequences termed as locked nucleic acid (LNA) in nucleic acid hairpin probe (NAH) also find their application in sensitive miR-21 detection (Yin et al., 2012). LNA, also referred as inaccessible RNA are synthetic ribonucleotides with locked confirmation due to a bridge between 2′ O and 4′ C, which enhances their affinity to complementary RNA or DNA. Hybridization of target gene with cDNA has been exploited in optical detection also (Chen et al., 2014b; Culha et al., 2004; Li et al., 2014b; Zhou et al., 2014). In an investigation, hybridized form of MUC1 protein specific aptamer (termed as AP) with its cDNA (termed as SP) in the absence of MCF-7 cells did not impart any enhancement to terbium (Tb^{3+}) fluorescence, but, in the presence of MCF-7 cells, fluorescence was greatly enhanced (because ssSP is left behind after association of AP with MUC1 protein on cell surface), equivalent to MCF-7 cells concentration (Cai et al., 2015b). Disruption of G-quadraplex structures due to cDNA association has also been exploited as bioassay principle (Zhou et al., 2014).

4. Biotransducers and CTC capturing devices

Biotransducer is the part of biosensor that converts the biological signal produced by the interaction of target analyte and bioreceptor into a measurable signal. The compatibility of biotransducer with the bioreceptor is the key feature for the success of biosensor. Basically, a uniform trend with respect to biotransducers has been observed in the development of breast cancer biosensors. Majorly, electrochemical and optical

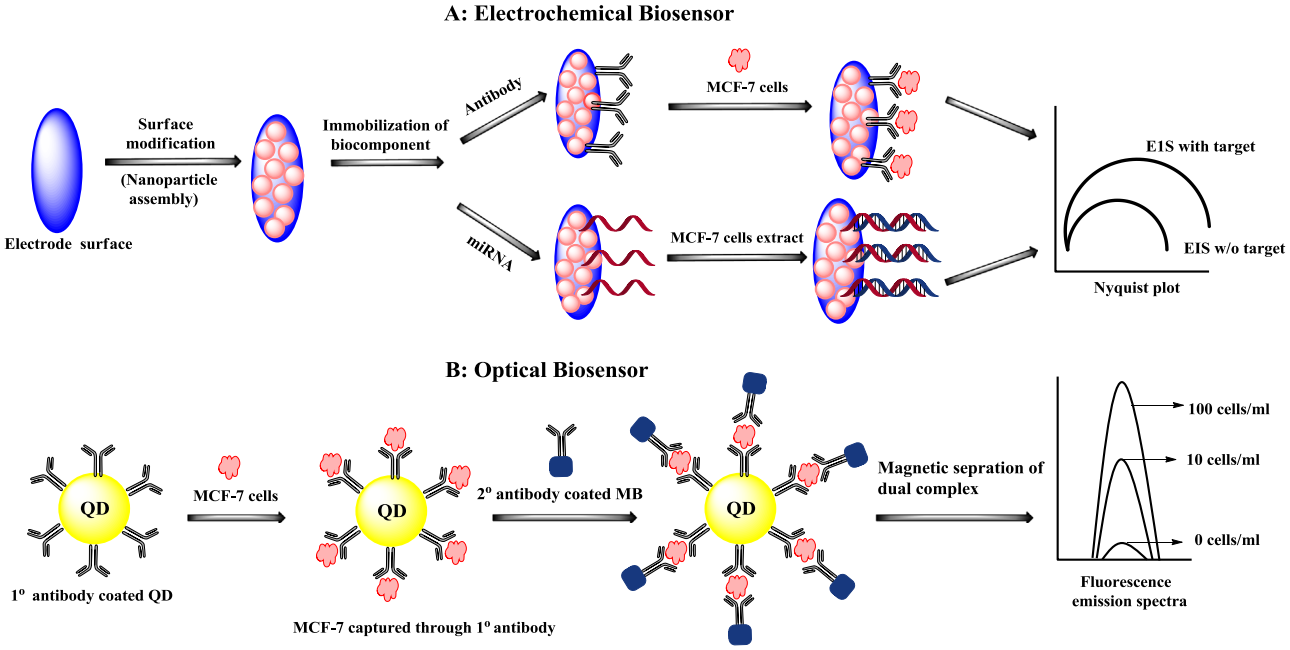
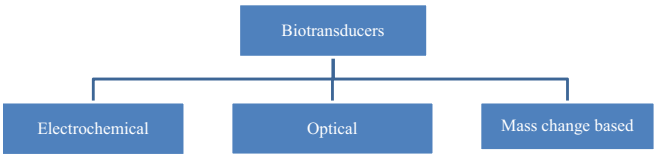


Fig. 3. Strategies to construct breast cancer biosensors: A) Electrochemical biosensor for detection of MCF-7 cells: Antibodies against surface proteins of MCF-7 cells are immobilized on nanoparticle assembled electrode to capture MCF-7 cells at the electrode surface which increases the interfacial resistance and hence enlarged semicircle in Nyquist plot. Alternatively cDNA complementary to miR can also be immobilized to capture target miR released from the cell extracts of MCF-7 cells. B) Quantum dot based optical biosensor for breast cancer: QDs are labelled with primary antibodies against MCF-7 cell surface proteins and subjected to sample containing MCF-7 cells. Addition of secondary antibody labelled magnetic beads (MB) enable their magnetic separation to obtain fluorescence emission spectra.



Scheme 2. Classification of biotransducers.

biotransducers have been employed for biosensor construction. The studies have been divided and discussed in three broad categories of biotransducers as per Scheme 2. In addition, the microfluidic assemblies employed to capture CTC have also been discussed under this section.

4.1. Electrochemical

Electrochemical biotransducers find wide application in biosensing due to their obvious high sensitivity, specificity, portability, ease of use and fast response. Electrochemical sensors offer a virtuous platform for immobilizing bioreceptors of various kinds that could deliver the signal with respect to analyte concentration. They also provide important information on the interaction of target molecule with the bioreceptor. Proteins, antibodies, aptamers and enzymes constitute wide array of components that could be attached to electrode surface through various means. Majority of electrochemical investigations take the advantage of this fact, and attempt to figure out the perfect combination of target – bioreceptor along with a conductivity enhancer (nanoparticles) to generate an amplified readout. A typical electrochemical workstation consists of three electrode system i.e. a working electrode, a reference electrode and an auxiliary electrode. The biochemical reactions pertaining to target-bioreceptor interaction occurs at the surface of working electrode. The electrochemical readout is obtained mainly through cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS) etc. Nowadays, EIS is a preferred technique because it provides insight to the bimolecular interaction through their effect on electron transfer resistance (R_{et}). The interfacial resistance increases due to the bulky assembly of mono-dispersed layers (Ag-Ab or protein–aptamer) on electrode surface. The increased resistance is evident by the enlarged semicircle in Nyquist plot obtained by EIS (Fig. 3A). The insulating effect of different layers of target- bioreceptor complex affect the mass transfer rate of electrons at the electrode surface and confer information on sequential binding of interacting biomolecules (Chun et al., 2013; Elshafey et al., 2013; Feng et al., 2011; Florea et al., 2013; Kong et al., 2011; Li et al., 2014a; Norouzi et al., 2011; Rafiee-Pour et al., 2016; Sonuç and Sezgintürk, 2014; Sun et al., 2011; Wang et al., 2015a). Due to improved signal-to-noise ratio and ability to work in high resistive solutions, microelectrodes are reported to offer better current change characteristics than large diameter electrodes (Arya et al., 2012; Arya et al., 2013; Prabhulkar et al., 2009; Prabhulkar and Li, 2009).

The various researchers have made attempts to develop POC for early diagnosis of breast cancer using electrochemical transducers and overexpressed biomarkers such as mutated *BRCA1* gene (Benvidi et al., 2015; Benvidi et al., 2016; Fan et al., 2013; Tiwari and Gong, 2009; Wang et al., 2015b), VEGF (Prabhulkar et al., 2009; Prabhulkar and Li, 2009; Sezgintürk, 2011; Zhao et al., 2011), EGFR (Vasudev et al., 2013; Zhao et al., 2011), MUC1 (Florea et al., 2013; Hu et al., 2014; Li et al., 2010a; Yan et al., 2013; Zhu et al., 2013), CEA (Kong et al., 2011; Norouzi et al., 2011; Sun et al., 2011; Yang et al., 2011; Zhong et al., 2010), HER-2 (Al-Khafaji et al., 2012; Arkan et al., 2015; Liu et al., 2014a; Marques et al., 2014; Qureshi et al., 2015; Seven et al., 2013), CA15–3 (Fragoso et al., 2011; Li et al., 2013b; Selwyna et al., 2013; Wang et al., 2014), miR-21 (Kilic et al., 2012; Meng et al., 2013; Miao et al., 2015; Rafiee-Pour et al., 2016; Torrente-Rodríguez et al., 2015; Torrente-Rodríguez et al., 2014; Yin et al., 2012). Table 3 enlists the specifications and detection limits of various electrochemical biosensors developed for breast cancer.

In addition to conventional electrochemical studies, a modified form of electrochemical investigation has been reported by da la Escosura-Muñiz and Merkoçi (2011). In this study, nano channel accompanied nanoporus membrane has been developed, that could be used as blocking agent for CA15-3 protein through immobilization of anti- CA15–3 antibodies on silver enhanced gold nanparticles (AuNP) inside the channels. The whole assembly has been converted in to a mini electrolytic cell with 300 μl capacity and detection limit of 52 U ml^{-1} for biomarker CA15-3 (de la Escosura-Muñiz and Merkoçi, 2011). In an another study, integration of flow injection system and fast Fourier transform with SWV provided sensitive estimation of minute levels of biomarker CEA (Norouzi et al., 2011). In this case, background noise was reduced by separating the voltametric signal and background signal in different frequency domains. Fragoso et al. (2011) also reported integration of microfluidic assembly with amperometric immunosensor for the breast cancer diagnosis (Fragoso et al., 2011). For the first time, HER-2 concentration was related to silver stripping by Zhu et al. (2012). In this case, AuNP – aptamer conjugate was armed with a reductant hydrazine to reduce silver ions to metallic silver for square wave stripping voltammetry (Zhu et al., 2012). Recently, the study of Swisher et al. demonstrated that metastatic cells have high Cathepsin B production, which could assist proteolytic cleavage of some specific tetrapeptides and generate ferrocene dependent electrochemical signals. The increased ferrocene signal revealed metastatic cell load in the sample (Swisher et al., 2015).

