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Rapid biosensing tools for cancer biomarkers

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ABSTRACT

The present review critically discusses the latest developments in the field of smart diagnostic systems for cancer biomarkers. A wide coverage of recent biosensing approaches involving aptamers, enzymes, DNA probes, fluorescent probes, interacting proteins and antibodies in vicinity to transducers such as electrochemical, optical and piezoelectric is presented. Recent advanced developments in biosensing approaches for cancer biomarker owes much credit to functionalized nanomaterials due to their unique opto-electronic properties and enhanced surface to volume ratio. Biosensing methods for a plenty of cancer biomarkers has been summarized emphasizing the key principles involved.

1. Introduction

At present, human mortality due to cancer is next to heart ailments. Moreover, it has been projected that the fatality rates from cancer may surpass heart attacks in the near future (Siegel et al., 2015). Various strategies that are currently being implemented to identify and fight against this disease include therapeutic approaches, molecular imaging techniques and ultrasensitive monitoring of cancer biomarkers using innovative biosensing tools. Therapeutic approaches mainly investigate potential inhibitors against cancer promoting factors. However, this approach is rather remedial instead of preventive. Difficulties in combating cancer in advanced stages strongly recommend precautionary approach via upcoming quick and reliable cancer diagnostics.

Therefore, focus on early monitoring of cancerous tumor is as important as the discovery of lifesaving anticancer drugs and treatments (Hussain and Nguyen, 2014).

Necessity of early cancer prognosis through convenient and economical in vitro diagnostic tests motivates to devise miniaturized, robust and rapid multiplexed analytical platform based point-of-care (POC) devices (Gubala et al., 2012) for monitoring various forms of cancer at preliminary stages. Presently, popular methods for cancer diagnostics include biopsy analysis, tumor imaging, cancer biomarker monitoring through approaches such as enzyme-linked/radio/electrophoretic immunosorbent assay (ELISA) and mass spectrometry based proteomics (Jin et al., 2014).

These complicated, expensive and cumbersome diagnostic methods discourage patients to undergo routine investigative consultation for early signs of cancer which often results in its progression, ultimately reaching incurable advanced stages. To eliminate this diagnostic delay, researchers are investigating auspicious cancer biomarkers and their ultrasensitive monitoring via different novel biosensing approaches. Monitoring of these biomarkers, whose abnormal concentration in blood or serum indicates early warning signs of cancer will prove highly useful in adopting preventive measures. Considering these points, multi-channeled microfluidics based diagnostic automation may result in miniaturized and smart POC devices for mass utility (Raamanathan et al., 2012). Microfluidics allows controlled laminar flow of participating reactants through microchannels during a diagnostic assay and offers benefits such as low material consumption, reduced sample size, real time analysis and high throughput screening (Zhang and Nagrath, 2013). Measurement of altered gene expression during the onset of cancer can be performed through the development of rapid DNA sequencing methods. Use of capillary array electrophoresis in form of microfluidic chip for building multiplexed analytical platform is one of

AbbreviationsAPTMS3-aminopropyltriethoxysilane8-OHdG8-hydroxydeoxyguanosineBREBio-recognition elementBRCA-1Breast cancer 1 geneCNTsCarbon nanotubesCLLChronic lymphocytic leukemiaEISElectrochemical impedance spectroscopyELISAEnzyme linked immunosorbent assayEGFREpidermal growth factor receptorFETField-effect transistorFPsfluorescent probesFRETFluorescence resonance energy transferGOxGlucose oxidaseGAGlutaraldehydeAuNCsGold nanoclustersAuNPsGold nanoparticlesAuNRsGold nanorodsGOGraphene oxideHERHuman epidermal growth factor receptorIL-6Human interleukin-6LODLimit of detectionLSPRLocalized surface plasmon resonancemiRNAsMicro RNAsOFRROpto-fluidic ring resonatorPDACPancreatic ductal adenocarcinomaPTHrPParathyroid hormone-related peptidePEMSPiezoelectric microcantilever sensorsPQCPiezoelectric quartz crystalsPtNPsPlatinum nanoparticlesPOCPoint-of-carePSAProstate specific antigenQDsQuantum dotsQCMQuartz crystal microbalanceRSRaman spectroscopyAgNPsSilver nanoparticlesSWSVSquare wave stripping voltammetrySERSSurface enhanced Raman scatteringSWVSquare wave voltammograms

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the prime interests in cancer research (Ying and Wang, 2013). Presently, popular POC devices are based on lateral flow immunological or colorimetric strips and glucose sensors. Recently, Su et al., 2012 came up with an innovative solution towards designing POC devices in cancer monitoring via the use of personal glucose sensors for detecting carcinoembryonic antigen. Magnetic beads labeled with secondary antibody were used as carriers for invertase enzyme to perform sandwiched immunoassay.

The present review in general is an attempt to explain the most recent advancements in the field of biosensors for cancer diagnostics and impeccable role of functionalized nanomaterials in improving the biosensor research.

2. Biosensing approach for cancer biomarkers

Recent years have witnessed extensive research in fabricating miniaturized biosensors for monitoring cancer biomarkers. Quick evaluation of biological fluids for probing potential disease biomarkers requires advanced analytical techniques.

In this aspect, bioluminescence based rapid approaches for evaluation of endotoxicosis in human serum was investigated more than a decade ago (Esimbekova et al., 1999). Monitoring the course of diseases viz. chronic cholecystitis, bronchitis or ulcerous disease in human beings through the analysis of biological fluid was an important study which markedly promoted the area of rapid bio-diagnostics. The adopted method was based on studying the bioluminescence decay pattern (A: normalized luminescence in control; B: observed difference of luminescence in test sample with respect to control) from bacterial luciferase system when incubated with blood serum of afflicted patients. Though the method could not justify disease type, nevertheless high sensitivity, ease of usage and rapidity of the method played a pivotal exploratory role for witnessing new horizons in rapid biosensing. Lately, a detailed review on various applications on bacterial coupled enzyme systems: NADH: FMN-oxidoreductase and bacterial luciferase is presented by Kratasyuk and Esimbekova (2015). The emphasis was laid on development of economic biosensors for environmental, medical and industrial applications. In medical field, this methodology was used to evaluate the state of patient's illness and usefulness of luciferase index as prognostic plan in diseased personnel.

Lately, functionalized nanomaterials inheriting overwhelmingly high surface to volume ratio, conductivity and opto-electronic properties such as localized surface plasmon resonance (LSPR) and surface enhanced Raman scattering (SERS) is being investigated for their widespread application in fabrication of smart biosensor systems and minimizing signal interferences during real sample analysis. Further, concerns associated with biorecognition element (BRE) such as instability and non-reusability invites ample scope and motivation for continuous improvements in biosensor research (Arya and Bhansali, 2011).

The BRE often employs antibody, enzyme, short DNA strand or aptamer against specific cancer biomarker in close association to a physical transducer (electrochemical, optical and piezoelectric) which amplifies the signal response manifold after which the data is digitalized to give results in form of display readout (Fig. 1). Therefore, research involved in this area is highly interdisciplinary and encourages close associations among researchers from the field of biochemistry, biotechnology, medical sciences, material sciences, computer programming and electronics, chemical sciences and nanotechnology.

The role of physical transducers in biosensor research is indeed, very crucial. Prime desired features of transducing electronic components include high signal to noise ratio, requirement of low input voltage, inexpensiveness and provision for their fabrication into a handheld sleek design. In cue of this, electrochemical transducers offer cost effective and portable biosensing of analytes. In one of such sensitive electrochemical sensing strategies; the voltage across the working electrode (fabricated bioprobe) is gradually increased in a cyclic manner and concomitant current measurement is taken during assay. However, precise functionalization of working electrode with biological component is a challenging task which often involves multiple layering with conductive chemical agents and nanomaterials for enhanced current measurement. Optical transducers have gained significant attention after the introduction of functional nanomaterials for biosensing purposes, which have greatly improved the assay sensitivity due to plasmonic interactions between nanomaterials and light waves (Rusling et al., 2010; Abhijith et al., 2014; Swierczewska et al., 2012). However, fine tuning of the multiple experimental conditions (e.g. distance between luminescence response and the nanomaterial, dielectric constant of the surrounding medium, shape, size and opto-electronic properties of the metal nanomaterial) for observing such phenomena is a perplexing task. Recently, ring resonator sensing technology is being investigated for its potential in biosensor research. A subclass of this technology, opto-fluidic ring resonator (OFRR) promises an inexpensive and multiplexed optical biosensing of cancer biomarkers (Gohring et al., 2010).

Another class of sensors, generally made from quartz crystals inheriting piezoelectric (mechanical stress induced electric charge) properties respond to mass changes at their surface and are used for biosensing purposes.

Further, biosensing automation via incorporation of microfluidics and multiplexed analytical platform is currently being the thrust area of biosensor research; will aid in revolutionizing POC devices for early cancer monitoring (Malhotra et al., 2012; Miyamoto et al., 2014).

2.1. Electrochemical biosensors for cancer biomarkers

Fabrication of biosensors using the techniques of field-effect transistor (FET), square wave voltammograms (SWV), square wave stripping voltammetry (SWSV), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) allow rapid biosensing of key analytes for different types of cancer. An electric field dependent transistor, FET sensors for biological applications allow interaction of biomolecules with the gate of the transistor resulting in re-distribution of charge, which leads to measurable conductance change in FET channels. In yet another electrochemical approach, quantitative analysis using SWV has received tremendous attention in the fabrication of biosensors due to their ability to perform experiments at much faster scan rate when compared to pulse voltammetric techniques. Meanwhile, to introduce, sensitivity of pulse voltammetric techniques towards faradic currents is more than direct current (dc) voltammetry due to their ability to discriminate against charging (capacitance) current which also obtain peaks for faradic currents instead of sigmoidal waveforms in case of normal pulse or dc voltammetric techniques. SWSV, an even more sensitive answer for rapid biosensing involve deposition of an analyte on the working electrode leaving its concentration lesser in the sample solution. After sufficient concentration, application of electrode potential strips the overladen analyte into the solution leading to increased current yielding higher sensitivity during electrochemical biosensing of analytes.

A sandwiched immunosensor based on the principle of SWSV was described by Zhu et al. 2013 in which they reported monitoring of human epidermal growth factor receptor-2 (HER-2) and cancerous breast cells expressing HER-2. Anti HER-2 antibodies were covalently immobilized on self-assembled nanocomposites of 2,5-bis(2-thienyl)−1*H*-pyrrole-1-(*p*-benzoic acid) and AuNPs which in the presence of HER-2/HER-2 overexpressing cells and hydrazine-AuNPs-aptamer conjugate; formed a sandwich-type format. Hydrazine could specifically and uniformly reduce silver ions during stripping voltammetric assay. The deposited silver was detected under microscope and the level of these analytes was quantified using SWSV.

