Recent advances on applications of immunosensing systems based

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REVIEW

Recent advances on applications of immunosensing systems based on nanomaterials for CA15-3 breast cancer biomarker detection

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Abstract

Breast cancer is one of the leading causes of death and is the most routine form of cancer in women in the world. Carbohydrate antigen 15–3 (CA15–3) tumor marker is a serum-based product of the MUC1 gene and different studies have determined the over-regulation of this tumor marker in breast cancer. Moreover, CA15-3 is overexpressed in a number of other types of cancers including lung, ovarian, pancreatic, and colon. The important role of CA-15–3 detection in the screening and diagnosis of breast cancer is proved. Some specific methods for the detection of CA15-3 have significant advantages and also suffer from some disadvantages. Biosensor tools as analytical devices measure biological or chemical reactions, by converting them into electrical signals. Biosensors based on antibody molecules as the detector are called immunosensors. Different types of immunosensors including electrochemical, various optical sensors such as fluorescent, SPR, and colorimetric with immobilization of nanomaterials for improving sensitivity and antibodies can be useful devices for the detection of CA15-3 biomarkers. In this review, we intend to focus on various new immunosensors to overcome the disadvantages of conventional methods for the detection of CA15-3 biomarkers.

Keywords Carbohydrate antigen 15–3 · CA15-3 · Optical · Immunosensor · Electrochemical · Fluorescent · Surface plasmon resonance

Abbreviations

CA15-3	Carbohydrate antigen 15–3
ELISA	Enzyme-linked immunosorbent assay
ECL	Electrochemiluminescent immunoassay
CLIA	Chemiluminescence immunoassay
SPE	Screen-printed electrode
CNT	Carbon nanotubes
GA	Graphene aerogels
PPy	Polypyrrole
SPR	Surface plasmon resonance

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Introduction

Breast cancer is one of the leading causes of death and is the most routine form of cancer in women [1]. Breast cancer has a significant health disorder and demonstrates a top biomedical research priority. The incidence of this offensive disorder with nearly 1,7000,000 new cases each year remains alarmingly high; these rates suggest the slow progress made in the prevention setting [2]. Moreover, researchers have predicted the global burden of breast cancer is expected over 2 million by 2030, with growing proportions from developing countries [3]. In cancerous people, specific protein molecules are detected in the blood serum and body tissues called tumor biomarkers. The approach biomarker detection plays a vital role in the monitoring of breast cancer patients, especially in the follow-up of the disease [4]. Carbohydrate antigen 15–3 (CA15-3) is a mucin-like glycoprotein (≈ 400 kDa) released from breast cancer cells [5]. CA15-3 is a large transmembrane glycosylated molecule aberrantly high-regulated in numerous adenocarcinomas in an underglycosylated form and then secreted into the circulation and serum [6]. Development of high-performance biomarker monitoring is of great meaning to bioinformatics and clinical medicine, and will provide more crucial information for different disease-specific detection [7]. CA 15–3 recognizes the soluble moiety of the transmembrane mucin 1 protein that is heterogeneously expressed on the apical surface of normal epithelial cell type such as those of the breast. CA 15–3 tumor marker is a serum-based product of the MUC1 gene and has a prominent role in the screening and diagnosing of breast cancer. Moreover, CA15-3 is overexpressed in the number of other types of cancers containing lung, ovarian, pancreatic cancer, and colon [8, 9]. During a malignant transformation, the membrane expression of the MUC1 cell surface correlated oncoprotein usually converts from apical to circumferential simultaneously with a loss of polarity of the epithelial cells, facilitating the dissociation of tumor cells and operating as anti-adhesive molecules, and enhancing the invasive and metastatic potential of cancer cells [10]. CEA is one of the tumor markers that is useful in some cases for the demonstrating of treatment in advanced breast cancer [11]. Previous clinical reports show that in the follow-up of breast cancer, CA15-3 is more sensitive than CEA. Finally, Duffy and his colleagues [12] approved that the most crucial role for CA15-3 is in exhibiting therapeutic approaches in patients with advanced breast cancer. There are many challenges, containing an early diagnosis of recurrence and guidance via the different lines of treatment in the controlling of disease and determination of high-risk recurrence population. Among them, the best method for the early cancer diagnosis is an important problem. The conventional approaches, such as biopsy and ultrasound, and magnetic resonance imaging, are poor ways for early CA 15–3 detection as these methods depend on the phenotypic features of the tumor. Some immunological methods for detection of CA 15–3 such as enzyme-linked immunosorbent assay (ELISA) (42.9%), electrochemiluminescent (ECL) (25.7%), chemiluminescence immunoassay (CLIA) (14.3%), and radioimmunoassay are developed [13]. These methods are the main diagnosis methods that have unique advantages but there are some defects that increased the need to alternative techniques. Some advantages and defects of ELISA, CLIA, and radioimmunoassay are summarized in Table 1. Hence, developing appropriate and safe analytical methods for specific and sensitive detection of CA 15–3 biomarker at very low concentrations in biological samples and physiological environments are highly demanded. Biosensor technology is based on a specific biological recognition factor in combination with a transducer for signal processing and has some excellent advantages and leads to the development of this method for CA 15–3 detection in recent years. In this study, we first reviewed the different types of immunoassay methods and their characteristics and disadvantages for measuring CA 15–3. Then, we discussed about the advances of the last few years in the field of biosensors for sensitive and accurate detection of CA 15–3 biomarker.