Now, from electrochemical electrodes, the sensing platforms have been advanced to capacitor sensors. Capacitor surface provide high sensitivity, label – free and cost effective testing. Direct effect of ligand (ssDNA and aptamer) binding and target protein (HER-2) on charge distribution at the surface has been exploited as the bioassay principle (Qureshi et al., 2015). Few conductivity based biosensors have been upgraded with the use of graphene based field effect transistors and silicon nanowires, which has enabled sensitive recognition of highly expressed biomarkers in breast cancer patients (Myung et al., 2011; Zhang et al., 2011).

4.2. Optical biotransducers

Light emission/ absorption based determination of diverse analytes is a widely researched field with major emphasis on plasmonic effect of nanoparticles (Cai et al., 2015a; Chang et al., 2011; Chen et al., 2015; Kim and Jeong, 2011; Manikandan et al., 2014). With reference to clinical diagnosis and prognosis, use of nanoparticles has gained interest in past couple of years. Nanoparticles have provided virtuous photostable synthesis and noise free fluorescence signal along with biocompatible environment (Chang et al., 2014b; Hun and Zhang, 2009). Their prolonged fluorescence is responsible for surface enhanced Raman spectroscopy (SERS) and has been utilized to differentiate between human breast normal cells, cancer cells and cancer stem cells based on scattering patterns (Manikandan et al., 2014). Shift in resonance frequency of gold nanoparticles and similar nanocomposits define the adsorption of aptamer or antibodies on their surface and correlate the shift with target density. The Ag-Ab interaction causes change in refractive index of gold nanorods (GNRs) microenvironment that can be qualitatively observed as shift in longitudinal plasmon wavelength [LPW] (Chen et al., 2015).

Diverse bioreceptors against distinct surface proteins, genes

Table 3
Specifications of electrochemical biosensors for breast cancer diagnosis.

Target molecule	Biosensor specification	Linear range of detection	Detection limit	Reference
BRCA1	cDNA/CHIT-*co*-PANI/ITO	0.05–25 fM	0.05 fM	(Tiwari and Gong, 2009)
	BRCA1/BSA/anti-BRAC1/BMIM · BF4/MCN–TB/GCE	0.01–15 ng ml^{-1}	3.97 ng ml^{-1}	(Fan et al., 2013)
	ssDNA probe/ Au electrode	1×10^{-19}–1×10^{7} M	4.6×10^{-20} M	(Benvidi et al., 2015)
	ssDNA probe /PEG/AuNP/GCE	50.0 fM–1.0	1.7 fM	(Wang et al., 2015b)
	ssDNA probe/PANHS/GCE	$10^{-16} \times 10^{-10}$ M	3.7×10^{-17} M	(Benvidi et al., 2016)
	ssDNA probe /PANHS/MWCNT/GCE	$10^{-17} \times 10^{-10}$ M	3.0×10^{-18} M	(Benvidi et al., 2016)
	ssDNA probe /PANHS/RGO/GCE	$10^{-18} \times 10^{-10}$ M	3.5×10^{-19} M	(Benvidi et al., 2016)
MUC1	Qdots/Apt/cDNA/Au electrode	1×10^{2}–1×10^{6} cells ml^{-1}	100 cells ml^{-1}	(Li et al., 2011a)
	DNA probe/gold SPE	0–10 ng ml^{-1}	0.95 ng ml^{-1}	(Florea et al., 2013)
	Aptamer/graphene/Au/GCE with Aptamer/TH/PtFe conjugate	100–5×10^{7} cells ml^{-1}	38 cells ml^{-1}	(Yan et al., 2013)
	Aptamer-HRP/MUC1/Aptamer1/Au electrode	100–1×10^{7} cells	100 cells	(Zhu et al., 2013)
	DNA probe-AuNP-HRP//Streptavidin/ MWCNT/GCE	8.8–353.3 nM	2.2 nM	(Hu et al., 2014)
MUC1 & CEA	MUC1 aptamer/MCF- 7/anti-CEA-CdS NPs	10^{4}–10^{7} cells ml^{-1}	3.3×10^{2} cells ml^{-1}	(Li et al., 2010a)
CEA	HRP-anti-CEA-AuNP-graphene/GCE	0.05–350 ng ml^{-1}	0.01 ng ml^{-1}	(Zhong et al., 2010)
	Anti-CEA/GNP–Thi–GR/GCE	10–500 pg ml^{-1}	4 pg ml^{-1}	(Kong et al., 2011)
	HRP/anti-CEA/AuNPs/ZnONPs/Au electrode	0.1–70 ng ml^{-1}	0.01 ng ml^{-1}	(Norouzi et al., 2011)
	Anti-CEA/GNPs/Azure I/ Nf -MWCNT	0.1–40 ng ml^{-1}	0.03 ng ml^{-1}	(Sun et al., 2011)
	Ag/HRP-Ab2–HPtNPs / BSA /Ab1/ Au–TiO2	0.02–120 ng ml^{-1}	12 pg ml^{-1}	(Yang et al., 2011)
CA15–3	Pt NCs/OrgSi@CS-CNTs/GCE	0.1–160 U ml^{-1}	0.04 U ml^{-1}	(Li et al., 2010b)
	Anti-CA15–3/ NGS-GCE	0.1–20 U ml^{-1}	0.012 U ml^{-1}	(Li et al., 2013b)
	antiCA15–3/ZnO nanorods on QCM	0.5–26 U ml^{-1}	0.5 U ml^{-1}	(Wang et al., 2014)
VEGF	R1-VEGF/EDC-NHS3-MPA/Au	10–70 pg ml^{-1}	10 pg ml^{-1}	(Sezgintürk, 2011)
	Aptamer-methylene blue/Au electrode	50 pM–0.15 nM	5 pM	(Zhao et al., 2011)
EGFR	EA/Anti-EGFRab/DTSP/Au electrode	1 pg–100 ng ml^{-1}	1 pg ml^{-1}	(Vasudev et al., 2013)
	PG/PDITC/Cys/AuNPs/Au electrode	1 pg–1 μg ml^{-1}	0.34 pg ml^{-1}	(Elshafey et al., 2013)
ER	ERα-ERE-SiNW	1 pM–10 fM	10 fM	(Zhang et al., 2011)
FA	FA-Folate/DNA probe/Au electrode	10^{2}–10^{6} cells ml^{-1}	67 cells ml^{-1}	(Zhao et al., 2013a)
BP 1	Anti- BP 1 aptamer with intercalated methylene blue	5.3–23.6 nM	1.2 nM	(Pérez et al., 2015)
EpCAM	Anti – EpCAM/LC-SPDP/Au electrode	1×10^{5}–1×10^{8} cells ml^{-1}	1×10^{5} cells ml^{-1}	(Arya et al., 2013)
HER-2	Anti c-erbB2/Polypyrrole-NHS/GCE	100–10,000 cells ml^{-1}	100 cells ml^{-1}	(Seven et al., 2013)
	S6 aptamer/Au/ZnO/G ITO electrode	1×10^{2}–1×10^{6} cells ml^{-1}	58 cells ml^{-1}	(Liu et al., 2014a)
	Anti-HER-2/HER-2/Anti-HER-2-S-AP/SPCE	15–100 ng ml^{-1}	4.4 ng ml^{-1}	(Marques et al., 2014)
	Ab/GNP/HDT/GNP@MW-CILE	10–110 ng ml^{-1}	7.4 ng ml^{-1}	(Arkan et al., 2015)
	Anti – HER-2 Aptamer- capacitor chip	0.2–2 ng ml^{-1}	0. 2 ng ml^{-1}	(Qureshi et al., 2015)
HER-2 ECD	AP-Ab2/HER-2 ECD/Ab1/Protein A/MB/SPE	0–15 ng ml^{-1}	6 ng ml^{-1}	(Al-Khafaji et al., 2012)
HER-3	Anti-HER-3/HER-3/BSA/HER-3/4-ATP/Au electrode	0.4–2.4 pg ml^{-1}	0.4 pg ml^{-1}	(Sonuç and Sezgintürk, 2014)
Choline	ZnO – ChO - GCE	0.3–5.1 mM	0.647 mM	(Thiagarajan et al., 2016)
miR- 21	Anti-miR probe/GPE	–	6 pM	(Kilic et al., 2012)
	HRP/AuNPs–barcode/miR–NAH/AuNPs/graphene/Au electrode	0.01–700 pM	6 fM	(Yin et al., 2012)
	Hemin/miR/DNA-Au/probe/AuNPs/Au electrode	0.01–500 pM	6 fM	(Meng et al., 2013)
	ALP/APBA-biotin-AuNP/miR/DNA probe/Au electrode + redox cycling	10 fM–5 pM	3 fM	(Liu et al., 2014b)
	HRP-antiMBP-p19-dsRNA-MBs/SPCE	1.4–1.0 nM	4.2 fM	(Torrente-Rodríguez et al., 2014)
	Anti-miR/CP-AP/ PDDA/GCE	100 aM–1 nM	30 aM	(Miao et al., 2015)
	ALP/DNA probe/Au electrode + redox cycling	0.5 fM–1 pM	0.2 fM	(Liu et al., 2015)
	ALP/miR/DNA probe/Au electrode + redox cycling	0.5 fM–1 pM	0.2 fM	(Xia et al., 2015)
	Ir (III) /MB-DNA probe 2-AuNP /miR/DNA probe1/Au electrode	5 fM–1 pM	1.6 fM	(Miao et al., 2016)
	Anti-miR/MWCNT- COOH/GCE	1.0–500 pM	84.3 fM	(Rafiee-Pour et al., 2016)
	ALP/DNA probe 2/miR/DNA probe 1-AuNPs/WO_3-graphene/GCE + redox cycling	0.0 fM–100 pM	0.05 fM	(Shuai et al., 2016)
miR – 21, miR – 205	P19 captured dual aptamer complex on SPCE	2–10 nM	0.6 nM	(Torrente-Rodríguez et al., 2015)
miR – 21, 32, 122, 141, 200	Anti-miR probe/SPE –p19 based HPD – sensor	10 aM–1 μM	5 aM	(Labib et al., 2013b)
miR – 155	Anti-miR probe/GNR/GO/GCE	2.0 fM–8.0 pM	0.6 fM	(Azimzadeh et al., 2016)
	Anti-miR probe/AuSPE	10 aM–1.0 nM	5.7 aM	(Cardoso et al., 2016)

and miRs have been reported for optical breast cancer biosensing such as, aptamers (Cai et al., 2015b; Hua et al., 2013; Kim and Jeong, 2011; Zhao et al., 2015a), antibodies (Chang et al., 2011; Chang et al., 2014b; Chen et al., 2015; Hun and Zhang, 2009), cDNA (Chen et al., 2014a), lectins (Park et al., 2013), folic acid (Zhang et al., 2014), cancer antigen GIPC-I (Salama et al., 2007) etc. Also, some optical labels such as thioflavin (Chen et al., 2014b) and terbium III (Tb^{3+}) (Cai et al., 2015b; Zhang et al., 2015) have been employed to produce target dependent fluorescence responses. Some important attributes of optical and mass change based breast cancer biosensors have been discussed in Table 4.