High counts of B-lymphocytes may indicate chronic lymphocytic leukemia (CLL) and unfortunately, are detected only at advanced cancer stages. In this direction, a single stranded DNA probe

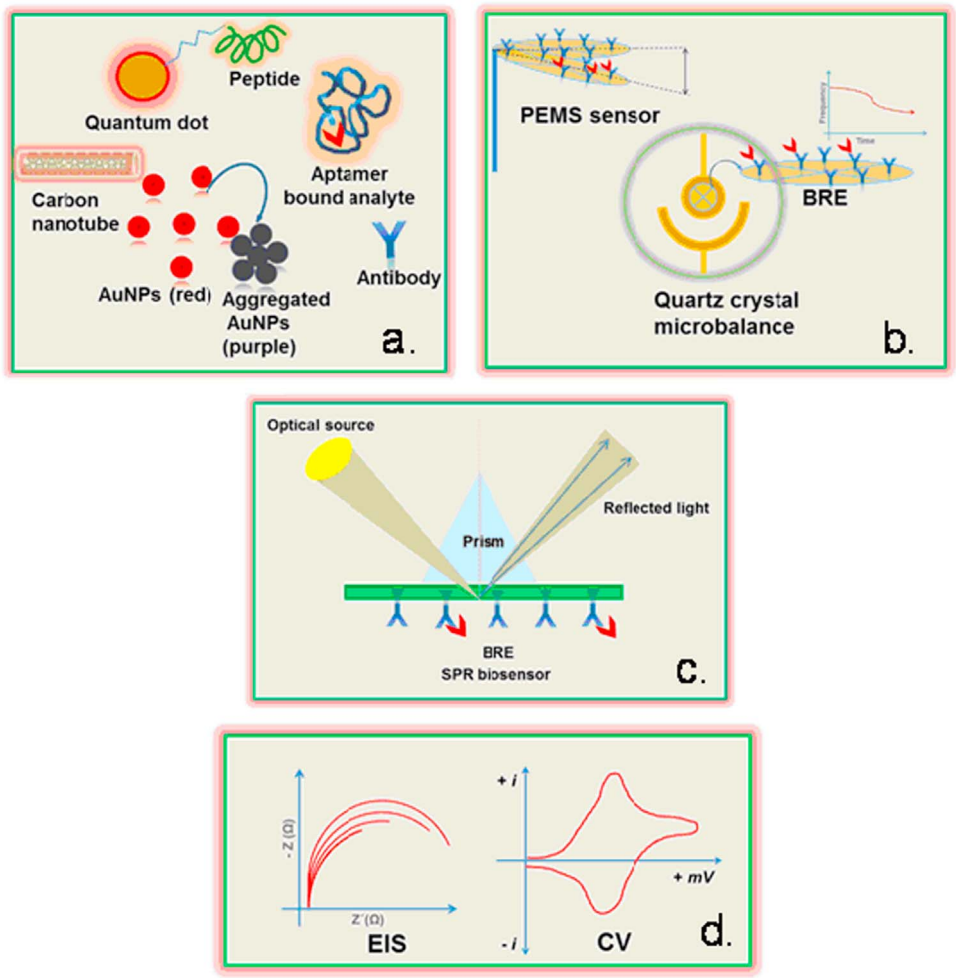

Fig. 1. Few biosensing strategies involve a.) Functionalization of nanomaterials (AuNPs, QDs, Carbon nanotubes) with biological molecules such as antibody, peptides and aptamers; b.) Microcantilever and QCM based piezoelectric immunosensing; c.) SPR based biosensing and d.) Arbitrary characteristic readout measurement from electrochemical biosensors (EIS and CV).

(ssDNA: Porphobilinogen deaminase) for CLL was used for the construction of biosensor. Electrode coated with functionalized AuNPs was found to increase the density of DNA probe which resulted in high sensitivity with a detection limit of 1.0 pM (Ensafi et al., 2011). High surface to volume ratio, scope for surface functionalization and excellent biocompatibility of AuNPs owes the credit for their popularity. In a similar study, AuNPs functionalized folic acid on the gold electrode surface was used to monitor folate receptor-rich HeLa cells upto 6 cells/mL. Folate receptor (α isoform) is one of the antigens associated with cancer which is more abundantly present on cancer cells than the normal ones and hence, a potential biomarker for cancer (Wang et al., 2012).

In the recent years, wide applications of functionalized and complex nanomaterials in biosensing have attracted much attention for the construction of versatile biosensors. Such an attempt uses highly fluorescent semiconductor nanocrystals (quantum dots: CdS, CdTe) for functionalization on graphene sheets as ultrasensitive and robust label for prostate specific antigen (PSA) detection using a sandwich-type electrochemical immunosensor (Yang et al., 2011a). The biosensor demonstrated linear response in the range of 0.005 ng/mL to 10 ng/mL with limit of detection (LOD) of 3 pg/mL as shown in Fig. 2.

Another important cancer biomarker, epidermal growth factor receptor (EGFR) was monitored using a label free and highly sensitive electrochemical impedimetric immunosensor. Electrochemical impedance spectroscopy (EIS) analyses dielectric properties of a medium in response to varying frequency while its prime advantage is the utility of label free and non-destructive techniques for monitoring analytes. The strategy utilized electrodeposition of AuNPs on gold electrode to increase the surface area by 68% followed by immobilization of anti-EGFR antibodies on the electrode in the presence of protein G; which acted as scaffold permitting proper orientation of these antibodies. The synergistic effects of AuNPs and protein G was believed to enhance the sensitivity of the immunosensor having LOD of 0.34 pg/mL in phosphate buffered saline and 0.88 pg/mL in human plasma (Elshafey et al., 2013).

Human breast cancer cells are known to release H_2O_2, the most stable reactive oxygen species and are known to cause severe effects in human beings. Therefore, its accurate and sensitive detection was carried out by Zhao et al. 2013 in which a sequence specific peptide was immobilized on gold electrode, which allowed specific binding with horseradish peroxidase to orient it in its catalytic active form to efficiently react with o-phenylenediamine and H_2O_2.

There are many other significant scientific reports in the fabrication of electrochemical biosensors (Table 1). However, functionalization of electrodes with bio-affinity probes such as enzymes, aptamers and antibodies often poses difficulties in maintaining stability and repeated use for a longer period of time. Further, fabrication of BRE requires considerable time and skilled personnel. However, researchers are trying to eliminate the present shortcomings of electrochemical biosensors through simplifying the procedures involved in the fabrication of BRE and involving nanomaterial assisted bio-fabrication (Chen et al., 2014).

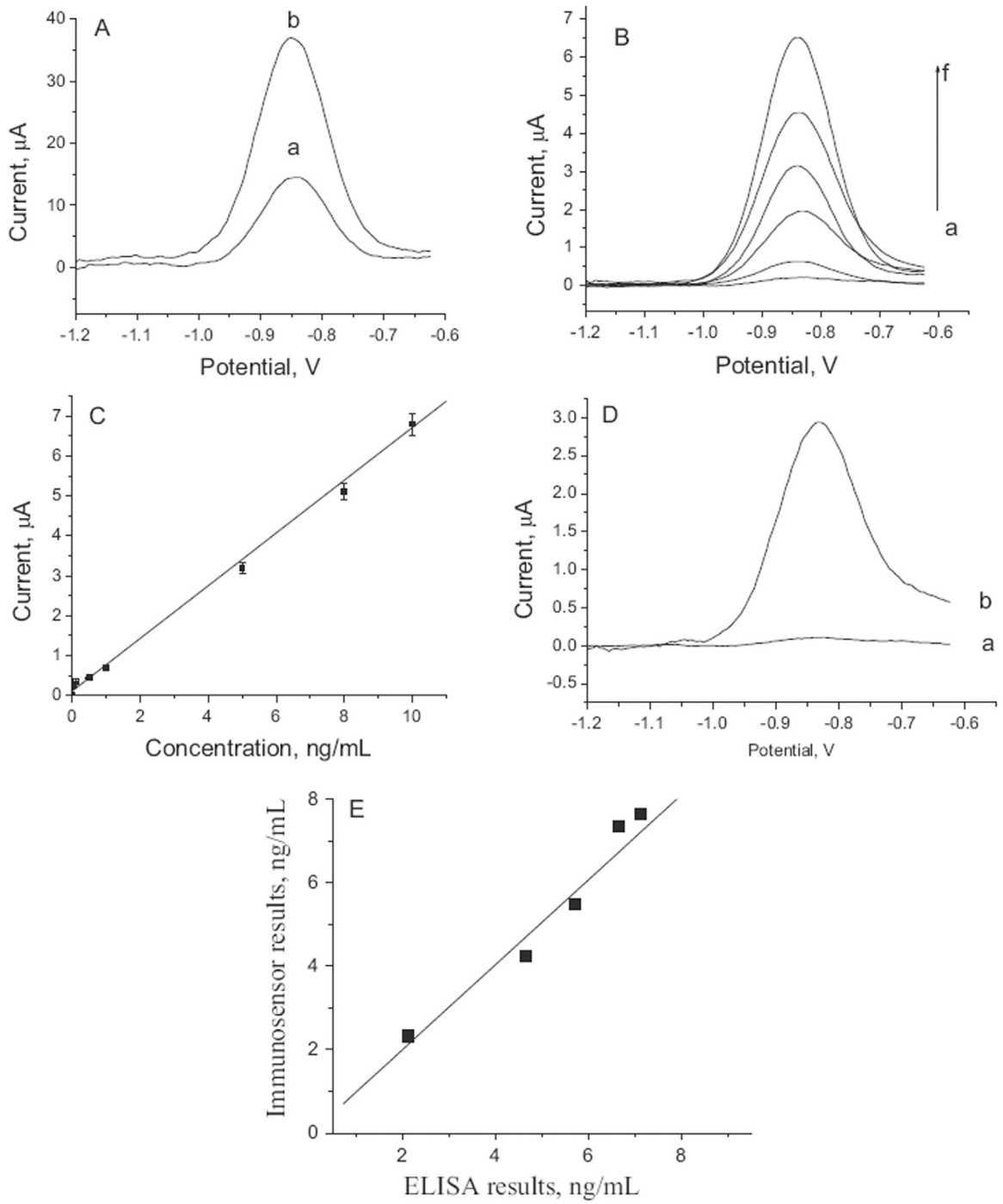

Fig. 2. SWV response of QD on (a) bare and (b) GS modified electrode. (B) SWV response of the immunosensor(C) Calibration curve of the immunosensor (D) SWV response of the immunosensor based on GO (a) and GS (b) towards 5 ng/mL of PSA. (E) Comparison of the PSA concentrations in patient serum samples determined with the new GS-based immunosensor and the ELISA method. Reproduced from Yang et al. (2011a) with permission from Elsevier publishers.