Table 1 Conventional methods for CA 15–3 detection

Detection method	Advantages	Defects	References
ELISA	Low cost, simple method, high level of selectivity	Low sensitivity, long incubation periods	[14, 15]
CLIA	Fewer false-positive results	Complexity, various sensitivity ranges in different samples	[16]
RIA	Well sensitivity, high precision and stability	Short shelf-life of the reagents, dangerous radioactivity	[13, 17, 18]

CA15-3 carbohydrate antigen 15–3, *ELISA* enzyme-linked immunosorbent assay, *CLIA* chemiluminescence immunoassay, *RIA* radioimmunoassay

Conventional immunoassay methods for CA15-3 biomarker

Enzyme-linked immunosorbentassay(ELISA)

ELISA (enzyme-linked immunosorbent assay) has a role in the selective analysis of antigens, including nucleic acids, hormones, proteins, peptides, biomarkers, herbicides, and plant secondary metabolites. To detect these factors, an antigen or antibody is labeled with enzymes, and the so-called enzyme immunoassay is employed [19]. In enzyme immunoassays, enzymes are linked to secondary (detection) antibodies (Abs), which bind to the primary antigen–antibody complex. The suitable substrate is incubated and added for the quantification and visualization of the production of a colored end-product by enzyme [15]. The most appropriate enzymes used for catalyzing are horseradish peroxidase, β-galactosidase, and alkaline phosphatase. In the traditional ELISA method for CA15-3 detection, utilizing a monoclonal Ab directed against a specific antigenic determinant on the CA15-3 molecule. This Ab can be utilized to bind CA15-3 in the serum when coated on a solid phase (i.e., microwells of assay plate). A secondary Ab conjugated to an enzyme is employed as a signal generator [14]. ELISA is a specific, sensitive, and readily available method, highly used in almost every laboratory. ELISA can be performed and adapted in different fashions. The test sample can react sequentially with the two Abs, resulting in the antigen molecules being sandwiched between the enzyme-linked Abs and the solid phase. In general, ELISA has some features such as a relatively high level of selectivity, and needs low concentrations of sample pre-treatment procedures; however, it has some disadvantages containing long incubation periods, multiple washing steps, and pipetting, and sometimes relatively low sensitivity unless signal enhancer is used. These mentioned advantages and defects are almost identical to all types of ELISA methods for detecting CA 15–3.

Chemiluminescence immunoassay (CLIA)

Electrogenerated chemiluminescence (electrochemiluminescence, ECL) is one type of luminescence occurred at/near the surface of an electrode derived from chemiluminescence (CL) and electrochemical reactions. It is various from electroluminescence (EL), CL as a result of chemical reactions, photoluminescence (PL) as a result of absorption of light and photons as a result of direct conversation of electric energy to light [20]. The CLIA method is highly employed in many various formats. In the chemiluminescence device, a substrate decays from an excited state to a ground state and generates an emission of light. Chemiluminescent energy is produced from a chemical reaction, often an oxidation reaction [15]. CLIA is a strong technique in analytical chemistry, especially for clinical diagnosis and immunoassays. Today, many classical CLIA and CL systems containing luminol, lucigenin, peroxalate, 1,2-dioxetanes, and tris(bipyridine) ruthenium (II) ([Ru(bpy)3] 2 +) have been developed [21]. CLIA immunoassay techniques have excellent sensitivity with detection limits as low as $10-18$ or $10-21$ and also have appropriate accuracy because of a low background of chemiluminescence [16]. Moreover, in comparison with ELISA, CLIA assays approach significantly fewer false-positive results. However, this sensitivity range is different for various types of samples, and the kind of sample can reduce the sensitivity of diagnosis. Also, another important limitation is the complexity of this method, and the development of a chemiluminescent immunoassay requires the optimization of several vital parameters.