4.2.1. *Fluorescence resonance energy transfer (FRET)*

FRET is a light emission phenomenon observed between two

Table 4
Specifications of optical and mass change based breast cancer biosensors.

Target molecule	Biosensor specification	Linear range of detection	Detection limit	Reference
BRCA1	MB based FRET biosensor	0.2–2.5 μM	70 nM	(Culha et al., 2004)
HER-2 /ErbB2	LSPCF based fluorescence biosensor	1–1000 pg ml^{-1}	1.8 pg ml^{-1}	(Chang et al., 2011)
	Thioflvain T induced quadraplex based fluorescent biosensor	100 fM–1 pM	20 fM	(Chen et al., 2014b)
	Longitudinal extension of microcantilever with scFv	0.06–0.6 nM	0.06 nM	(Capobianco et al., 2011)
MUC1	Three component DNA system with QD	0–2 μM	250 nM	(Cheng et al., 2009)
	LSAW aptasensor based piezoelectric biosensor	1×10^2 –1×10^7 cells ml^{-1}	32 cells ml^{-1}	(Chang et al., 2014a)
	Anti MUC 1 aptamer with Tb^{3+}	500–500,000 cells ml^{-1}	70 cells ml^{-1}	(Cai et al., 2015b)
	Fluorescent anti-MUC 1 aptamer	–	10 ± 5 cells ml^{-1}	(Zhao et al., 2015a)
CA15–3	Antibody – lectin sandwich assay	1.25–25 U ml^{-1}	0.4 U ml^{-1}	(Park et al., 2013)
	Gold nanorods based plasmonic biosensor	2–32 nM	10^{-10} M	(Chen et al., 2015)
CEA	Biobarcode and G- quadraplex/hemin based colorimetric assay	0.025–40 ng ml^{-1}	0.025 ng ml^{-1}	(Zhou et al., 2014)
miR – 21	miR-21 tagged R. luciferase competitive assay	1×10^{-11}–1×10^{-15} M	1 fM	(Cissell et al., 2008)
	Anti- miR – 21 aptamer with Tb^{3+}	50 fM–1.0 pM	8.0 fM	(Zhang et al., 2015)
	MB/GDNA/ Seal probe mediated TIRCA	10 aM–1.0 nM	5 aM	(Li et al., 2016)

fluorophores. Fluorophores are the chemical compounds that absorb a particular wavelength of light, undergo excitation and then emit light at a higher wavelength. To observe FRET, the fluorophores should be selected wisely so as the emission wavelength of the donor should overlap the excitation wavelength of the acceptor. Certain compounds that act as acceptors decrease the fluorescence intensity and are called quenchers. FRET is widely used in optical studies of biosensing and diverse compounds have been used as fluorophores or quenchers (Chang et al., 2011; Cheng et al., 2009; Culha et al., 2004; Degliangeli et al., 2014; He et al., 2012; Zhao et al., 2015a). For example, conjugated polymers with high quantum yield and multiple color emission capable of simultaneous multiple targeting have been used to differentiate between breast cancer live cells from other cancer cell lines (Li et al., 2011b). Graphene oxide (GO) as a fluorescence quencher has been employed to detect MUC1 through MUC1-aptamer association (He et al., 2012). In this study, fluorophore labelled aptamer in the absence of MUC1 made close association with GO, which resulted in fluorescence quenching of the fluorophore. But in the presence of MUC1, aptamer–MUC1 association disrupted aptamer interaction with GO and restored fluorescence. Enhanced fluorescence output of Tb^{3+} bound to ssDNA has also been investigated to obtain target dependent response (Cai et al., 2015b; Zhang et al., 2015). Tb^{3+} is insensitive to duplex formation and hence, display no or very low change in fluorescence intensity. It was chosen due to its long fluorescence lifetime, large Strokes shift and sharp emission spectra. In addition to this, it is nontoxic to biological systems and has simple synthesis. It was demonstrated that the Tb^{3+} based method is more versatile and efficient detection system due to higher simplicity and sensitivity in comparison to other fluorophores (Cai et al., 2015b).

Apart from the direct fluorescence studies, optical fibre is the most convenient platform for photosensitive recognition of target moiety with the bioreceptor (Chang et al., 2011; Salama et al., 2007). In an initial investigation, autoantibodies (human monoclonal IgM antibody 27. B1) against cancer protein GIPC-1 (GIPC PDZ domain containing family member 1) were captured on optical fibre through recombinant GIPC-1 (r-GIPC-1) association which enabled 50 and 500 fold more sensitive detection than chemiluminscent ELISA and colorimetric ELISA, respectively (Salama et al., 2007). Modified surface of the fibre proved efficient platform for biomolecule immobilization to produce chemiluminscent signals. In an another study localized surface plasmon coupled fluorescence fibre optic biosensor (LSPCF – FOB) was developed for total prostate specific antigen (*t*-PSA) evaluation of breast cancer patient's serum samples (Chang et al., 2011). The bioassay characterization was based on enhancement of fluorophore fluorescence in the vicinity of gold nanoparticles which were employed in the bioassay as detection antibody carriers (Chang et al., 2011).

4.2.2. Quantum dots (QD)

Use of QD has gained momentum in past few years. The enhanced FRET, electrochemi- luminescence (ECL) and photoluminescence (PL) of QDs have made them preferred labelling options. Easy surface modification of QDs provided better opportunities for bioconjugate formation with aptamers or antibodies (Algar et al., 2010; Li et al., 2011a). Quantum dots (QD) offer advantage of size dependent fluorescence emission which is generated by capturing the target cell (breast cancer cells) on QD through alternate aptamers against two different surface proteins or dual antibody complexes (Fig. 3B). Taking the advantage of inherent properties of QDs, Cheng et al., designed an assembly of three DNA sequences to relate fluorescence quenching with MUC1 concentration in the sample. Decrease in fluorescence was observed in the presence of target protein when all the three DNA components got hybridized resulting in close association of fluorophore and quencher (Cheng et al., 2009). QD have also been used to visualize cancer cells through Erb2 specific aptamer assembly (Kim and Jeong, 2011). Immobilization of QD on silicon dioxide (SiO_2) nanoparticles offered enhanced PL emission and signal amplification when conjugated with target MCF-7 cells (Hua et al., 2013). Generally, cadmium (Cd) core QD are synthesized and their dissolution released Cd is quantified and related to antigen concentration by optical and voltametric techniques (Hua et al., 2013; Li et al., 2011a).

4.2.3. Surface plasmon resonance (SPR)

SPR biotransducers provide an opportunity of real time sensing of bimolecular interaction in a rapid, sensitive and label-free way. It works on simple principle of change in refractive index of the sensing medium on gold surface generally due to bimolecular interactions that result in change in reflected light or resonance angle. It has profound application in breast cancer biosensing and has been employed for the detection of point mutations in breast cancer related genes (Carrascosa et al., 2009; Li et al., 2006; Li et al., 2014b). But, SPR suffer from low sensitivity issues. Inclusion of nanoparticles has overcome the limitations of conventional SPR technique and worked as substrate enhancement and amplification tags (Nguyen et al., 2015; Zhao et al., 2015b). Nanoparticle based SPR has been employed to detect some breast cancer biomarkers such as HER-2 (Martin et al., 2006), PR (Yuan et al., 2007), ER (Neo et al., 2009), CA15-3 (Chang et al., 2010; Liang et al., 2012) and PSA (Uludag and Tothill, 2012). SPR analysis has been applied

to serum as well as saliva samples confirming its plasticity and adaptability to different sample matrixes (Liang et al., 2012; Uludag and Tothill, 2012).

4.2.4. Colorimetric assays

Color development based assays are preferred for commercialization purpose due to simple interpretation and easy handling. Color development is usually coupled with formation of target-bioreceptor complex that helps in realization of target concentration proportional to color intensity. AuNP have been used as color developing moiety in numerous studies. Aggregation of AuNPs (labelled with anti-target antibodies or aptamers) in the presence of target cells result in color change of AuNPs and hence enable target detection (Lu et al., 2010). HRP or platinum-gold nanoparticles (PtAuNP) catalysed conversion of 3,3′,5,5′- tetramethylbenzidine (TMB) to a colored product is an another strategy commonly engaged as an indicator of aptamer or antibody reaction with the target protein (Wang et al., 2015a; Zhang et al., 2015). Conversion of luminol–piodophenol–H_2O_2 to its colored product through alkaline phosphatase has also been reported (Wei et al., 2011). Very recently, Li et al. applied the phenomenon of strand displacement amplification (SDA) and toehold initiated rolling circle amplification (TIRCA) to design a novel amplification machine for colorimetric estimation of miR-21 in MCF-7 cell lysates (Li et al., 2016).

4.3. Mass change based biotransducers

Some investigators have endeavored to develop mass change based biotransducres for breast cancer biosensors, through interaction of Ag-Ab on mass sensitive platform. The complex formation is associated with change in refractive index or shift in resonance in accordance with target concentration. Participation of nanocomposits to enhance target effect has also been investigated (Gruhl et al., 2010; Wang et al., 2014). Surface acoustic wave biosensors with surfaces modified with nanomaterials are reported to produce sensitive detection of breast cancer (Chang et al., 2014a; Crivianu-Gaita et al., 2016; Gruhl et al., 2010). Piezoelectric microcantilever sensors (PEMS) provide sensitive platform for Ag-Ab interaction, and peptide 18–4 based shift in resonance frequency (Etayash et al., 2015; Loo et al., 2011). But longitudinal extension of piezoelectric microcantilever containing H3 single-chain variable fragment (scFv) antibody as bioreceptor is reported to offer lower detection limit for HER-2 than lower frequency flexural mode (Capobianco et al., 2011). Technical attributes of relevant studies are discussed in Table 4. A cost effective, silicon microring resonator that enabled estimation of resonance wavelength shift upon Ag-Ab interaction has also been reported (Kim et al., 2013). A very prominent technology pertaining to early diagnosis of breast cancer through detection of autoantibodies against ATP6AP1 proteins in saliva has been postulated (Arif et al., 2015). This study has given a blue print for construction of a quartz crystal microbalance biosensor which could provide an easy and reliable (70% accuracy) detection method for regular and timely breast cancer check-ups.

4.4. Devices to capture and analyse circulating tumor cells (CTC) in breast cancer

Microfluidic chip technology is the science of controlling fluids in multi-microchannels for analytical purposes. It has played a pivotal role in recognition and capture of breast cancer antigens because of restricted entry of cells through multichannel chip minimize cell loss and contamination (Kellner et al., 2011). Therefore, in addition to fluorescence based diagnostic techniques, some highly advanced automated microfluidic devices have also been applied for isolation of CTC from serum samples (Hyun et al., 2013; Moon et al., 2011; Nagrath et al., 2007; Tan et al., 2009; Wei et al., 2011). Diamagnetic property of cancer cells as compared to paramagnetic behavior of normal RBCs have also been exploited as isolation strategy (Han et al., 2006).