2.2. Optical Biosensors in cancer monitoring

Diagnostics based on various optical phenomena often involve intricate optical properties of nanomaterials such as SPR, LSPR and SERS; which when functionalized with fluorescent, chemiluminescent or bioluminescent probes open up even wider range of biosensing strategies.

2.2.1. SERS based biosensing of cancer cells and biomarkers

Raman spectroscopy (RS) is a powerful tool which can provide details about the structure and conformation of macromolecules such as lipids, nucleic acids and proteins by sensing signature vibrational modes of molecules. Laser RS has already been investigated to differentiate cancerous cells from the normal ones and its application has also been successfully demonstrated in the diagnosis of Alzheimer's disease, diabetes and atherosclerosis. However, RS setbacks due to emission of very weak Raman scattering signals and requires either long duration for data acquisition or high power laser beams which affects the biological sample. Secondly, interference from auto fluorescence while dealing with biological samples leads to great difficulty in identifying Raman signals. Enhancement of weak Raman signals is generally carried out either by resonance via use of laser beam of specific wavelengths or SERS which has emerged as an excellent

Table 1
Cancer biomarker and their monitoring using different biosensing approaches.

No.	Cancer biomarker	Method of detection or biorecognition element	Sensitivity, stability	Assay time (min/ s*)	Reference
1.	*TP53* gene	EIS using MWCNT/Aunanoparticles Electrode	1.0×10^{-17} M,	120	(Fayazfar et al., 2014)
2.	Alpha-fetoprotein (AFP)	Optical microfiber with Au as amplifying label	0.2 ng/mL	30–40	(Li et al., 2014a)
3.	HER-3	Impedimetric biosensor composed of aminothiophenol self assembled monolayers on gold electrodes	0.4 pg/mL, 10 days at 4 °C	60	(Sonuç and Sezgintürk, 2014)
4.	miRNAs	Quantitative reverse transcriptase polymerase chain reaction	–	–	(Shen et al., 2014; Li et al., 2014b)
5.	8-OHdG	anti-8 hydroxyguanosine (8-OHdG) antibody functionalized silicon nanowire biosensor	1 ng/mL	–	(Azmi et al., 2014)
6.	Cancer cells (SW48)	Silicon nanograssed impedance biosensor	25 cancer cells per 10,000 cells	–	(Abdolahad et al., 2014)
7.	MCF-7 cells	Leaky surface acoustic wave MUC1 aptasensor	32 cells/mL, upto 4 weeks	30	(Chang et al., 2014)
8.	Vascular endothelial growth factor (VEGF)	Magnetic graphene oxide modified Au electrode	31.25 pg/mL	30	(Lin et al., 2015)
9.	Vacuolar ATPase (ATP6AP1) Auto antibodies	Probed novel high-density custom protein microarrays	–	–	(Anderson et al., 2010)
10.	Intracellular pH (pHi)	Gold nanocluster (AuNC) based fluorescence biosensor	pH 6.0, highly stable	30*	(Ding and Tian, 2015)
11.	Carcinoembryonic antigen (CEA)	poly(3,4ethylenedioxythiophene): poly(styrene sulfonate) PEDOT: PSS coated paper based electrochemical biosensor	25.8 mA ng/(mL cm^2), 3–4 weeks	1	(Kumar et al., 2015)
12.	Prostate-specific antigens (PSA)	Anti-PSA antibody functionalized Molybdenum disulfide nanosheets based field effect biosensor	375 femtomolar (fM)	Real-time detection	(Wang et al., 2014a)
13.	Human leukemic lymphoblasts (CCRF-CEM cells)	Aptamer based peroxidase active DNAzyme as colorimetric biosensor	500 cells	30	(Zhu et al., 2010)
14.	Interleukin-8 (IL-8) from head and neck squamous cell carcinoma cells	Glutathione-protected gold nanoparticle (GSH-AuNP) and amperometry based immunosensor coupled to superparamagnetic beads-horseradish peroxidase labels	100 attomolar (aM)	2	(Munge, et al., 2011)
15.	Single-nucleotide polymorphism (SNP) associated with mutation in Breast Cancer Gene *BRCA1*	DNA-gold nanoparticle conjugates (DNA-AuNPs) based complementary binding colorimetric assay	–	>7	(Oh and Lee., 2011)
16.	Arylamine N-Acetyltransferase 1 (NAT-1)	Naphthoquinone-1 based pH dependent colorimetric biosensing	5–20 μM	–	(Laurieri et al., 2010)
17.	*TP53* gene	Electrochemical DNA hairpin beacon based DNA biosensor	–	–	(Farjami et al., 2011)

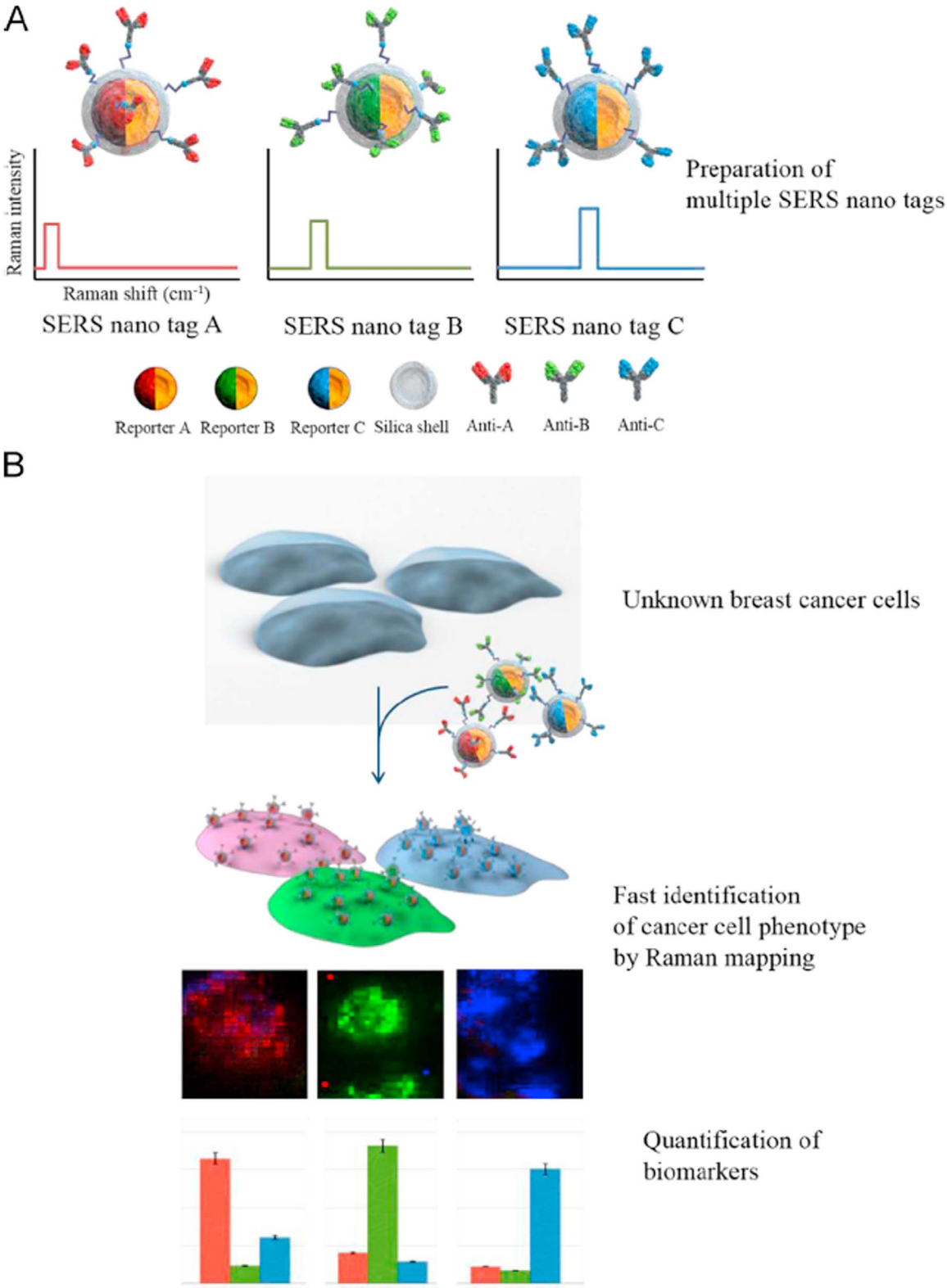

Fig. 3. SERS-based cell imaging for quick monitoring and quantification of growth factor receptors on cell membranes of breast cancer cells. (A) SERS nanotags bearing three different functional structures. Raman reporter-antibody sets were conjugated to silica-encapsulated HGNs to target specific breast cancer phenotypic markers. (B) Evaluation of phenotypic markers on cell surface membranes using the SERS imaging technique. Reproduced from Lee et al. (2014) with permission from Elsevier publishers.

biosensing tool for the development of multiplexed analysis platform and optical imaging systems.

In a recent scientific report, cell surfaces expressing phenotypic markers for breast cancer were imaged and quantified simultaneously using SERS technique. Specific antibodies conjugated silica-encapsulated hollow gold nanospheres were used as nanotags as shown in Fig. 3 for multiplexed imaging of breast cancer cell lines (MDA-MB-468, KPL4 and SK-BR-3) carrying specific markers viz. EGFR, receptor tyrosine-protein kinase (ErbB2) and IGF-1 R (Lee et al., 2014).

Monitoring of nasopharyngeal cancer by means of silver nanopar-

ticles (AgNPs) based SERS was carried out in samples containing blood plasma (Feng et al., 2010b). In the study, AgNPs were directly mixed with the blood plasma for RS enhancement. Further, they applied this approach for non-invasive nasopharyngeal cancer detection (Feng et al., 2012). The role of nanomaterials in different biosensing methods is indispensable due to its unique opto-electronic properties which have revolutionized the field of biosensors. In an another similar investigation, antibodies functionalized hollow gold nanospheres based SERS imaging was developed by Lee et al. (2011); wherein, SERS mapping images was measured after the formation of sandwiched immunocomplex on gold patterned wells with a broad imaging range of (10^{-4}–10^{-12} g/mL) with LOD of 0.1 pg/mL. Mucin protein (MUC4), a potential pancreatic cancer biomarker was detected by SERS based immunoassay when other diagnostic tools such as ELISA and radio-immunoassay (RIA) proved unsuccessful, which possibly highlights the higher sensitivity and applicability of SERS based biosensors (Wang et al., 2011a).