Radioimmunoassay

Radioimmunoassay (RIA) is a heterogeneous assay that uses radiolabeled drugs and the radioimmunoassay format is correlated with high analytical specificity, and in combination with a sensitive biomarker detection, resulting in a high performing measurement [22]. This technique needs the antibody with a high affinity constant to utilize a particular, and labeled antigen. Specificity is related to the ability of the antibody to bind subtle structural features of the analytes. Vizcarra et al. [23] have measured the concentration of CA 15–3 with 30U/ml with RIA. The benefits of the radioimmunoassay containing the ease of isotope conjugation, signal detection without optimization, extremely sensitive assay as it can measure antigen up to picogram quantities, appropriate specific test as the antibody-antigen reaction, and stability against other interferences in the sample have caused this method to evaluate exactly target molecules. On the other hand, the defects of the radioimmunoassay including shielding for protection against dangerous radioactivity, the short shelf-life of the reagents, requiring special arrangements for storage of radioactive material, radiation hazardous, the high cost of waste disposal, lengthy counting time, the reaction time being long because of the use of highly diluted reagent, and having some difficulties in the automation of this assay, have increased the importance of alternative methods [13, 17, 18].

Biosensor technology and tumor marker

The focus of clinical cancer diagnosis is creating and improving analytical techniques, which are explicitly capable of parallel and sensitive detection of cancer-related markers rendering excellent point-of-care testing. Biosensor devices have highly positive effects on the quality of human life by their operation for specific, sensitive, and accurate detection of different markers in patients [24]. Also, biosensors are step by step employed to develop advanced detection methods. A biosensor contains three necessary parts: signal-processing unit, a bio receptor, and a transducer in excellent connection with the bioreceptor [25]. Moreover, the biosensors have suitable values of reproducibility, sensitivity, assay time, specificity, accuracy, and robustness. In recent years, biosensors attract high attention in developing cancer detection as they exhibit real-time measurement and superior analytical operation. Due to their lower minimum detection limits, they can evaluate very low levels of biomarkers in real and spike samples that can assist in the detection of cancer at an early stage [24]. Biosensors are mainly classified according to the biological specificity conferring mechanism (catalytic or affinity biosensors) or, alternatively, to the mode of physical–chemical signal transduction: optical, electrochemical, piezoelectric, thermometric, or magnetic [26].

Electrochemical biosensor for CA 15–3 detection

Electrochemical sensors operate by reacting with the target biomarker of interest to create an electrical signal proportional to the specific biomarker concentration. The most typical part of electrochemical biosensors is an appropriate enzyme in the biorecognition layer preparing electroactive substances for diagnosis through the physico-chemical transducer and subsequently providing the detectable signal [27, 28]. The basic performance of an electrochemical biosensor is that the biological reaction between bioreceptor and target molecule can consume or create electrons or an ion that changes the electric potential, current, or other electrical features of the solution [29]. Electrochemical biosensors, a member of chemical sensors, combine the specificity, as demonstrated by low detection limits (LOD), of electrochemical transducers with the high sensitivity of biological recognition processes are summarized in Table 2 [30].

Table 2 Various types of electrochemical immunosensor and CA15-3 detection

Electrochemical techniques	Sensing platform	LOD	Types of electrode	References
CV, EIS	CuS/RGO	0.3U/ml	SPE	[31]
CV, EIS	TH–NPG–GN	5×10^{-6} U/ml	GCE	[32]
DPV	poly(toluidine blue)	0.10 U/ml	SPE	[33]
DPV	Pβ-CD/GAs	0.03 mU/ml	GCE	[34]
CV, EIS	Fe3O4 NPs	5.2 µU/mL	Gold electrode	[35]
DPV	NGS	0.012U/ml	GCE	[36]
EIS, CV	Streptavidin/biotinylated Abs/magnetic beads	15×10^{-6} U/ml	Gold electrode	[37]
DPV	Graphite/Abs	15U/ml	GCE	[38]
SWV	PAMAM dendrimer-QD nanocomposites	0.005U/ml	Film electrode	[39]
EIS, CV	Ferrocenyl/SNPs	0.64U/ml	Gold electrode	[40]
SWV	GQDs/CysA	0.011U/ml	GCE	[41]
DPV	GO/MWCNT	0.07U/ml	Gold electrode	[42]
SWV	AuNPs/GQDs	0.11U/ml	GCE	[43]
EIS	PPy NWs	0.02U/ml	SPE	[44]
DPV, SWV	AuNPs	5.0U/ml	SPCE	[45]
EIS	Ab-AuNPs	7.8 mU/ml	Gold electrode	[46]
EIS	Ag/TiO2/rGO NPs	0.07 U/ml	GCE	[47]