The basic idea for microfluidic based biosensing was formulated by Nagrath et al. (2007). They designed a microchip (CTC chip) for isolation of CTC from varied metastatic cancers including breast cancer. Flow rate through the device under shear forces was manifested as important parameter to attain capture efficiency of 65% (Nagrath et al., 2007). Fabrication of high throughput micro – sampling unit (HTMSU) for prospective capture of CTC, equipped with conductivity sensor for enumeration has also been reported (Adams et al., 2008). The device provided the advantage over other sensors, as it omitted the need of sample pre-treatment (centrifugation) or post treatment (staining) for inventory. This also reduced the interference problems due to erythrocytes or leucocytes which are responsible for false positive or negative results. Workers were able to process 1 ml untreated whole blood in 30 min that could be reduced to 2.7 min by designing the capture channel with different aspect ratio. CTC capture on solid surface is the primary requirement for CTC enumeration and initial studies were based on EpCAM realization on cell surface (Chung et al., 2011; Kim et al., 2015; Nagrath et al., 2007; Song et al., 2013; Zhao et al., 2013b). Recently, employment of fluorescent nanoparticles ($Ru(bpy)_3$ – SiNP) have been reported for recognition of CEA (Wei et al., 2011), HER-2 and MUC1 protein (Jo et al., 2015).

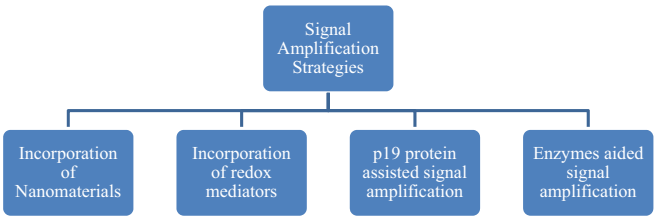
Some dimension optimized flow channel devices have also been fabricated that allow size dependent segregation of CTC from normal WBC. Generally CTC are larger than WBC. Taking advantage of this fact, multiple microchips have been designed to provide high throughput filtration efficiency (Moon et al., 2011). Highly efficient (99.24%) separation of CTC from RBC and WBC has been attained using multi orifice flow fractionation (MOFF) followed by dielectrophoresis (DEP) (Hyun et al., 2013; Moon et al., 2011). Recently, a silicon nanowire platform (SiNW) has been fabricated to specifically isolate breast BT20 carcinoma through EpCAM antibody labelling (Kim et al., 2015). Specific peptide 18–4 assisted isolation of CTC is demonstrated through micro cantilever arrays with a detection limit of 50–100 cells ml^{-1} (Etayash et al., 2015).

5. Signal amplification strategies

Although the perfect combination of bioreceptor and the biotransducer decides the sensitivity of the developed biosensor but, incorporation of some signal amplification modules is necessary to magnify the output signal especially when dealing with very minute quantities of the target entities. This section is dedicated to discuss all such modifications adapted to enhance biosensor efficiency and lower down the detection limits. The section is further divided in to subsections as per the Scheme 3, to entail various signal amplification strategies employed in electrochemical and optical biosensing.

5.1. Nanomaterials

Surface chemistry plays a pivotal role in biosensing, especially in electrochemical investigations, and it need to be managed to produce desired signal outcome. Certain inorganic components have been explored to functionalize the electrode surface that facilitate rapid electron transfer and improve recognition molecule adsorption on the electrode surface. The prerequisite for surface modifiers are that these should be biocompatible, thermally stable, easily functionalizable and provide antifouling effect. AuNPs or gold nanoclusters, graphene oxide (GO), multiwall carbon



Scheme 3. Signal amplification strategies.

nanotubes (MWCNT) or carbon nanorods are among the recognized nanomaterials famed for their highly conductive nature and enlarged surface area (Arkan et al., 2015; Benvidi et al., 2015; Kumar et al., 2015; Wang et al., 2015a; Wang et al., 2015b). Such materials not only facilitate biomolecule immobilization on transient platforms but also expand electrochemical properties such as low background current, high signal to noise ratio and amplified signals. These properties help to attain surface characteristics that is ought to deliver sensitive output signals. Among all the nanomaterials, AuNPs are laced with all attributes required for electrochemical biosensing. An elaborate account of AuNP participation in cancer biomarker detection has been already discussed (Devi et al., 2015). Non-toxic, simple and rapid synthesis, convenient functionalization, large surface area to volume ratio and high electrical conductivity have raised their preference for clinical diagnosis of breast cancer (Chun et al., 2013; Elshafey et al., 2013; Florea et al., 2013; Li et al., 2011c; Liu et al., 2014a; Selwyna et al., 2013; Wang et al., 2015a; Wang et al., 2015b; Yan et al., 2013; Zhu et al., 2013). Size of nanoparticles is an important parameter considered for their easy functionalization which is necessary for stable adsorption of DNA probes, aptamers or antibodies. Also, electrodeposition or direct adsorption of AuNPs on gold platform decreases interfacial resistance offered by biomolecule adsorption on electrode surface (Arya et al., 2012; Arya et al., 2013; Chun et al., 2013; Florea et al., 2013; Li et al., 2011a; Li et al., 2010a; Pérez et al., 2015; Zhao et al., 2011). Association of AuNPs with some other nanocomposits escalate the signal amplification effect. For instance, amalgamating Zinc Oxide (ZnO) (superior photoelectric properties) with AuNP (better electron transportation property) results in more sensitive response against HER-2 (Liu et al., 2014a) and CEA (Norouzi et al., 2011). Gold – Titanium Oxide (Au-TiO_2) nano composites offered fast electron transfer with biocompatible interface for biomolecule immobilization (Yang et al., 2011). Not only for electrochemical analysis, AuNPs have also crested their place in optical studies, such as introduction of AuNP to enhance sensitivity of ELISA (Ambrosi et al., 2009) as well as fluorescent and colorimetric assays (Cai et al., 2015b; Chang et al., 2011; Chang et al., 2014b; Degliangeli et al., 2014; Lu et al., 2010; Manikandan et al., 2014; Zhang et al., 2014).

Along with AuNPs, carbon based nanomaterials (MWCNT and graphene) have also been extensively applied as electron transfer facilitators in designing breast cancer biosensors (Arkan et al., 2015; Hu et al., 2014; Li et al., 2011c; Li et al., 2010b; Myung et al., 2011; Rafiee-Pour et al., 2016; Sun et al., 2011; Xiang et al., 2011; Zhang et al., 2011). CNT has shown their potential in research areas ranging from aerospace engineering to nanotechnology based applications. Due to their large surface area and hydrophobic nature, these offer high adsorption affinities for aromatic and aliphatic molecules including proteins and antibodies along with enhanced conductivity (Benvidi et al., 2016; Feng et al., 2011). Reduced, porous GO or N-doped graphene integrated with other nanocomposits such as AuNP and ZnO have been postulated to increase the sensitivity and dynamic range of detection as compared to unmodified or MWCNT modified electrodes (Azimzadeh et al., 2016; Kong et al., 2011; Li et al., 2013b; Liu et al., 2014a; Myung et al., 2011; Yan et al., 2013; Zhong et al., 2010). This could be because of sp^2 bonded carbon atoms densely packed in honeycomb lattice to form one atom thick planar sheet which provide minimum resistance to electron flow. The $\pi - \pi$ interaction of GO with ssDNA makes it an excellent acceptor in resonance energy transfer and provides valuable tool to conduct fluorescence based studies (He et al., 2012).

Owing to better photoelectrical properties and high isoelectric point of ZnO, it has been a valuable adsorption platform for biomolecule immobilization (Liu et al., 2014a; Norouzi et al., 2011; Thiagarajan et al., 2016; Wang et al., 2014). Wet chemical route of ZnO synthesis directly on gold electrode provides strong affinity with better sensitivity for biomolecule adsorption (Wang et al., 2014). Interestingly, platinum nanoparticles (PtNPs) that own peroxidase like activity, find their application as HRP substitute to carry out either oxidation of hydroquinone (Yang et al., 2011) or TMB in the presence of H_2O_2 (Wang et al., 2015a; Zhang et al., 2014) leading to electrochemical or optical signals. It is customary to integrate them with other nanomaterials such as AuNP, CNT or graphene that either maximize their catalytic activity or act as stabilizer and reductant. PtNPs have also been explored as electron transfer facilitators to enhance analytical performance. Assembly of Pt nanoclusters and glucose oxidase on organosilica@chitosan nano spheres has been demonstrated as an efficient, mediator less and reagent less method to detect CA15–3 in serum samples (Li et al., 2010b). Employment of photostable, fluorescent tris (2,2′-bipyridyl) dichlororuthenium (II) hexahydrate (Ru(BPY)3)-doped SiNPs ($Ru(bpy)_3$ – SiNP) are reported to provide distinct fluorescence when encountered HER-2 (+) and MUC1 (+) cells (Jo et al., 2015). Recently application of modified nanomaterials with improved electrochemical and optical properties is encouraged to achieve the desired specificity and sensitivity in breast cancer diagnosis (Shuai et al., 2016).

5.2. Redox mediators

Electrochemical biosensors are dependent on the flow of electrons from the bio-complex to the electrode surface. More efficient is the electron transfer, more will be the sensitivity of the developed system. To increase the rate of electron transport, external mediators are introduced in the system that can proficiently increase the electron flow from the bio-complex activity to the electrode surface without undergoing side reactions (Chaubey and Malhotra, 2002). $Fe(CN)_6^{3-/4}$ complex is the most extensively exploited mediator system for impedance studies in aqueous phase (Arkan et al., 2015; Arya et al., 2012; Arya et al., 2013; Benvidi et al., 2015; Chun et al., 2013; Elshafey et al., 2013; Fan et al., 2013; Florea et al., 2013; Norouzi et al., 2011; Sonuç and Sezgintürk, 2014; Vasudev et al., 2013), but in few cases hydroquinone has also been experimented (Meng et al., 2013; Seven et al., 2013; Yang et al., 2011; Yin et al., 2012). Oxidation of hydroquinone is integrated with reduction of H_2O_2, followed by regeneration of hydroquinone at electrode surface by electrochemical reduction of quinone. These mediators not only lower down the working redox potential to avoid interfering species (serum protein components), but also make the system free from oxygen and pH sensitivity. Apart from this, number of electrochemically active agents have been incorporated with bio-complexes to enhance electron flow. Out of these, methylene blue is the most extensively used electrochemically active agent in breast cancer biosensors. This is because methylene blue associates with ssDNA and dsDNA distinctly which produces change in peak current after hybridization. Taking the advantage of this feature, methylene blue has been assimilated in aptamer based biosensor construction where its assembly with surface protein or miR results in close association of methylene blue with electrode surface

(Miao et al., 2016; Mucelli et al., 2008; Rafiee-Pour et al., 2016; Yang et al., 2014; Zhao et al., 2011) or retains it away from electrode surface (Florea et al., 2013; Pérez et al., 2015). The length of DNA employed plays the crucial role in deciding the frequency of electron transfer rate. Shorter is the length, rapid will be the transfer.