2.2.2. SPR/LSPR based biosensors for cancer detection

Recent advancements in the area of functional nanomaterials have largely improved the art of synthesizing LSPR/SPR based sensing materials and has offered tunable surface plasmon characteristics meant for specific purposes. When conductive nanoparticles' (size less than incident wavelength) conduction band electrons intermingles with electromagnetic wave of the incident light, an optical phenomenon called LSPR occurs which produces coherent localized plasmon oscillations with a resonant frequency which strongly depends on dielectric environment, geometry, composition, dimension and particle–particle separation distance of NPs (Petryayeva and Krull, 2011). Visible range LSPR is often exhibited by metallic AgNPs and AuNPs due to d-d transitions of their energy levels. Due to exceedingly extreme localized electromagnetic field stimulated LSPR; nanoparticles become ultra-sensitive transducers of even insignificant refractive index change allowing development of smart and robust optical sensors.

In vitro and in vivo imaging and detection systems based on gold nanorods (AuNRs) have recently caught considerable attention due to tunable optical properties and near infrared optical absorption. Utilization of fluorescence and optical properties of AuNRs for cancer cells and biomarkers is gaining popularity in recent years. Lately, Guo et al. (2014) have reported a versatile optical probe consisting of folic acid-AuNRs conjugate for identification, imaging and quantification of cancerous cells (human cervical carcinoma, HeLa cells, LOD for absorption assay: 10 cells/mL and 70 cells/mL for fluorometric assay) among normal cells (African green monkey kidney, Vero cells).

Imaging of metastatic cancer cells and apoptotic events via a new class of nanomaterials called theragnostic (therapeutic and diagnostic potential) is described in the research work of Lee et al. (2010); wherein they functionalized AuNPs with heparin labeled with fluorescent dye. Heparanase/heparinase secretions from metastatic cancerous cells led to the cleavage of heparin bound AuNPs which resulted in fluorescence enhancement and simultaneous targeted induction of apoptotic events in metastatic cancerous cells via heparin interaction with various transcription factors.

One more form of cancer called pancreatic ductal adenocarcinoma (PDAC) goes undetected until the patient reaches an advanced stage. Circulating miRNAs are often associated with proliferation, invasion, survival and metastasis of different types of cancer including PDAC. In view of this, a solid state LSPR sensor was constructed via the attachment of gold nanoprism on glass coverslip functionalized with 3-mercaptopropyltriethoxysilane followed by surface modification of gold nanoprism with poly(ethyleneglycol)6-thiols (PEG_6-SH) as spacer and the cancer biomarker (complementary probe for miRNA: C6-ssDNA-21). The extinction spectrum was analyzed in presence and absence of different miRNAs concentrations. LSPR dipole peak shift (ΔλLSPR) correlated with levels of miRNA. The method could detect the presence of miRNAs at sub femtomolar range which is over 2 fold more sensitive than conventional methods (Joshi et al., 2014). A similar approach was reported by Zhao et al. (2014) where they targeted monitoring of squamous cell carcinoma antigen (SCCa) as cervical cancer biomarker using an array of silver nanoparticles (triangular shaped).

The trend for the use of label free and reusable functionalized nanomaterials has greatly improved sensing capabilities of different cancer biomarkers and has opened doors to design multiplexed analysis platforms. In this direction, eight microfluidic channels based thirty-two sensing sites was designed for real time monitoring of cancer biomarkers (prostate specific antigen and human alpha-feto-protein) upto a concentration of 500 pg/mL (Aćimović et al., 2014).

2.2.3. Colorimetric biosensing of cancer biomarkers

Much simpler procedures for cancer biomarker monitoring often involve color change based on the aggregation of AuNPs in presence or absence of analytes.

One of such analyte, cationic protein kinase C (PKC) plays an essential role in apoptosis, differentiation and proliferation of cells and has been linked to many diseases which signify its monitoring in clinical samples. In this regard, specific peptide substrate against PKC-α was used to design a AuNPs based colorimetric biosensing assay for monitoring cancerous cells. In case of phosphorylated substrate as a result of PKC-hyperactivity, no aggregation of AuNPs was observed indicating cancerous stage of cell lysates. However, in non-cancerous cells; the activity of PKC-α remains limited thereby non phosphorylation of peptide substrate which aggregates the AuNPs based on the principle of non-crosslinking aggregation mechanism leading to blue color (Kang et al., 2010). In this type, stability of AuNPs depends upon sufficient charge distribution on their exterior surface which acts as a screening layer. Salt induced aggregation of AuNPs hampers the extent of this shielding due to charged chemical species such as citrate ions (Sun et al., 2016). A similar approach was adopted for monitoring a factor called cyclin A_2, involved in cell cycle whose hyperactivation causes tumor proliferation (Wang et al., 2011b). In case of crosslinking aggregation mechanism, quick AuNP agglomeration is induced by biological probes such as DNA, antibodies and aptamers. The demonstration of this kind of approach can be noted in the work of Borghei et al. (2016); wherein overexpressed nucleolin receptors (AS 1411) in cancer cells showed high affinity towards specific aptameric sequence. Solution containing single stranded DNA (ssDNA) functionalized AuNPs (ssDNA-AuNPs: red color) had complementary binding sites on the aptamer for AS 1411. Nucleolin receptor exhibiting cancer cells entrapped the aptamers. In paucity of free aptamers in solution, ssDNA-AuNPs remained dispersed in the solution displaying red color. However, in the opposite case, binding of ssDNA-AuNPs to the free aptamers brought AuNPs together to agglomerate resulting in blue color. Qualitative analysis is rapid, however, automation and quantitative measurement of this procedure for POC device application is still far from realization.

Another immunological colorimetric strategy is reported where; prostate specific antigen (PSA), a common cancer biomarker was detected based on the inherent peroxidase activity of graphene oxide (GO). The labeling probe i.e. secondary antibodies (Ab_2) functionalized GO emitted color in presence of PSA and anti-PSA antibodies (Ab_1) functionalized magnetic beads when external magnetic field was applied due to the migration of the sandwiched immunocomplex in the region containing hydroquinone and H_2O_2. The intensity of color varied with the quantity of PSA in the sample (Qu et al., 2011).

Lately, an interesting research work regarding the development of a paper based colorimetric biosensor for wide monitoring of analytes was investigated by Zhou et al. (2014). The work describes the use of siloxane 3-aminopropyltriethoxysilane (APTMS) as a colorimetric probe when cross linked with glutaraldehyde (APTMS-GA: brick red

color). After integration of the APTMS-GA probe with filter paper, color change was observed in the presence of H_2O_2. This colorimetric probe when immobilized with glucose oxidase (GOx); liberated H_2O_2 in presence of glucose resulting in color change.

Substantiation of this biosensing approach was utilized for monitoring of PSA through immobilization of GOx with AuNRs and anti-PSA antibody (Ab_2) followed by adsorption of primary anti-PSA antibody (Ab_1) on AuNPs. Immunosensor consisted of chitosan assisted adsorption of AuNPs and Ab_1 on colorimetric probe (APTMS-GA) attached to paper surface. Then the filter paper was blocked using bovine serum albumin and incubated with different concentrations of PSA followed by repeated washing to remove unbound PSA. Then, AuNR-GOx-Ab_2 was incubated onto the paper surface followed by repeated washing and addition of glucose solution. Color change was observed after sometime as an indication of PSA content in the sample in the linear range of 0.1–10 ng/mL of PSA.

An important biomarker, human interleukin-6 (IL-6) plays a crucial role in eliciting inflammatory responses such as inflammatory bowel diseases, arthritis and cardiovascular diseases. In pathological state, the IL-6 levels elevates more than thousand times than its normal level (10–75 pg/mL) in serum. High level of IL-6 has also been linked to prostate and breast cancer which necessitates its monitoring at sensitive levels for early diagnosis. In this regard, Peng et al. (2016) used ceria spheres as labels for sensitive monitoring of IL-6 through magnetic colorimetric immunoassay. Ceria spheres inherit outstanding oxidase activity and can directly oxidize the substrate, o-phenylenediamine to an oxidized product called 2,3-diaminophenazine having a stable yellow color. The differential absorption of this chromogenic substrate reveals the levels of IL-6 with the broad linear range of 0.0001–10 ng/mL with sensitivity up to 0.04 pg/mL.

A class of regulatory elements for messenger RNA; called miRNA are single stranded oligoribonucleotides which was recently discovered to circulate in the bloodstream and was found to be associated with most of the human malignancies and therefore, its early monitoring has opened up a new avenue in the area of biosensor research for cancer biomarkers. In this context, a functional amplification machine for nucleic acids was designed for ultrasensitive colorimetric detection of miRNA which consisted of probe (Trigger template complex, cytosine rich molecular beacon, guanine rich DNA), nicking and polymerase enzymes and dumbbell shaped amplification template. The present approach could monitor miRNAs in a wide dynamic range of 10 attomolar (aM) to 1.0 nanomolar (nM) concentrations (Li et al., 2016).

A recent biosensing approach demonstrates dual signal amplified colorimetric assay (release of multi-DNA from the aptamer scaffold and cyclic enzymatic amplification) for cancer cells (Yu et al., 2016). The approach utilizes conjugation of cancer recognizing aptamers to magnetic beads followed by alignment of five messenger DNAs (mDNAs) on these aptamers in such a way that they get released altogether after aptamers binds to the receptors of cancerous cells. The released mDNAs were introduced to cyclic enzymatic pathway to produce more single stranded DNA (ssDNA) fragments. These ssDNAs being negatively charged, bind strongly to the AuNPs and shield them from possible aggregation in presence of high salt concentration and hence the color of the AuNPs remain red.

2.2.4. Fluorescence based probes in cancer detection

Fluorophores undergo excitation via photons of comparatively shorter wavelength to subsequently emit photons with a characteristic longer wavelength. The energy loss during this event is due to vibrational relaxation and is stated as Stoke's shift. Precisely, the process of fluorescence can be described in three steps. Firstly, the high energy photons excite the electrons present in the fluorophores to shift them from ground level to the excited state followed by one of the several photophysical events (radiative emission, nonradiative emission, intersystem crossing i.e. transfer of electron from singlet stage to triplet stage, vibrational relaxation and internal conversion). Ultimately, the electrons in the excited state return back to the ground level releasing energy in form of photons, usually of higher wavelengths carrying less energy (Chinen et al., 2015). Underperformance of conventional fluorophores for biosensing applications became evident over years when their shorter fluorescence lifetime, low molar absorption coefficients, narrow absorption spectra and susceptibility to photodegradation achieved much lower signal to noise ratio during bioassays.

In this regard, upcoming fluorescent probes (FPs) such as aggregation-induced emission based fluorogens (Liang et al., 2015), water-soluble fluorescent conjugated polymers (Feng et al., 2010c), ditopic zinc sensor (Ghosh et al., 2010) and red-emissive bioprobe TPE-red-2AP2H (Hu et al., 2014) have largely improved over the pitfalls associated with their conventional equivalents and have emerged as highly specific and sensitive optical sensing tool for on-site visualization of biochemicals in a dynamically active live cell. Fluorescent biosensors constitute different classes of FPs as advanced monitoring tool to decipher minute information through cellular imaging and biochemical assays for useful biomarkers (Morris, 2013).

One of the most popular candidates for FPs in cancer biomarker detection are quantum dots (QDs); highly fluorescent semiconducting nanoparticles (Wagner et al., 2010; Wang et al., 2015). QDs resist photobleaching or photoblinking unlike conventional organic fluorophores and possess broad excitation range accompanied with provision for presenting size tunable narrow emission range, thereby, making it an ideal probe to promote multiplexed analytical platform for wide biosensing purposes in confined space and time. Additionally, multifunctionalization abilities of QDs encourage researchers in designing novel multifaceted biosensing platforms.

One of the most important biosensing strategy, fluorescence resonance energy transfer (FRET) involves nonradiative energy transfer between two chromophores at very short distances (10–100 Å) wherein emission spectrum of the donor overlaps with absorption spectrum of acceptor and is independent of the chromophore concentration and externally triggered light intensity (Thakur et al., 2013; Lu and Wang, 2010). FRET based analytical investigations are widely reported in cancer biodiagnostics (Aoki et al., 2012) and often employ FPs such as cationic conjugated polymer/ fluorescein pair (Feng et al., 2010a) and quantum dots as labeling agents (Li et al., 2011; Chi et al., 2012) in conjugation to proteins, antibodies (Yang et al., 2011b; Liu et al., 2016), aptamers (Liu et al., 2013b), peptides (Liu et al., 2011) and small molecules. Undoubtedly, FRET based applications in biodiagnostics is huge, however suffers from certain drawbacks. Possibilities for employing a random pair of fluorescent probe to observe FRET is ruled out due to non-overlapping of donor emission spectral range with acceptor's excitation range and therefore, “FRET pairs” are highly selective. Another flaw associated with FRET based assays is auto-fluorescence from the background during investigation (Zadran et al., 2012).

In a relatively newer approach, surface plasmon coupled emission (SPCE) based biosensing of analytes via the use of QDs claim to offer much higher sensitive and efficient biosensor systems than its counterparts (Jin et al., 2012). However, toxic nature of commonly synthesized QDs risks human health and environmental safety. In this regard, report on cadmium free QDs (Mandal et al., 2013) and other potential FPs in cancer biodiagnostics such as hyperbranched conjugated polyelectrolyte (Pu et al., 2011), fluorescent silicon nanoparticle (Peng et al., 2014) and core–shell near-infrared fluorescent nanoparticles offer excellent properties of exhibiting negligible background fluorescence, minimal toxicity and interferences in complex assay sample during experimental procedures, thereby improving the biosensing approach (Deng et al., 2010). A handful of related reports describe the use of silica-coated fluorescent nanoparticles (Tao et al., 2012), bovine serum albumin (BSA) functionalized gold nanoclusters (Ding and Tian,

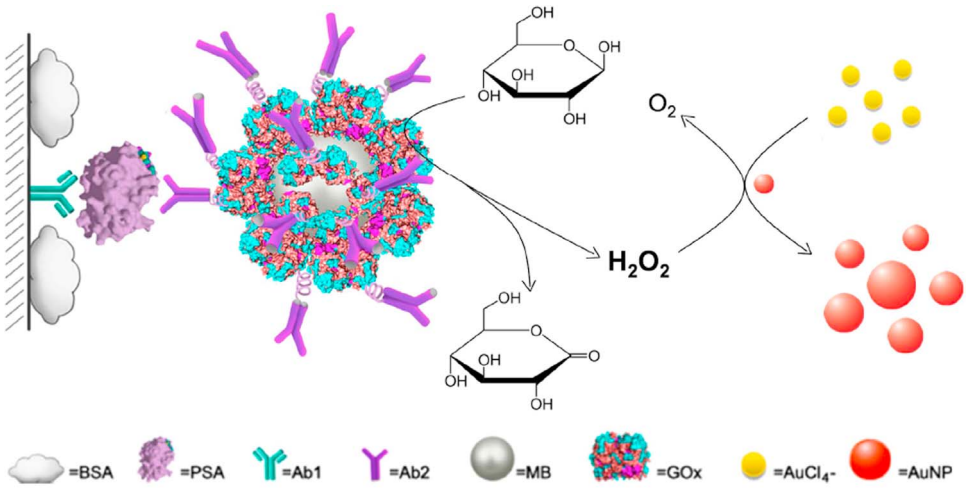

Fig. 4. PSA antigen detection via sandwich immunoassay using GOx mediated GNP synthesis as quantitative colorimetric label. Reproduced from Liu et al. (2014) with permission from American Chemical Society.

2015), cerium oxide nanoparticles (Asati et al., 2011), photonic crystals as fluorescence enhancing entity (Huang et al., 2011; Cunningham and Zangar, 2012), gold nanoclusters stabilized with fluorescent trypsin (Liu et al., 2013a) and persistent-luminescence nanoparticles (Wu et al., 2011) in designing novel biosensing schemes for cancer biomarkers. Therefore, application of complex functionalized nanomaterials can be considered as hotspot for research in the area of rapid cancer biodiagnostics which has potential to offer miniaturized POC devices for early monitoring of cancer.

2.3. Complexed nanomaterial based sensors for cancer prognosis

Artificial and complexed hybrid nanostructures such as electrochemical, or optical immunoarrays (e.g. electrochemiluminescence, fluorescence, colorimetric) offer large surface area and decent support system for many catalytic processes among which carbon nanotubes (CNTs) (Sardesai et al., 2011; Chen et al., 2011b), porous platinum nanoparticles (PtNPs)-metal hybrid (Wang et al., 2014b), silicon nanomaterials (Peng et al., 2014), graphene/graphene oxide (Tong et al., 2013) and zinc oxide nanoparticles are emerging alternatives for cancer biosensing.

CNTs offer exceptional electrical conductivity, biocompatibility and functionalization capabilities which are the pre-requisites for constructing smart diagnostic systems. Measurement of signal response in form of altered electrical conductivity due to binding of biological entities on the side walls of CNTs can be a useful tool in designing biosensing tools for cancer biomarkers. These conducting materials consist of only a few layers of carbon which can be synthesized as long as several micrometers in length. Further improvements in the architecture of conventional nanotubes for minimizing loose electrical connections between improperly aligned CNTs with biological receptors was reported for monitoring folate receptors as cancer biomarker (Zanganeh et al., 2016).

Nanocomposites involving PtNPs and GO offer enhanced peroxidase like catalytic abilities due to the presence of multiple active sites and large surface area with impressive biocompatibility. In this regard, a colorimetric assay was developed for quick biosensing of cancer cells which display folate receptor (Zhang et al., 2014).

Further, water soluble GO and gold nanoclusters (AuNCs) are prime candidates which have contributed immensely as enzyme mimics for wide applications in cancer research. Biocompatibility of AuNCs along with intrinsic peroxidase like activity, small size and high stability contribute greatly to bioassays. In a research, GO was hybridized with lysozyme stabilized AuNCs to obtain GO-AuNCs hybrid possessing synergistic peroxidase like activities confirmed through catalysis of 3,3′,5,5′-tetramethylbenzidine in presence of H_2O_2. Further, GO-AuNCs was conjugated to folic acid (FA) to get FA-GO-AuNCs hybrid which was utilized as ultrasensitive colorimetric probe for diagnosis of cancer via binding to folate receptors present on cancer cells (Tao et al., 2013).

An another interesting complex nanostructured biosensing approach witness monitoring of cyclin A_2; an early cancer prognostic marker using glassy carbon electrode modified graphene functionalized porphyrin, hexapeptide P_0 (RWIMYF) and Tween 20. Cyclin A_2 has a surface pocket to which P_0 binds strongly while Tween 20 prevents the probe from non-specific binding while performing assay. Attachment of Cyclin A_2 (as low as 0.32 pM) causes hindrance to the redox probe $[Fe(CN)_6]^{3-/4-}$ in vicinity to surface of the electrode resulting in detectable change in EIS i.e. ΔEIS (Feng et al., 2012).

Though the above mentioned methods seem promising, efficient functionalization of biological components with nanomaterials to form complexed nanosensor is a challenging task.

In continuation to our previous discussion (Section 2.2.3), GNPs based colorimetric detection methods for cancer biomarkers are comparatively much simpler and can provide economically feasible solution to devise POC diagnostic systems.

However, ultrasensitive detection of cancer biomarkers in clinical samples through conventional AuNPs based colorimetric assays wherein color change is observed from red to blue or purple is difficult.

To solve this issue, a reverse colorimetric assay approach is described by Liu et al. (2014) in which colorless AuNPs turns red through GOx catalyzed size enlargement of colorless AuNPs (5 nm) leading to appearance of red color. 5 nm sized AuNPs have low extinction coefficient which are colorless at picomolar levels. However, large sized particles in the same concentration range are colored. The assay method utilizes conjugation of GOx on detection antibodies (Ab_2) which were immobilized on the surface of magnetic beads. The amount of GOx-Ab_2-magnetic beads was proportional to the concentration of the analyte (PSA antigen) and subsequently generated H_2O_2 which in presence of $AuCl^{4-}$ ions formed AuNPs of large size resulting in appearance of visible red color as described in Fig. 4.