Another mediator, thionine has found its application in electrochemical breast cancer biosensors. Being a positively charged ion, thionine is usually amalgamated with negatively charged polymers or cross linkers such as PSS to attain rapid transfer rate along with higher adsorption of bioreceptor. Association of thionine with discrete nanomaterials have helped in amplification of voltametric signals (Kong et al., 2011; Li et al., 2011c; Yan et al., 2013). Electrode surface modification with some infrequent mediators such as perylene tetracarboxylic acid [PTCA] (Feng et al., 2011), ferrocene monocarboxylic acid (Prabhulkar and Li, 2009), osmium VI (Bartosik et al., 2014; Bartosik et al., 2013), oracet blue (Azimzadeh et al., 2016) and azure I (Sun et al., 2011) have also been reported in breast cancer biosensors. In addition, $[Ru(NH_3)_6]^{3+}$ has been reported as DNA binding agent to decipher MCF-7 cells concentration on electrode surface through folate assisted capture of cells on probe DNA. In the study, reduction in peak current of the $[Ru(NH_3)_6]^{3+}$ mediator was observed with decrease in number of MCF-7 cells on electrode surface (Zhao et al., 2013a).

5.3. p19 protein assisted signal amplification

A very important criterion of a successful biosensor is the ability to amplify the signals. An array of strategies has been devised to somehow improve the target- bioreceptor association. Recently, an organic moiety has been observed to display its potential as an indicator of recognition reaction and has ability to bind the complex. A viral protein p19 regarded as RNA silencing suppressor, capable to specifically bind 20–23 nucleotide long miR has been used as label to capture miR-21 bound to its aptamer. The importance of this protein lies in the fact that it does not bind to ssRNA or ss/dsDNA and hence describes its specific nature towards dsRNA. Although p19 has been widely used as complexing molecule, but it suffers from drawbacks like requirement of qPCR of isolated RNA and its loss due to solution based hybridization (Kilic et al., 2013; Labib et al., 2013a, 2013b). To overcome the above drawbacks, few researchers have immobilised p19 protein on magnetic beads (Campuzano et al., 2014; Torrente-Rodríguez et al., 2015; Torrente-Rodríguez et al., 2014). It enabled the easy isolation of miR complex at electrode surface from the solution and lead to many fold increase in detection limit as illustrated in Fig. 4A.

In another study, the ingenious production of p19 fusion protein with maltose binding protein (MBP) at N- terminal and chitin binding domain (CBD) at the C- terminal has been employed. This enabled the binding of HRP labelled anti-MBP antibodies to the whole complex and lead to dose-dependent analysis of miR-21 through HRP/H_2O_2/HQ system. Although in this study, the assay time (1 h) was less but the detection limit for the analyte was higher than previous p19 based studies (Torrente-Rodríguez et al., 2014). Further, Torrente-Rodríguez et al. extended the approach by targeting two biomarkers miR-21 (over expressed) and miR-205 (under expressed) simultaneously in metastatic breast cancer (Torrente-Rodríguez et al., 2015).

5.4. Enzyme aided signal amplification strategies

Enzymes have special importance in biosensing, either as bioreceptor or as amplification tools. This section is dedicated to discuss the role of enzymes as signal amplification tools. Although a lot of studies are reported on various enzyme based amplification strategies, the present review is focussed on duplex specific nucleases (DSN) and redox cycling studies pertaining to breast cancer detection.

5.4.1. Duplex specific nuclease (DSN) based recycling and amplification of target moieties

Recycling of target moiety is desired while designing the bioassay principle. In this case, sequence specific or hybrid specific cleavage vehicles such as endonucleases play an important role. The selective cleavage preference of duplex specific nucleases (DSN) to cleave ssDNA in dsDNA or RNA – DNA hybrid has been explored as an amplification strategy for recycling of target miRs. The detection method involves immobilization of a DNA probe complementary to miR-21 (capture probe) on the electrode surface. Addition of miR-21 activates DSN and cleaves the capture probe hybridized with miR-21. This leads to removal of the capture probe from electrode surface leading to decreased resistance in EIS studies. DSN assisted cleavage of capture probe facilitates binding of target miRs to next DNA probe and initiation of another round of cleavage reaction resulting in an amplified response (Degliangeli et al., 2014; Miao et al., 2015; Yang et al., 2014; Zhang et al., 2016; Zhang et al., 2015; Zhou et al., 2014). Diagrammatic presentation of the concept is illustrated in Fig. 4B. Adsorption of separate capture probes (labelled with methylene blue and ferrocene) against two different miRs could deliver better sensitivity towards breast cancer (Yang et al., 2014).

5.4.2. Redox cycling based signal amplification

Recently, a new phenomenon called redox cycling has been engaged in the biosensor development which has been reported to amplify the detection levels by many folds (Shuai et al., 2016; Xia et al., 2015). As per the basic bioassay principle, alkaline phosphatase (ALP) enzyme is employed for the production of either p-aminophenol (*p*-AP) or ascorbic acid (AA) from p- aminophenol phosphate (*p*-APP) and ascorbic acid phosphate (AAP) respectively. These products are electro-oxidized to produce equivalent anodic current and the presence of reducing agent induces cyclic reduction of the oxidized product and amplifies corresponding signal by many folds. Reducing agents such as tris(2-carboxyethyl) phosphine (TCEP), nicotinamide adenine dinucleotide (NADH), cystamine, vitamin C, sodium borohydride ($NaBH_4$), hydrazine, etc. are the preferred one (Liu et al., 2015; Liu et al., 2014b; Xia et al., 2013). Among these, cystamine, TCEP and NADH have been reported as the best reducing agents for redox cycling of p-AP due to their chemical stability, strong reducing properties and display of low background current on SAM modified gold electrodes (Xia et al., 2013). Further, the study by Xia et al. also concluded that in addition to above advantages, cystamine and TCEP have no inhibitory effect on enzyme activity of ALP (Xia et al., 2013). In a later study, Xia et al. utilized TCEP as the reducing agent to analyse miR-21 by redox cycling of AA and achieved detection limit of 0.2 fM (Xia et al., 2015). Advancement in the same approach has been demonstrated by Shuai et al. (2016). In addition to redox cycling of AA, they used two hair pin probes, one to capture target miR and other to replace miR from the duplex which can bind to the next capture probe. This system displayed ten times better detection limit for miR-21 than the former one. It is very much evident from all these studies that the capture of miR through complementary probe is linked with enzyme (ALP) loading on the electrode surface which enabled production of redox active products and further signal enhancement (Shuai et al., 2016). Recently, redox cycling has attracted many researchers and has been engaged in mechanism elucidation of many processes such as ROS mediated DNA damage (Ensafi et al., 2016). This approach could further be extended to explain the process of cancer induction by various carcinogenic agents.

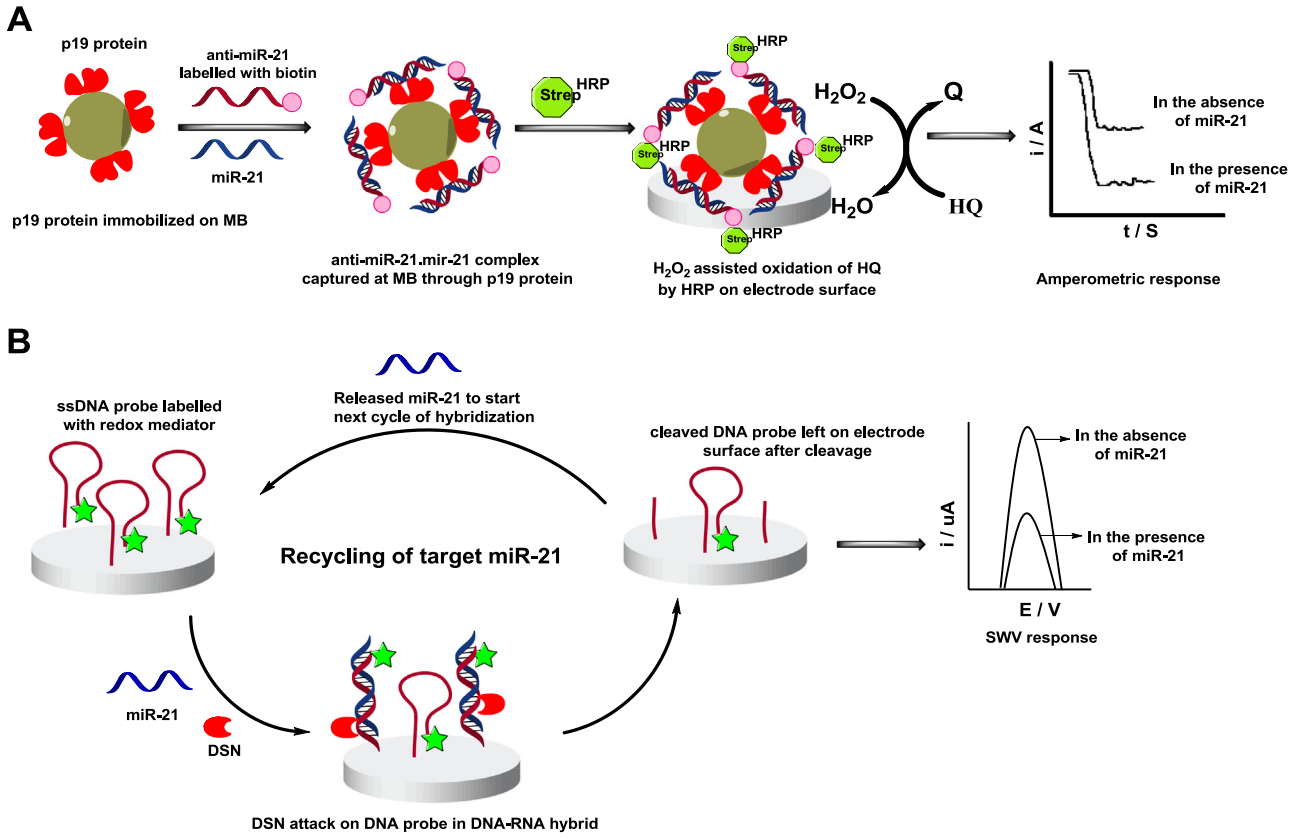


Fig. 4. Signal amplification strategies for miR detection: A) p19 assisted amplified detection of miR-21: p19 protein labelled magnetic beads brings biotin labelled *anti*-miR - miR hybrid at the electrode surface. Addition of streptavidin labelled HRP enzyme facilitates oxidation of hydroquinone to quinine leading to miR concentration dependent amperometric response. B) DSN aided recycling of target miRNA-21 to produce amplified signals: Hybridization of ssDNA probe (labelled with redox mediator) with complementary miR at electrode surface activates DSN, which cleaves the capture probe and releases target miR for an another round of hybridization leading to the signal amplification. Cleaved DNA probes left at the electrode surface produces miR concentration dependent and mediator related responses (modified from Yang et al., 2014).