Another class of nanomaterial composed of silicon, possesses unique optical, electronic and mechanical properties apart from their biocompatibility, high surface to volume ratio and cost economy. Utility of silicon based nanomaterials in bioimaging and biosensing of analytes is highly promising after their surface modification with hydrophilic functional groups allowing their solubility in water (Peng et al., 2014). Further, use of nanoporous silica chips in concentrating specific proteins and peptides as proteomic biomarkers of cancer from a pool of biochemicals was presented (Fan et al., 2012). Reports on the

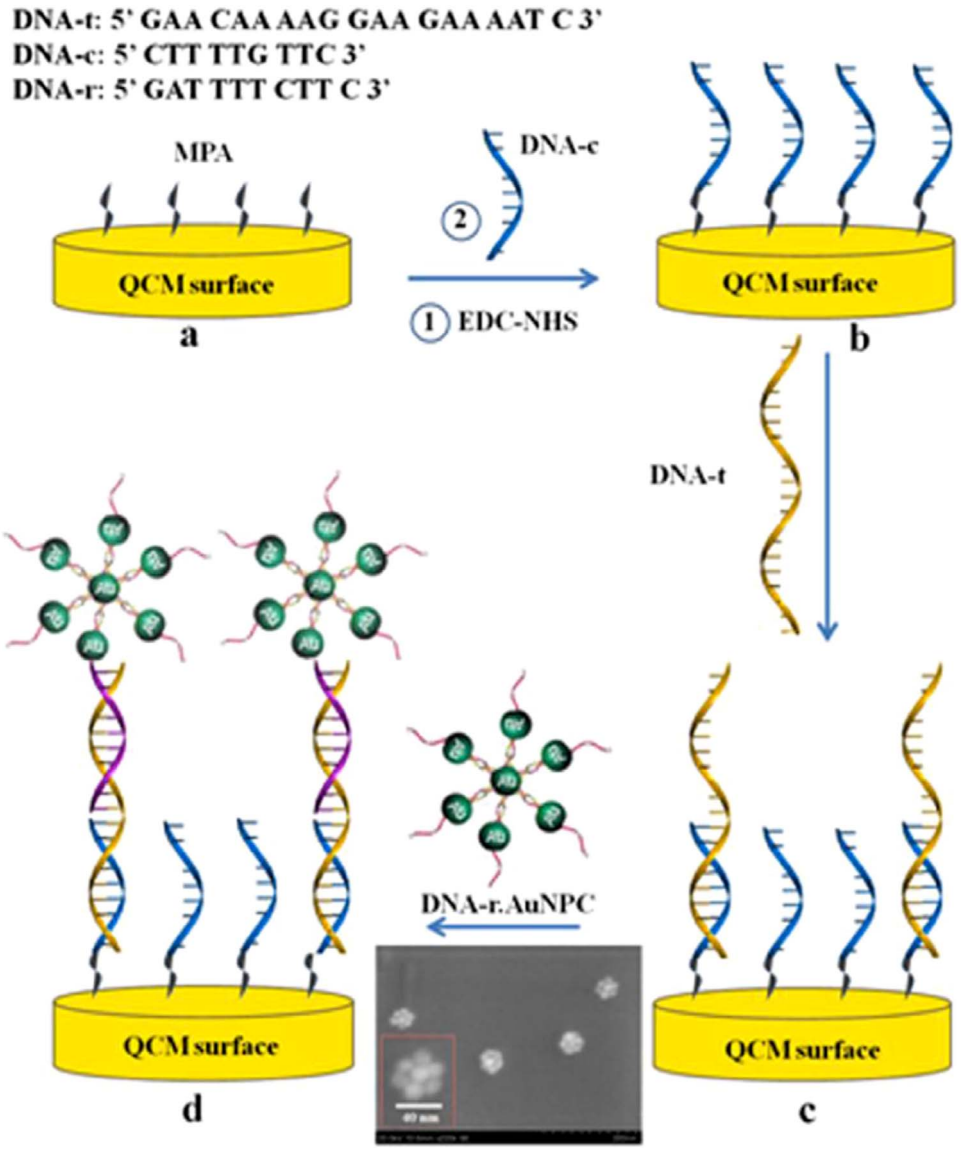

Fig. 5. Sensor fabrication using BRCA-1 and specific DNA sequence. The unhybridized DNA-r on the outer gold nanoparticles of the cluster undergoes hybridization with DNA-t. Reproduced from Rasheed and Sandhyarani (2016) with permission from Elsevier publishers.

use of silicon nanowires in the fabrication of reusable FET based biosensors for cancer monitoring hold many advantages over carbon nanomaterials (Lerner et al., 2012) and metal oxide based FET sensors (Chen et al., 2011a).

Fabrication of electrochemical and electrochemiluminescence (electrochemical reaction led light emission) based biosensors using zinc oxide nanoparticles have gained significant attention due to few salient and exclusive features such as quick electron transfer kinetics, high electrochemical activity, enhanced chemical stability and decent biocompatibility (Cheng et al., 2012). Application of zinc oxide nanorods and their highly fluorescent polymeric derivatives has also been shown to be very useful in biosensing of cancer associated biomarkers (Hu et al., 2015).

Elimination from a) expensive and cumbersome instrumentation b) tedious sample preparation and c) manual bioassays by skilled personnel requires designing of 1) miniaturized low cost physical transducer, in vicinity to a 2) stable, ultrasensitive and robust biosensing component and a recommended 3) microfluidics based multiplexed analytical array for high throughput screening (Sardesai et al., 2013). These prerequisites are highly crucial in fabricating potential POC devices (Chan et al., 2013).

The idea of "lab on a paper" is an emerging platform to design economic and convenient paper based diagnostic platforms for a wide range of analytes. In one such attempt, miRNA detection as potential cancer biomarker with unaided eye is described by Yildiz et al. (2013) via impregnation of poly(3-alkoxy-4-methylthiophene) on poly(vinylidene fluoride) thin paper to construct a luminescent probe. The assay is based on the formation of dual/triple complex system between "reporter and miRNA-peptide and nucleic acid (PNA) hybrid" and "reporter and miRNA" which was reported to work in a wide range (10–10 mM) of clinical samples.

Scientific reports on the use of complexed nanomaterial based sensors for cancer biomarkers have provided researchers with ample novel strategies to build auspicious ultrasensitive POC devices (Kumar et al., 2016; Azmi et al., 2014; Rusling, 2013; Yu et al., 2014; Kadimisetty et al., 2015).

2.4. Piezoelectric biosensors for cancer biomarkers

A piezoelectric device is sensitive to mass changes at nanoscale and is very helpful in bioanalytical applications.

Piezoelectric quartz crystals (PQC) are thin slices of quartz derived

from a single crystal with optimal chemical, electrical and mechanical properties suitable for analytical applications. The sensor fabrication is done by vapor deposition of gold or silver which serve as electrodes on both sides of PQC surface. Then, potential difference is applied across the electrodes to generate electric field which results in altered physical orientation of the crystal lattice. Consequently, mechanical oscillations across the bulk of the quartz disk takes place at a characteristic vibrational frequency which depends upon the physical properties of the crystal and phase associated parameters.

Decline of this resonance frequency in quartz crystals induced by mass changes at their surface leads to detectable signals and is used to monitor analytes at nanogram levels (Fig. 1b). The principle was utilized for fabrication of genosensors built on quartz crystal microbalance (QCM). Further, minimal mass threshold for unit change of frequency in conventional QCM was improved through the use of nanoparticles, which led to ultrasensitive detection of analytes up to few attomoles. Key features of piezoelectric sensors such as simplicity, provisional on-site monitoring and ultrasensitivity have led to their popularity. However, the present biosensing approach generally utilizes the basic principle of sandwich immunoassays or DNA hybridization techniques for cancer biodiagnostics.

A cancer associated gene, breast cancer 1 gene (BRCA-1) helps in the maintenance and destruction of abnormal cells and thus plays a key role in initiating preventive measures against abnormal proliferation of cells in humans. Presence of BRCA-1 gene mutants in cells alarms an early sign of cancer in afflicted patients. Therefore, detection of mutated versions of BRCA-1 is very useful in diagnosis of breast cancer. In this regard, AuNPs conjugated reporter probe DNA (DNA-r) based sandwich immunoassay format (Fig. 5) was designed coupled with T7 endonuclease I led selectivity and cleavage of mismatched DNA during the BRCA-1 gene assay (Rasheed and Sandhyarani, 2016)..

Recently, a receptor-ligand based QCM sensor is reported by Atay et al. (2016) which describes detection of metastatic breast cancer cells (MDA-MB-231). Requirement of high amount of iron by cancerous cells adopts a large number of transferrin receptors on their cell surface which have high affinity for transferrin, a molecule which supplies iron to the cells. Sensor fabrication involved adsorption of poly(2-hydroxyethyl methacrylate) nanoparticles on QCM surface followed transferrin attachment by covalent modification.

There is a lot of scope for continuous improvements in biosensing methods for cancer biomarkers and currently facing many challenges in designing an easy to use, inexpensive, robust and ultrasensitive biosensor for real time monitoring of cancer biomarkers. In such an attempt, Crivianu-Gaita et al. (2016) developed an acoustic wave biosensor equipped with ultra-high frequency modulator for sensing elevated levels of a biomarker for prostate and breast cancer viz. parathyroid hormone-related peptide (PTHrP). The label free biosensor utilized the conventional approach of immobilizing anti-PTHrP antibodies and Fab' fragments on ultra-high frequency electromagnetic piezoelectric acoustic sensor. The biorecognition element was tested using sandwich type secondary antibody for mass amplification. A similar approach is realized in a recent work of Yang et al. (2016), in which a biomarker for cervical cancer ($p16^{INK4a}$) was detected at nanogram levels.

Another class of piezoelectric biosensor, called piezoelectric microcantilever sensors (PEMS) is composed of lead magnesium niobate-lead titanate layer which has enhanced piezoelectric properties; was utilized for fabrication of biosensor for HER-2 (Loo et al., 2011). Functionalization of microcantilevers with biologically active ligands (e.g. antibody) against specific biomarkers (i.e. antigen) followed by their incubation with sample allow precise interactions at the cantilever surface, resulting in mass increase with concomitant detectable resonance frequency shifts (Fig. 1b). Improvements in the sensitivity of PEMS sensors can be further achieved through the use of novel hybrid materials in designing cantilevers and AuNPs led coating of the sensor. In this aspect, miRNA (*hsa-let-7a*) detection using cantilever piezoelectric sensor coated with AuNPs has been devised for analyte monitoring in a wide range (10 fM–1 nM) based on the principle of DNA hybridization as described earlier (Johnson and Mutharasan, 2012).

A novel piezoelectric transducer called lead titanate zirconate ceramic resonator was reported for PSA and α-fetoprotein using a dual sensing approach. Very small size of the transducer could be utilized for multiplexed analysis (Su et al., 2013).

Label free probe is always considered as an improvement in biosensor development which was seen in the work of Zhang et al. (2010) wherein cancer cell trapping mechanism and their detection was defined using super paramagnetic micro-beads with a physically controlled magnetic field approach. A human lung cancer cell line (A549) as a model was used for the study having wheat germ agglutinin protein-functionalized paramagnetic micro-beads as the biosensing element.

However, it is not a denying fact that almost all the sensing methodologies utilize fragile biochemicals and their successful functionalization remains a challenging task.

Few more scientific reports on biosensing of cancer biomarker are summarized in Table 1.

3. Conclusion and future prospects

Early monitoring and indication of warning alarm for cancer susceptibility in patients is of prime importance in order to save lives through timely treatment; in which the role of easy to use, affordable, highly selective and reliable biosensor is indispensable. The role of functionalized nanomaterials in improving biosensing platform is inevitable especially the versatile role of AuNPs, magnetic nanoparticles, nanomaterials based on silicon and metal oxides, nano graphene and its oxide, aptamers, antibodies, peptides, fluorescent and chromogenic labels. These functionalized nanomaterials are capable of enhancing signal response and few show intrinsic enzyme like properties as well. Functionalization capacity, biocompatibility, ease of synthesis and their inexpensiveness are the reasons for their ubiquitous and mass utility in the fabrication of advanced nanomaterials.

Indeed, AuNPs have played a pivotal role in designing quick colorimetric and LSPR/SERS based ultrasensitive biosensors. However, the fundamental role of enzymes, peptides, aptamers and antibodies in designing affinity based assays deserves huge credits. The major challenge associated with construction of BRE is the perishable nature of biologicals. Biological components require specific ambient environment and their bio-functionalization may seriously affect their activity. Therefore, extensive research in this area is always welcome. Further, new discoveries of promising cancer biomarkers followed by diagnostic automation through designing miniaturized real time, ultrasensitive and reliable POC devices owe much attention in designing smart diagnostic tools. A plethora of advanced biosensing tools and techniques for cancer biodiagnostics promises a bright future in mass utility of biosensors for cancer prognosis.

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References

Abdolahad, M., Shashaani, H., Janmaleki, M., Mohajerzadeh, S., 2014. Biosens. Bioelectron. 59, 151–159.
Abhijith, K.S., Sharma, R., Ranjan, R., Thakur, M.S., 2014. Photochem. Photobiol. Sci. 13, 986–991.
Aćimović, S.S., Ortega, M.A., Sanz, V., Berthelot, J., Garcia-Cordero, J.L., Renger, J., Maerkl, S.J., Kreuzer, M.P., Quidant, R., 2014. Nano Lett. 14, 2636–2641.