6. Conclusion

Biosensors have changed the world of breast cancer diagnosis offering rapid, simple and cost effective routes. For low detection limits, glycoproteins are the most preferred targets for breast cancer diagnosis among miRs and CTC due to low abundance of latter ones. Among bioreceptors, aptamers have advantage of synthetic and thermo stable nature over antibodies that make them the perfect candidates for bio-detection. Electrochemical and optical routes of biosensing have enabled sensitive and specific detection in the past years. Latest improvement in signal amplification through nanomaterials, redox mediators or enzyme mediated cleavage or redox cycling has lead to10^2–10^5 fold enhancement in detection limits. Still, realization of on-line application in complex biological matrices is a challenge and manifests major purpose of biosensors. Also, there is a lack of specific early stage biosensing breast cancer tools, and further research is needed in this area to reduce high mortality rate associated with the high risk breast cancer.

Acknowledgment

Dr. Hardeep Kaur is thankful to the Department of Science and Technology (DST), New Delhi, India for providing financial assistance under DST-Disha/SoRF Programme [Ref. No. DST/Disha/SoRF-PM/011/2015(G)]. Further, due to limited focus of the article, some relevant and appropriate references could not be included, for which we apologize.

References

Adams, A.A., Okagbare, P.I., Feng, J., Hupert, M.L., Patterson, D., Göttert, J., McCarley, R.L., Nikitopoulos, D., Murphy, M.C., Soper, S.A., 2008. J. Am. Chem. Soc. 130 (27), 8633–88641.
Algar, W.R., Tavares, A.J., Krull, U.J., 2010. Anal. Chim. Acta 673 (1), 1–25.
Al-Khafaji, Q., Harris, M., Tombelli, S., Laschi, S., Turner, A., Mascini, M., Marrazza, G., 2012. Electroanalysis 24 (4), 735–742.
Ambrosi, A., Airo, F., Merkoçi, A., 2009. Anal. Chem. 82 (3), 1151–1156.
Andorfer, C.A., Necela, B.M., Thompson, E.A., Perez, E.A., 2011. Trends Mol. Med. 17 (6), 313–319.
dos Anjos Pultz, B., da Luz, F.A.C., de Faria, P.R., Lima, A.P., Oliveira, R.Ad.A., Silva, M.J. B., 2014. J. Cancer 5 (7), 559–571.
Arif, S., Qudsia, S., Urooj, S., Chaudry, N., Arshad, A., Andleeb, S., 2015. Biosens. Bioelectron. 65, 62–70.
Arkan, E., Saber, R., Karimi, Z., Shamsipur, M., 2015. Anal. Chim. Acta 874, 66–74.
Arya, S.K., Lee, K.C., Rahman, A.R.A., 2012. Lab. Chip 12 (13), 2362–2368.
Arya, S.K., Wang, K.Y., Wong, C.C., Rahman, A.R.A., 2013. Biosens. Bioelectron. 41, 446–451.
Azimzadeh, M., Rahaie, M., Nasirizadeh, N., Ashtari, K., Naderi-Manesh, H., 2016. Biosens. Bioelectron. 77, 99–106.
Bartosik, M., Hrstka, R., Palecek, E., Vojtesek, B., 2014. Anal. Chim. Acta 813, 35–40.
Bartosik, M., Trefulka, M., Hrstka, R., Vojtesek, B., Palecek, E., 2013. Electrochem. Commun. 33, 55–58.
Benvidi, A., Tezerjani, M.D., Jahanbani, S., Ardakani, M.M., Moshtaghioun, S.M., 2016. Talanta 147, 621–627.
Benvidi, A., Firouzabadi, A.D., Tezerjani, M.D., Moshtaghiun, S., Mazloum-Ardakani, M., Ansarin, A., 2015. J. Electroanal. Chem. 750, 57–64.
Brody, E.N., Gold, L., 2000. Rev. Mol. Biotechnol. 74 (1), 5–13.
Burgess, A.W., Cho, H.-S., Eigenbrot, C., Ferguson, K.M., Garrett, T.P., Leahy, D.J., Lemmon, M.A., Sliwkowski, M.X., Ward, C.W., Yokoyama, S., 2003. Mol. Cell 12 (3), 541–552.
Cai, H.-H., Pi, J., Lin, X., Li, B., Li, A., Yang, P.-H., Cai, J., 2015a. Biosens. Bioelectron. 74, 165–169.
Cai, S., Li, G., Zhang, X., Xia, Y., Chen, M., Wu, D., Chen, Q., Zhang, J., Chen, J., 2015b. Talanta 138, 225–230.
Campuzano, S., Torrente-Rodríguez, R.M., López-Hernández, E., Conzuelo, F., Granados, R., Sánchez-Puelles, J.M., Pingarrón, J.M., 2014. Angewandte Chemie Int. Ed. 53(24), pp. 6168–6171.