Anderson, K.S., Sibani, S., Wallstrom, G., Qiu, J., Mendoza, E.A., Raphael, J., Hainsworth, E., Montor, W.R., Wong, J., Park, J.G., Lokko, N., Logvinenko, T., Ramachandran, N., Godwin, A.K., Marks, J., Engstrom, P., LaBaer, J., 2010. J. Proteome Res. 10, 85–96.
Aoki, K., Komatsu, N., Hirata, E., Kamioka, Y., Matsuda, M., 2012. Cancer Sci. 103, 614–619.
Arya, S.K., Bhansali, S., 2011. Chem. Rev. 111, 6783–6809.
Asati, A., Kaittanis, C., Santra, S., Perez, J.M., 2011. Anal. Chem. 83, 2547–2553.
Atay, S., Piskin, K., Yilmaz, F., Çakir, C., Handan, Y., Denizli, A., 2016. Anal. Methods 8, 153–161.
Azmi, M.A.M., Tehrani, Z., Lewis, R.P., Walker, K.A.D., Jones, D.R., Daniels, D.R., Doak, S.H., Guy, O.J., 2014. Biosens. Bioelectron. 52, 216–224.
Borghei, Y., Hosseini, M., Dadmehr, M., Hosseinkhani, S., Ganjali, M.R., Sheikhnejad, R., 2016. Anal. Chim. Acta 904, 92–97.
Chan, C.P.Y., Mak, W.C., Cheung, K.Y., Sin, K.K., Yu, C.M., Rainer, T.H., Renneberg, R., 2013. Annu. Rev. Anal. Chem. 6, 191–211.
Chang, K., Pi, Y., Lu, W., Wang, F., Pan, F., Li, F., Jia, S., Shi, J., Deng, S., Chen, M., 2014. Biosens. Bioelectron. 60, 318–324.
Chen, K., Li, B., Chen, Y., 2011a. Nano Today 6, 131–154.
Chen, Z., Zhang, X., Yang, R., Zhu, Z., Chen, Y., Tan, W., 2011b. Nanoscale 3, 1949–1956.
Chen, X., He, Y., Zhang, Y., Liu, M., Liu, Y., Li, J., 2014. Nanoscale 6, 11196–11203.
Cheng, Y., Yuan, R., Chai, Y., Niu, H., Cao, Y., Liu, H., Bai, L., Yuan, Y., 2012. Anal. Chim. Acta 745, 137–142.
Chi, X., Huang, D., Zhao, Z., Zhou, Z., Yin, Z., Gao, J., 2012. Biomaterials 33, 189–206.
Chinen, A.B., Guan, C.M., Ferrer, J.R., Barnaby, S.N., Merkel, T.J., Mirkin, C.A., 2015. Chem. Rev. 115, 10530–10574.
Crivianu-Gaita, V., Aamer, M., Posaratnanathan, R.T., Romaschin, A., Thompson, M., 2016. Biosens. Bioelectron. 78, 92–99.
Cunningham, B.T., Zangar, R.C., 2012. J. Biophotonics 5, 617–628.
Deng, T., Li, J., Zhang, L., Jiang, J., Chen, J., Shen, G., Yu, R., 2010. Biosens. Bioelectron. 25, 1587–1591.
Ding, C., Tian, Y., 2015. Biosens. Bioelectron. 65, 183–190.
Elshafey, R., Tavares, A.C., Siaj, M., Zourob, M., 2013. Biosens. Bioelectron. 50, 143–149.
Ensafi, A.A., Taei, M., Rahmani, H.R., Khayamian, T., 2011. Electrochim. Acta 56, 8176–8183.
Esimbekova, E.N., Kratasyuk, V.A., Abakumova, V.V., 1999. Luminescence 14, 197–198.
Fan, J., Deng, X., Gallagher, J.W., Huang, H., Huang, Y., Wen, J., Ferrari, M., Shen, H., Hu, Y., 2012. Philos. Trans. R. Soc. A 370, 2433–2447.
Farjami, E., Clima, L., Gothelf, K., Ferapontova, E.E., 2011. Anal. Chem. 83, 1594–1602.
Fayazfar, H., Afshar, A., Dolati, M., Dolati, A., 2014. Anal. Chim. Acta 836, 34–44.
Feng, F., Liu, L., Wang, S., 2010a. Nat. Protoc. 5, 1255–1264.
Feng, S., Chen, R., Lin, J., Pan, J., Chen, G., Li, Y., Cheng, M., Huang, Z., Chen, J., Zeng, H., 2010b. Biosens. Bioelectron. 25, 2414–2419.
Feng, X., Liu, L., Wang, S., Zhu, D., 2010c. Chem. Soc. Rev. 39, 2411–2419.
Feng, L., Wu, L., Wang, J., Ren, J., Miyoshi, D., Sugimoto, N., Qu, X., 2012. Adv. Mater. 24, 125–131.
Ghosh, S.K., Kim, P., Zhang, X., Yun, S., Moore, A., Lippard, S.J., Medarova, Z., 2010. Cancer Res. 70, 6119–6127.
Gohring, J.T., Dale, P.S., Fan, X., 2010. Sens. Actuat. B-Chem 146, 226–230.
Gubala, V., Harris, L.F., Ricco, A.J., Tan, M.X., Williams, D.E., 2012. Anal. Chem. 84, 487–515.
Guo, Y., Sun, G., Zhang, L., Tang, Y., Luo, J., Yang, P., 2014. Sens. Actuat. B-Chem 191, 741–749.
Hu, F., Huang, Y., Zhang, G., Zhao, R., Yang, H., Zhang, D., 2014. Anal. Chem. 86, 7987–7995.
Hu, W., Liu, Y., Chen, T., Liu, Y., Li, C.M., 2015. Adv. Mater. 27, 181–185.
Huang, C., George, S., Lu, M., Chaudhery, V., Tan, R., Zangar, R.C., Cunningham, B.T., 2011. Anal. Chem. 83, 1425–1430.
Hussain, T., Nguyen, Q.T., 2014. Adv. Drug Deliv. Rev. 66, 90–100.
Jin, L., Li, S., Cho, Y., 2012. Biosens. Bioelectron. 33, 284–287.
Jin, B., Wang, P., Mao, H., Hu, B., Zhang, H., Cheng, Z., Wu, Z., Bian, X., Jia, C., Jing, F., Jin, Q., Zhao, J., 2014. Biosens. Bioelectron. 55, 464–469.
Johnson, B.N., Mutharasan, R., 2012. Anal. Chem. 84, 10426–10436.
Joshi, G.K., Deitz-McElyea, S., Johnson, M., Mali, S., Korc, M., Sardar, R., 2014. Nano Lett. 14, 6955–6963.
Kadimisetty, K., Malla, S., Sardesai, N.P., Joshi, A.A., Faria, R.C., Lee, N.H., Rusling, J.F., 2015. Anal. Chem. 87, 4472–4478.
Kang, J., Asami, Y., Murata, M., Kitazaki, H., Sadanaga, N., Tokunaga, E., Shiotani, S., Okada, S., Maehara, Y., Niidome, T., Hashizume, M., Mori, T., Katayama, Y., 2010. Biosens. Bioelectron. 25, 1869–1874.
Kratasyuk, V.A., Esimbekova, E.N., 2015. Comb. Chem. High Throughput Screen. 18, 952–959.
Kumar, S., Kumar, S., Srivastava, S., Yadav, B.K., Lee, S.H., Sharma, J.G., Doval, D.C., Malhotra, B.D., 2015. Biosens. Bioelectron. 73, 114–122.
Kumar, S., Sharma, J.G., Maji, S., Malhotra, B.D., 2016. Biosens. Bioelectron. 78, 497–504.
Laurieri, N., Crawford, M.H.J., Kawamura, A., Westwood, I.M., Robinson, J., Fletcher, A.M., Davies, S.G., Sim, E., Russell, A.J., 2010. J. Am. Chem. Soc. 132, 3238–3239.
Lee, K., Lee, H., Bae, K.H., Park, T.G., 2010. Biomaterials 31, 6530–6536.
Lee, M., Lee, S., Lee, J., Lim, H., Seong, G.H., Lee, E.K., Chang, S., Oh, C.H., Choo, J., 2011. Biosens. Bioelectron. 26, 2135–2141.
Lee, S., Chon, H., Lee, J., Ko, J., Chung, B.H., Lim, D.W., Choo, J., 2014. Biosens. Bioelectron. 51, 238–243.
Lerner, M.B., D'Souza, J., Pazina, T., Dailey, J., Goldsmith, B.R., Robinson, M.K., Johnson, A.T.C., 2012. ACS Nano 6, 5143–5149.
Li, H., Cao, Z., Zhang, Y., Lau, C., Lu, J., 2011. Analyst 136, 1399–1405.
Li, K., Liu, G., Wu, Y., Hao, P., Zhou, W., Zhang, Z., 2014a. Talanta 120, 419–424.
Li, N., Ma, J., Guarnera, M.A., Fang, H., Cai, L., Jiang, F., 2014b. J. Cancer Res. Clin. Oncol. 140, 145–150.
Li, D., Cheng, W., Yan, Y., Zhang, Y., Yin, Y., Ju, H., Ding, S., 2016. Talanta 146, 470–476.
Liang, J., Zhong, B.T., Liu, B., 2015. Chem. Soc. Rev. 44, 2798–2811.
Lin, C., Wei, K., Liao, S., Huang, C., Sun, C., Wu, P., Lu, Y., Yang, H., Ma, C., 2015. Biosens. Bioelectron. 67, 431–437.
Liu, H., Liang, G., Abdel-Halim, E.S., Zhu, J., 2011. Anal. Methods 3, 1797–1801.
Liu, J., Chen, J., Yan, X., 2013a. Anal. Chem. 85, 3238–3245.
Liu, H., Xu, S., He, Z., Deng, A., Zhu, J., 2013b. Anal. Chem. 85, 3385–3392.
Liu, D., Yang, J., Wang, H., Wang, Z., Huang, X., Wang, Z., Niu, G., Walker, A.R.H., Chen, X., 2014. Anal. Chem. 86, 5800–5806.
Liu, L., Wu, S., Jing, F., Zhou, H., Jia, C., Li, G., Cong, H., Jin, Q., Zhao, J., 2016. Biosens. Bioelectron. 80, 300–306.
Loo, L., Capobianco, J.A., Wu, W., Gao, X., Shih, W.Y., Shih, W., Pourrezaei, K., Robinson, M.K., Adams, G.P., 2011. Anal. Chem. 83, 3392–3397.
Lu, S., Wang, Y., 2010. Clin. Cancer Res. 16, 3822–3824.
Malhotra, R., Patel, V., Chikkaveeraiah, B.V., Munge, B.S., Cheong, S.C., Zain, R.B., Abraham, M.T., Dey, D.K., Gutkind, J.S., Rusling, J.F., 2012. Anal. Chem. 84, 6249–6255.
Mandal, G., Darragh, M., Wang, Y.A., Heyes, C.D., 2013. Chem. Commun. 49, 624–626.
Miyamoto, D.T., Sequist, L.V., Lee, R.J., 2014. Nat. Rev. Clin. Oncol. 11, 401–412.
Morris, M.C., 2013. Biochim. Biophys. Acta 1834, 1387–1395.
Munge,, B.S., Coffey, A.L., Doucette, J.M., Somba, B.K., Malhotra, R., Patel, V., Gutkind, S.J., Rusling, J.F., 2011. Angew. Chem. 123, 8061–8064.
Oh, J., Lee, J., 2011. Anal. Chem. 83, 7364–7370.
Peng, F., Su, Y., Zhong, Y., Fan, C., Lee, S., He, Y., 2014. Acc. Chem. Res. 47, 612–623.
Peng, J., Guan, J., Yao, H., Jin, X., 2016. Anal. Biochem. 492, 63–68.
Petryayeva, E., Krull, U.J., 2011. Anal. Chim. Acta 706, 8–24.
Pu, K., Shi, J., Cai, L., Li, K., Liu, B., 2011. Biomacromolecules 12, 2966–2974.
Qu, F., Li, T., Yang, M., 2011. Biosens. Bioelectron. 26, 3927–3931.
Raamanathan, A., Simmons, G.W., Christodoulides, N., Floriano, P.N., Furmaga, W.B., Redding, S.W., Lu, K.H., Bast, R.C., Jr, McDevitt, J.T., 2012. Cancer Prev. Res. 5, 706–716.
Rasheed, P.A., Sandhyarani, N., 2016. Anal. Chim. Acta 905, 134–139.
Rusling, J.F., Kumar, C.V., Gutkind, J.S., Patel, V., 2010. Analyst 135, 2496–2511.
Rusling, J.F., 2013. Anal. Chem. 85, 5304–5310.
Sardesai, N.P., Barron, J.C., Rusling, J.F., 2011. Anal. Chem. 83, 6698–6703.
Sardesai, N.P., Kadimisetty, K., Faria, R., Rusling, J.F., 2013. Anal. Bioanal. Chem. 405, 3831–3838.
Shen, J., Liao, J., Guarnera, M.A., Fang, H., Cai, L., Stass, S.A., Jiang, F., 2014. J. Thorac. Oncol. 9, 33–40.
Siegel, R.L., Miller, K.D., Jemal, A., 2015. CA Cancer J. Clin. 65, 5–29.
Sonuç, M., Sezgintürk, M., 2014. Talanta 120, 355–361.
Su, J., Xu, J., Chen, Y., Xiang, Y., Yuan, R., Chai, Y., 2012. Chem. Commun. 48, 6909–6911.
Su, L., Zou, L., Fong, C., Wong, W., Wei, F., Wong, K., Wue, R.S.S., Yang, M., 2013. Biosens. Bioelectron. 46, 155–161.
Sun, M., Liu, F., Zhu, Y., Wang, W., Hu, J., Liu, J., Dai, Z., Wang, K., Wei, Y., Bai, J., Gao, W., 2016. Nanoscale 8, 4452–4457.
Swierczewska, M., Liu, G., Lee, S., Chen, X., 2012. Chem. Soc. Rev. 41, 2641–2655.
Tao, L., Zhang, K., Sun, Y., Jin, B., Zhang, Z., Yang, K., 2012. Biosens. Bioelectron. 35, 186–192.
Tao, Y., Lin, Y., Huang, Z., Ren, J., Qu, X., 2013. Adv. Mater. 25, 2594–2599.
Thakur, M.S., Ranjan, R., Vinayaka, A.C., Abhijith, K.S., Sharma, R., 2013. Nanoparticles and biophotonics as efficient tools in resonance energy transfer-based biosensing for monitoring food toxins and pesticides. In: Park, B., Appell, M. (Eds.), Advances in Applied Nanotechnology for Agriculture. American Chemical Society, Washington DC, 55–84.
Tong, S., Zhou, X., Zhou, C., Li, Y., Li, W., Zhou, W., Jia, Q., 2013. Analyst 138, 1549–1557.
Wagner, M.K., Li, F., Li, J., Li, X., Le, X.C., 2010. Anal. Bioanal. Chem. 397, 3213–3224.
Wang, G., Lipert, R.J., Jain, M., Kaur, S., Chakraboty, S., Torres, M.P., Batra, S.K., Brand, R.E., Porter, M.D., 2011a. Anal. Chem. 83, 2554–2561.
Wang, X., Wu, L., Ren, J., Miyoshi, D., Sugimoto, N., Qu, X., 2011b. Biosens. Bioelectron. 26, 4804–4809.
Wang, R., Di, J., Ma, J., Ma, Z., 2012. Electrochim. Acta 61, 179–184.
Wang, L., Wang, Y., Wong, J.I., Palacios, T., Kong, J., Yang, H.Y., 2014a. Small 10, 1101–1105.
Wang, Z., Liu, N., Ma, Z., 2014b. Biosens. Bioelectron. 53, 324–329.
Wang, L., Peng, C., Chen, C., Li, Y., 2015. Breast Cancer Res. Treat. 151, 7–17.
Wu, B., Wang, H., Chen, J., Yan, X., 2011. J. Am. Chem. Soc. 133, 686–688.
Yang, M., Javadi, A., Gong, S., 2011a. Sens. Actuat. B-Chem 155, 357–360.
Yang, Q., Gong, X., Song, T., Yang, J., Zhu, S., Li, Y., Cui, Y., Li, Y., Zhang, B., Chang, J., 2011b. Biosens. Bioelectron. 30, 145–150.
Yang, L., Huang, X., Sun, L., Xu, L., 2016. Sens. Actuat. B-Chem 224, 863–867.
Yildiz, U.H., Alagappan, P., Liedberg, B., 2013. Anal. Chem. 85, 820–824.
Ying, L., Wang, Q., 2013. BMC Biotechnol. 13, 76–86.
Yu, X., Li, Y., Wu, J., Ju, H., 2014. Anal. Chem. 86, 4501–4507.
Yu, T., Dai, P., Xu, J., Chen, H., 2016. Appl. Mater. Interfaces 8, 4434–4441.
Zadran, S., Standley, S., Wong, K., Otiniano, E., Amighi, A., Baudry, M., 2012. Appl. Microbiol. Biotechnol. 96, 895–902.
Zanganeh, S., Khodadadei, F., Tafti, S.R., Abdolahad, M., 2016. J. Mater. Sci. Technol.

32, 617–625.
Zhang, K., Zhao, L., Guo, S., Shi, B., Lam, T., Leung, Y., Chen, Y., Zhao, X., Chan, H.L.W., Wang, Y., 2010. Biosens. Bioelectron. 26, 935–939.
Zhang, Z., Nagrath, S., 2013. Biomed. Microdevices. 15, 595–609.
Zhang, L., Deng, H., Lin, F., Xu, X., Weng, S., Liu, A., Lin, X., Xia, X., Chen, W., 2014. Anal. Chem. 86, 2711–2718.
Zhao, J., Yan, Y., Zhu, L., Li, X., Li, G., 2013. Biosens. Bioelectron. 41, 815–819.
Zhao, Q., Duan, R., Yuan, J., Quan, Y., Yang, H., Xi, M., 2014. Int. J. Nanomed. 9, 1097–1104.
Zhou, M., Yang, M., Zhou, F., 2014. Biosens. Bioelectron. 55, 39–43.
Zhu, X., Cao, Y., Liang, Z., Li, G., 2010. Protein Cell 1, 842–846.
Zhu, Y., Chandra, P., Shim, Y., 2013. Anal. Chem. 85, 1058–1064.