Capobianco, J.A., Shih, W.Y., Adams, G.P., Shih, W.-H., 2011. Sens. Actuators B-Chem 160 (1), 349–356.
Cardoso, A.R., Moreira, F.T., Fernandes, R., Sales, M.G.F., 2016. Biosens. Bioelectron. 80, 621–630.
Carrascosa, L.G., Calle, A., Lechuga, L.M., 2009. Anal. Bioanal. Chem. 393 (4), 1173–1182.
Chang, C.-C., Chiu, N.-F., Lin, D.S., Chu-Su, Y., Liang, Y.-H., Lin, C.-W., 2010. Anal. Chem. 82 (4), 1207–1212.
Chang, K., Pi, Y., Lu, W., Wang, F., Pan, F., Li, F., Jia, S., Shi, J., Deng, S., Chen, M., 2014a. Biosens. Bioelectron. 60, 318–324.
Chang, Y.-F., Hung, S.-H., Su, L.-C., Chen, R.-C., Chou, C., 2014b. Sens. Biosens. Res. 2, 1–7.
Chang, Y.-F., Hung, S.-H., Lee, Y.-J., Chen, R.-C., Su, L.-C., Lai, C.-S., Chou, C., 2011. Anal. Chem. 83 (13), 5324–5328.
Chaubey, A., Malhotra, B., 2002. Biosens. Bioelectron. 17 (6), 441–456.
Chen, J., Lin, J., Zhang, X., Cai, S., Wu, D., Li, C., Yang, S., Zhang, J., 2014b. Anal. Chim. Acta 817, 42–47.
Chen, J.-K., Zhou, G.-Y., Chang, C.-J., Lee, A.-W., Chang, F.-C., 2014a. Biosens. Bioelectron. 54, 35–41.
Chen, S., Zhao, Q., Zhang, L., Wang, L., Zeng, Y., Huang, H., 2015. Sens. Actuators B-Chem 221, 1391–1397.
Cheng, A.K., Su, H., Wang, Y.A., Yu, H.-Z., 2009. Anal. Chem. 81 (15), 6130–6139.
Chun, L., Kim, S.-E., Cho, M., Choe, W.-s, Nam, J., Lee, D.W., Lee, Y., 2013. Sens. Actuators B- Chem 186, 446–450.
Chung, Y.-K., Reboud, J., Lee, K.C., Lim, H.M., Lim, P.Y., Wang, K.Y., Tang, K.C., Ji, H., Chen, Y., 2011. Biosens. Bioelectron. 26 (5), 2520–2526.
Cissell, K.A., Rahimi, Y., Shrestha, S., Hunt, E.A., Deo, S.K., 2008. Anal. Chem. 80 (7), 2319–2325.
Crivianu-Gaita, V., Aamer, M., Posaratnanathan, R.T., Romaschin, A., Thompson, M., 2016. Biosens. Bioelectron. 78, 92–99.
Culha, M., Stokes, D.L., Griffin, G.D., Vo-Dinh, T., 2004. Biosens. Bioelectron. 19 (9), 1007–1012.
Degliangeli, F., Kshirsagar, P., Brunetti, V., Pompa, P.P., Fiammengo, R., 2014. J. Am. Chem. Soc. 136 (6), 2264–2267.
DeSantis, C., Ma, J., Bryan, L., Jemal, A., 2014. CA Cancer J. Clin. 64 (1), 52–62.
Deutscher, S.L., Dickerson, M., Gui, G., Newton, J., Holm, J.E., Vogeltanz-Holm, N., Kliethermes, B., Hewett, J.E., Kumar, S.R., Quinn, T.P., 2010. BMC Cancer 10 (1), 1.
Devi, R.V., Doble, M., Verma, R.S., 2015. Biosens. Bioelectron. 68, 688–698.
Diaconu, I., Cristea, C., Hârceagă, V., Marrazza, G., Berindan-Neagoe, I., Săndulescu, R., 2013. Clin. Chim. Acta 425, 128–138.
Eletxigerra, U., Martinez-Perdiguero, J., Merino, S., Barderas, R., Torrente-Rodríguez, R., Villalonga, R., Pingarrón, J., Campuzano, S., 2015. Biosens. Bioelectron. 70, 34–41.
Elshafey, R., Tavares, A.C., Siaj, M., Zourob, M., 2013. Biosens. Bioelectron. 50, 143–149.
Ensafi, A.A., Jamei, H.R., Heydari-Bafrooei, E., Rezaei, B., 2016. Bioelectrochemistry 111, 15–22.
Etayash, H., Jiang, K., Azmi, S., Thundat, T., Kaur, K., 2015. Sci. Rep. . http://dx.doi.org/10.1038/srep13967
Fan, H., Zhang, Y., Wu, D., Ma, H., Li, X., Li, Y., Wang, H., Li, H., Du, B., Wei, Q., 2013. Anal. Chim. Acta 770, 62–67.
Feng, L., Chen, Y., Ren, J., Qu, X., 2011. Biomaterials 32 (11), 2930–2937.
Florea, A., Taleat, Z., Cristea, C., Mazloum-Ardakani, M., Săndulescu, R., 2013. Electrochem. Commun. 33, 127–130.
Fragoso, A., Latta, D., Laboria, N., von Germar, F., Hansen-Hagge, T.E., Kemmner, W., Gärtner, C., Klemm, R., Drese, K.S., O'Sullivan, C.K., 2011. Lab. Chip. 11 (4), 625–631.
Friedenson, B., 2007. BMC Cancer 7 (1), 1.
Gao, J., Zhang, Q., Xu, J., Guo, L., Li, X., 2013. Chin. J. Cancer Res. 25 (6), 743–748.
Gao, X., Zhang, Y., Chen, H., Chen, Z., Lin, X., 2011. Anal. Biochem. 414 (1), 70–76.
Gruhl, F.J., Rapp, M., Länge, K., 2010. Procedia Eng. 5, 914–917.
Han, K.-H., Han, A., Frazier, A.B., 2006. Biosens. Bioelectron. 21 (10), 1907–1914.
He, Y., Lin, Y., Tang, H., Pang, D., 2012. Nanoscale 4 (6), 2054–2059.
Hedau, S., Batra, M., Singh, U.R., Bharti, A.C., Ray, A., Das, B.C., 2015. J. Cancer Res. Ther. 11 (1), 158.
Heneghan, H.M., Miller, N., Lowery, A.J., Sweeney, K.J., Newell, J., Kerin, M.J., 2010. Ann. Surg. 251 (3), 499–505.
Hollingsworth, M.A., Swanson, B.J., 2004. Nat. Rev. Cancer 4 (1), 45–60.
Hu, R., Wen, W., Wang, Q., Xiong, H., Zhang, X., Gu, H., Wang, S., 2014. Biosens. Bioelectron. 53, 384–389.
Hua, X., Zhou, Z., Yuan, L., Liu, S., 2013. Anal. Chim. Acta 788, 135–140.
Huang, T.-H., Wu, F., Loeb, G.B., Hsu, R., Heidersbach, A., Brincat, A., Horiuchi, D., Lebbink, R.J., Mo, Y.-Y., Goga, A., 2009. J. Biol. Chem. 284 (27), 18515–18524.
Hun, X., Zhang, Z., 2009. Spectrochim. Acta A Mol. Biomol. Spectrosc. 74 (2), 410–414.
Hyun, K.-A., Kwon, K., Han, H., Kim, S.-I., Jung, H.-I., 2013. Biosens. Bioelectron. 40 (1), 206–212.
Jo, H., Her, J., Ban, C., 2015. Biosens. Bioelectron. 71, 129–136.
Kellner, C., Botero, M.L., Latta, D., Drese, K., Fragoso, A., O'Sullivan, C.K., 2011. Electrophoresis 32 (8), 926–930.
Keshavarz, M., Behpour, M., Rafiee-pour, H.-A., 2015. RSC Adv. 5 (45), 35651–35660.
Kilic, T., Topkaya, S.N., Ozsoz, M., 2013. Biosens. Bioelectron. 48, 165–171.
Kilic, T., Topkaya, S.N., Ariksoysal, D.O., Ozsoz, M., Ballar, P., Erac, Y., Gozen, O., 2012. Biosens. Bioelectron. 38 (1), 195–201.
Kim, D.-J., Lee, W.-Y., Park, N.-W., Kim, G.-S., Lee, K.-M., Kim, J., Choi, M.-K., Lee, G.H., Han, W., Lee, S.-K., 2015. Biosens. Bioelectron. 67, 370–378.
Kim, K.W., Song, J., Kee, J.S., Liu, Q., Lo, G.-Q., Park, M.K., 2013. Biosens. Bioelectron. 46, 15–21.
Kim, M.Y., Jeong, S., 2011. Nucleic Acid Ther. 21 (3), 173–178.
King, M.-C., Marks, J.H., Mandell, J.B., Group, N.Y.B.C.S., 2003. Science 302 (5645), 643–646.
Kohler, C., Barekati, Z., Radpour, R., Zhong, X.Y., 2011. Anticancer Res. 31 (8), 2623–2628.
Kong, F.-Y., Xu, M.-T., Xu, J.-J., Chen, H.-Y., 2011. Talanta 85 (5), 2620–2625.
Kumar, S., Ahlawat, W., Kumar, R., Dilbaghi, N., 2015. Biosens. Bioelectron. 70, 498–503.
de la Escosura-Muñiz, A., Merkoçi, A., 2011. Small 7 (5), 675–682.
Labib, M., Ghobadloo, S.M., Khan, N., Kolpashchikov, D.M., Berezovski, M.V., 2013a. Anal. Chem. 85 (20), 9422–9427.
Labib, M., Khan, N., Ghobadloo, S.M., Cheng, J., Pezacki, J.P., Berezovski, M.V., 2013b. J. Am. Chem. Soc. 135 (8), 3027–3038.
Lacombe, J., Mangé, A., Solassol, J., 2014. J. Immunol. Res., 2014
Laidi, F., Bouziane, A., Errachid, A., Zaoui, F., 2015. Asian Pacific J. Cancer Preven. 17 (1), 335–339.
Le Naour, F., Misek, D.E., Krause, M.C., Deneux, L., Giordano, T.J., Scholl, S., Hanash, S. M., 2001. Clin. Cancer Res. 7 (11), 3328–3335.
Li, D., Cheng, W., Yan, Y., Zhang, Y., Yin, Y., Ju, H., Ding, S., 2016. Talanta 146, 470–476.
Li, F.-R., Li, Q., Zhou, H.-X., Qi, H., Deng, C.-Y., 2013a. Nanomed. Nanotechnol. Biol. Med. 9 (7), 1106–1113.
Li, H., He, J., Li, S., Turner, A.P., 2013b. Biosens. Bioelectron. 43, 25–29.
Li, J., Xu, M., Huang, H., Zhou, J., Abdel-Halimb, E., Zhang, J.-R., Zhu, J.-J., 2011a. Talanta 85 (4), 2113–2120.
Li, K., Zhan, R., Feng, S.-S., Liu, B., 2011b. Anal. Chem. 83 (6), 2125–2132.
Li, Q., Tang, D., Tang, J., Su, B., Huang, J., Chen, G., 2011c. Talanta 84 (2), 538–546.
Li, R., Wang, C., Hu, Y., Zheng, O., Guo, L., Lin, Z., Qiu, B., Chen, G., 2014a. Biosens. Bioelectron. 58, 226–231.
Li, T., Fan, Q., Liu, T., Zhu, X., Zhao, J., Li, G., 2010a. Biosens. Bioelectron. 25 (12), 2686–2689.
Li, W., Yuan, R., Chai, Y., Chen, S., 2010b. Biosens. Bioelectron. 25 (11), 2548–2552.
Li, Y., Wark, A.W., Lee, H.J., Corn, R.M., 2006. Anal. Chem. 78 (9), 3158–3164.
Li, Y., Yan, Y., Lei, Y., Zhao, D., Yuan, T., Zhang, D., Cheng, W., Ding, S., 2014b. Colloids Surf. B Biointerfaces 120, 15–20.
Liang, Y.-H., Chang, C.-C., Chen, C.-C., Chu-Su, Y., Lin, C.-W., 2012. Clin. Biochem. 45 (18), 1689–1693.
Liu, F., Zhang, Y., Yu, J., Wang, S., Ge, S., Song, X., 2014a. Biosens. Bioelectron. 51, 413–420.
Liu, L., Gao, Y., Liu, H., Xia, N., 2015. Sens. Actuators B: Chem. 208, 137–142.
Liu, L., Xia, N., Liu, H., Kang, X., Liu, X., Xue, C., He, X., 2014b. Biosens. Bioelectron. 53, 399–405.
Loo, L., Capobianco, J.A., Wu, W., Gao, X., Shih, W.Y., Shih, W.-H., Pourrezaei, K., Robinson, M.K., Adams, G.P., 2011. Anal. Chem. 83 (9), 3392–3397.
Lu, W., Arumugam, S.R., Senapati, D., Singh, A.K., Arbneshi, T., Khan, S.A., Yu, H., Ray, P.C., 2010. ACS Nano 4 (3), 1739–1749.
Lusi, E., Passamano, M., Guarascio, P., Scarpa, A., Schiavo, L., 2009. Anal. Chem. 81 (7), 2819–2822.
Lv, M., Zhu, X., Chen, W., Zhao, J., Tang, J., 2013. Cancer Biomark. 13 (5), 395–401.
Mahfoud, O.K., Rakovich, T.Y., Prina-Mello, A., Movia, D., Alves, F., Volkov, Y., 2014. RSC Adv. 4 (7), 3422–3442.
Manikandan, M., Abdelhamid, H.N., Talib, A., Wu, H.-F., 2014. Biosens. Bioelectron. 55, 180–186.
Marques, R.C., Viswanathan, S., Nouws, H.P., Delerue-Matos, C., González-García, M. B., 2014. Talanta 129, 594–599.
Martin, V., Sullivan, B., Walker, K., Hawk, H., Sullivan, B., Noe, L., 2006. Appl. Spectrosc. 60 (9), 994–1003.
Menard, S., Tagliabue, E., Campiglio, M., Pupa, S.M., 2000. J. Cell. Physiol. 182 (2), 150–162.
Meng, X., Zhou, Y., Liang, Q., Qu, X., Yang, Q., Yin, H., Ai, S., 2013. Analyst 138 (12), 3409–3415.
Miao, P., Wang, B., Yu, Z., Zhao, J., Tang, Y., 2015. Biosens. Bioelectron. 63, 365–370.
Miao, X., Wang, W., Kang, T., Liu, J., Shiu, K.-K., Leung, C.-H., Ma, D.-L., 2016. Biosens. Bioelectron. 86, 454–458.
Michaelson, J., Satija, S., Moore, R., Weber, G., Halpern, E., Garland, A., Puri, D., Kopans, D.B., 2002. Cancer 94 (1), 37–43.
Misek, D.E., Kim, E.H., 2011. Int. J. Proteom. . http://dx.doi.org/10.1155/2011/343582
Mishra, A., Verma, M., 2010. Cancers 2 (1), 190–208.
Moon, H.-S., Kwon, K., Kim, S.-I., Han, H., Sohn, J., Lee, S., Jung, H.-I., 2011. Lab. Chip 11 (6), 1118–1125.
Mucelli, S.P., Zamuner, M., Tormen, M., Stanta, G., Ugo, P., 2008. Biosens. Bioelectron. 23 (12), 1900–1903.
Myung, S., Solanki, A., Kim, C., Park, J., Kim, K.S., Lee, K.B., 2011. Adv. Mat. 23 (19), 2221–2225.
Nadal, R., Lorente, J.A., Rosell, R., Serrano, M.J., 2013. Expert Rev. Mol. Diagn. 13 (3), 295–307.
Nagrath, S., Sequist, L.V., Maheswaran, S., Bell, D.W., Irimia, D., Ulkus, L., Smith, M.R., Kwak, E.L., Digumarthy, S., Muzikansky, A., 2007. Nature 450 (7173), 1235–1239.
Nath, S., Mukherjee, P., 2014. Trends Mol. Med. 20 (6), 332–342.
Neo, S.J., Su, X., Thomsen, J.S., 2009. Anal. Chem. 81 (9), 3344–3349.
Nguyen, H.H., Park, J., Kang, S., Kim, M., 2015. Sensors 15 (5), 10481–10510.
Norouzi, P., Gupta, V.K., Faridbod, F., Pirali-Hamedani, M., Larijani, B., Ganjali, M.R., 2011. Anal. Chem. 83 (5), 1564–1570.
O'Donovan, P.J., Livingston, D.M., 2010. Carcinogenesis 31 (6), 961–967.

Park, Y.M., Kim, S.J., Kim, K., Han, Y.D., Yang, S.S., Yoon, H.C., 2013. Sens. Actuators B-Chem 186, 571–579.
Pérez, W.I., Soto, Y., Ramirez-Vick, J.E., Meléndez, E., 2015. J. Electroanal. Chem. 751, 49–56.
Piura, E., Piura, B., 2010. J. Oncol. . http://dx.doi.org/10.1155/2010/264926
Prabhulkar, S., Alwarappan, S., Liu, G., Li, C.-Z., 2009. Biosens. Bioelectron. 24 (12), 3524–3530.
Prabhulkar, S., Li, C.-Z., 2009. Electrical Immuno Nanosensor for Breast Cancer Biomarker Assay. Bioinfo. Biomed. Eng. 2009. ICBBE 2009. 3rd International Conference pp. 1–4. (IEEE).
Qin, W., Gui, G., Zhang, K., Twelves, D., Kliethermes, B., Sauter, E.R., 2012. BMC Cancer 12 (1), 1.
Qureshi, A., Gurbuz, Y., Niazi, J.H., 2015. Sens. Actuators B-Chem. 220, 1145–1151.
Rafiee-Pour, H.-A., Behpour, M., Keshavarz, M., 2016. Biosens. Bioelectron. 77, 202–207.
Raina, D., Kharbanda, S., Kufe, D., 2004. J. Biol. Chem. 279 (20), 20607–20612.
Salama, O., Herrmann, S., Tziknovsky, A., Piura, B., Meirovich, M., Trakht, I., Reed, B., Lobel, L.I., Marks, R.S., 2007. Biosens. Bioelectron. 22 (7), 1508–1516.
Schwarzenbach, H., 2013. Breast Cancer Res. 15 (5), 211.
Schwarzenbach, H., Pantel, K., 2015. Breast Cancer Res. 17 (1), 1–9.
Selwyna, P., Loganathan, P., Begam, K., 2013. Development of electrochemical biosensor for breast cancer detection using gold nanoparticle doped CA 15–3 antibody and antigen interaction. Signal Processing Image Processing & Pattern Recognition (ICSIPR), 2013 International Conference, pp. 75–81. (IEEE).
Seven, B., Bourourou, M., Elouarzaki, K., Constant, J.F., Gondran, C., Holzinger, M., Cosnier, S., Timur, S., 2013. Electrochem. Commun. 37, 36–39.
Sezgintürk, M.K., 2011. Biosens. Bioelectron. 26 (10), 4032–4039.
Shuai, H.-L., Huang, K.-J., Xing, L.-L., Chen, Y.-X., 2016. Biosens. Bioelectron. 86, 337–345.
Song, Y., Zhu, Z., An, Y., Zhang, W., Zhang, H., Liu, D., Yu, C., Duan, W., Yang, C.J., 2013. Anal. Chem. 85 (8), 4141–4149.
Sonuç, M.N., Sezgintürk, M.K., 2014. Talanta 120, 355–361.
Sun, A.-L., Chen, G.-R., Sheng, Q.-L., Zheng, J.-B., 2011. Biochem. Eng. J. 57, 1–6.
Sun, H., Zu, Y., 2015. Molecules 20 (7), 11959–11980.
Swisher, L.Z., Prior, A.M., Gunaratna, M.J., Shishido, S., Madiyar, F., Nguyen, T.A., Hua, D.H., Li, J., 2015. Nanomed. Nanotechnol. Biol. Med. 11 (7), 1695–1704.
Tan, S.J., Yobas, L., Lee, G.Y.H., Ong, C.N., Lim, C.T., 2009. Biomed. Micro. 11 (4), 883–892.
Tangvarasittichai, O., Jaiwang, W., Tangvarasittichai, S., 2015. Indian J. Clin. Biochem. 30 (1), 55–58.
Thiagarajan, V., Madhurantakam, S., Sethuraman, S., Rayappan, J.B.B., Krishnan, U. M., 2016. J. Colloid Interface Sci. 462, 334–340.
Tiwari, A., Gong, S., 2009. Talanta 77 (3), 1217–1222.
Torrente-Rodríguez, R., Campuzano, S., López-Hernández, E., Granados, R., Sánchez-Puelles, J., Pingarrón, J., 2014. Electroanalysis 26 (10), 2080–2087.
Torrente-Rodríguez, R., Campuzano, S., López-Hernández, E., Montiel, V.R.-V., Barderas, R., Granados, R., Sánchez-Puelles, J.M., Pingarrón, J., 2015. Biosens. Bioelectron. 66, 385–391.
Tothill, I.E., 2009. Semin. Cell Dev. Biol. 20 (1), 55–62.
Uludag, Y., Tothill, I.E., 2012. Anal. Chem. 84 (14), 5898–5904.
Vasudev, A., Kaushik, A., Bhansali, S., 2013. Biosens. Bioelectron. 39 (1), 300–305.
Vidi, P.-A., Leary, J.F., Lelièvre, S.A., 2013. Integr. Biol. 5 (9), 1110–1118.
Wang, K., Fan, D., Liu, Y., Wang, E., 2015a. Biosens. Bioelectron. 73, 1–6.
Wang, W., Fan, X., Xu, S., Davis, J.J., Luo, X., 2015b. Biosens. Bioelectron. 71, 51–56.
Wang, X., Yu, H., Lu, D., Zhang, J., Deng, W., 2014. Sens. Actuators B-Chem 195, 630–634.
Wei, W., Zhang, C., Qian, J., Liu, S., 2011. Anal. Biooanal. Chem 401 (10), 3269–3274.
Weigel, M.T., Dowsett, M., 2010. Endocr. Relat. Cancer 17 (4), R245–R262.
Won, J.Y., Choi, J.-W., Min, J., 2013. Biosens. Bioelectron. 40 (1), 161–166.
Wu, J., Lu, L.-Y., Yu, X., 2010. Protein Cell 1 (2), 117–123.
Xia, N., Zhang, Y., Wei, X., Huang, Y., Liu, L., 2015. Anal. Chim. Acta 878, 95–101.
Xia, N., Ma, F., Zhao, F., He, Q., Du, J., Li, S., Chen, J., Liu, L., 2013. Electrochim. Acta 109, 348–354.
Xiang, Y., Zhang, Y., Jiang, B., Chai, Y., Yuan, R., 2011. Sens. Actuators B-Chem 155 (1), 317–322.
Yadav, N., Chandra, D., 2013. Biochim. Biophys. Acta-Rev. Cancer 1836 (2), 336–344.
Yan, M., Sun, G., Liu, F., Lu, J., Yu, J., Song, X., 2013. Anal. Chim. Acta 798, 33–39.
Yang, C., Dou, B., Shi, K., Chai, Y., Xiang, Y., Yuan, R., 2014. Anal. Chem. 86 (23), 11913–11918.
Yang, H., Yuan, R., Chai, Y., Mao, L., Su, H., Jiang, W., Liang, M., 2011. Biochem. Eng. J. 56 (3), 116–124.
Yang, Q., Sakurai, T., Mori, I., Yoshimura, G., Nakamura, M., Nakamura, Y., Suzuma, T., Tamaki, T., Umemura, T., Kakudo, K., 2001. Cancer 92 (1), 54–60.
Yang, Z., Chevolot, Y., Géhin, T., Solassol, J., Mange, A., Souteyrand, E., Laurenceau, E., 2013. Biosens. Bioelectron. 40 (1), 385–392.
Yin, H., Zhou, Y., Chen, C., Zhu, L., Ai, S., 2012. Analyst 137 (6), 1389–1395.
Yuan, J., Oliver, R., Li, J., Lee, J., Aguilar, M., Wu, Y., 2007. Biosens. Bioelectron. 23 (1), 144–148.
Zhang, G.-J., Huang, M.J., Liu, E.T., Desai, K.V., 2011. Biosens. Bioelectron. 26 (7), 3233–3239.
Zhang, J., Wu, D.-Z., Cai, S.-X., Chen, M., Xia, Y.-K., Wu, F., Chen, J.-H., 2016. Biosens. Bioelectron. 75, 452–457.
Zhang, J., Wu, D., Chen, Q., Chen, M., Xia, Y., Cai, S., Zhang, X., Wu, F., Chen, J., 2015. Analyst 140 (15), 5082–5089.
Zhang, L.-N., Deng, H.-H., Lin, F.-L., Xu, X.-W., Weng, S.-H., Liu, A.-L., Lin, X.-H., Xia, X.-H., Chen, W., 2014. Anal. Chem. 86 (5), 2711–2718.
Zhao, B., Wu, P., Zhang, H., Cai, C., 2015a. Biosens. Bioelectron. 68, 763–770.
Zhao, J., Zhu, L., Guo, C., Gao, T., Zhu, X., Li, G., 2013a. Biosens. Bioelectron. 49, 329–333.
Zhao, S., Yang, W., Lai, R.Y., 2011. Biosens. Bioelectron. 26 (5), 2442–2447.
Zhao, S., Yang, H., Zhang, M., Zhang, D., Liu, Y., Liu, Y., Song, Y., Zhang, X., Li, H., Ma, W., 2013b. Cell Biochem. Biophys. 65 (2), 263–273.
Zhao, X., Wong, M.M.-K., Chiu, S.-K., Pang, S.W., 2015b. Biosens. Bioelectron. 74, 799–807.
Zhong, Z., Wu, W., Wang, D., Wang, D., Shan, J., Qing, Y., Zhang, Z., 2010. Biosens. Bioelectron. 25 (10), 2379–2383.
Zhou, W., Su, J., Chai, Y., Yuan, R., Xiang, Y., 2014. Biosens. Bioelectron. 53, 494–498.
Zhu, X., Yang, J., Liu, M., Wu, Y., Shen, Z., Li, G., 2013. Anal. Chim. Acta 764, 59–63.
Zhu, Y., Chandra, P., Shim, Y.-B., 2012. Anal. Chem. 85 (2), 1058–1064.