RGO reduced graphene oxide, *CuS* copper sulfide, *EIS* electrochemical impedance spectroscopy, *CV* cyclic voltammetry, *TH–NPG–GN* thionine–nanoporous gold–graphene, *SPE* screen-printed electrode, *GCE* glassy carbon electrode, *Pβ-CD* β-cyclodextrin polymer, *GAs* graphene aerogel, *NGS* N-doped graphene sheets, *SWASV* square wave anodic stripping voltammetric, *GQDs/CysA* graphene quantum dots, *MWCNTs* multiwalled carbon nanotube, *PPy NWs* polypyrrole nanowires, *SPCE* screen-printed carbon electrode, *Ag/TiO2/rGO* silver/titanium dioxide/reduced graphene

Different types of electrochemical immunosensors

A number of new materials were fabricated for biomolecule immobilization on electrochemical electrodes to access sensitive, facile, and fast detection. Nanomaterials are mostly utilized to develop electrochemical sensors because of their perfect catalytic properties, such as noble metal nanoparticles, carbon-based nanomaterials [48], metal, oxides nanoparticles [49], and metal nitride nanoparticles [50]. Different strategies have been approved to enhance the catalytic activity of nanomaterials, including using carbon-based nanomaterials as the support matrix to improve the electrochemical operation [51]. Graphene is a novel class of carbon material with carbon atoms packed in a two-dimensional honeycomb lattice. Graphene has attracted high attention in recent years from researchers in different fields including biotechnologies, electronic tools, conversion devices, energy storage, solar cells, and biosciences, due to their unique features, especially excellent surface area and appropriate electronic conductivity [52]. Amani et al. [31] have produced a new label-free immunosensor based on a modified screen-printed electrode (SPE) with copper sulfides with reduced graphene oxide (CuS-RGO) nanocomposites. This biosensor could detect CA15-3 in serum samples with 0.3 U ml^{-1} LOD. This new device is rapid for marker detection, and has a low detection limit without the need for an enzyme, appropriate sensitivity, specificity, wide linear range, and sufficient stability and reproducibility. A combination of GO and carbon nanotubes (CNT) has been exhibited as effective nanomaterials for electrochemical biosensors due to their excellent electrical conductivity [53]. However, GO and CNT require to be functionalized for introducing surface anchoring groups and sufficient binding sites. To solve this problem, covalent functionalization of multiwalled carbon nanotubes (MWCNTs) is used as acid oxidation for shortening the tube length to the best size, enhancing the population density of carboxylic groups, and preparing more concentrations of labels to be linked. Akter and his colleagues [42] have constructed a new electrochemical immunosensor that used an Au electrode with MWCNT-ferritin (labeled probe) and GO/Py-COOH sensor probe (sensor probe). This prepared immunosensor demonstrated excellent specificity and selectivity for CA15-3 detection in human serum samples with 0.01×0.07 U/ml LOD. TiO2, a type of semiconductor, attracted further attention because of its excellent activity and excellent chemical stability, as well as low toxicity [54]. However, the decreased electronic and ionic conductivities limit its practical capacity and functions. To prevent the above disadvantages, noble metals, for example, silver (Ag) materials, can be loaded on the surface of TiO2 (Fig. 1A) [55]. Combining reduced graphene oxide and TiO2 could generate an appropriate situation on the glassy carbon electrode (GCE) for easy loading of anti-CA15-3 Abs and this immunosensor detected CA15-3 with more convenience and accuracy [47] (Fig. 1B).

Moreover, as has been approved, the use of highly conductive N-doped graphenesheets (NGS) remarkably enhanced amplified signals by increasing electron transfer [56]. Unique electronic features of the highly conductive graphene have caused an excellent replacement for conventional nanomaterials in biosensing and label-free detection. The use of NGS is simple and prevents the need for labels. High-surface free energy of Au nanoparticles (AuNPs) can be the best reason for using these nanomaterials along with NGS for modifying electrodes. In a study, the glassy carbon electrode (GCE) was modified with anti-CA15-3 NGS/AuNPs, and this label-free electrochemical biosensor detected the CA15-3 with a linear range of 0.1–2[illegible] U/ml [36] (Fig. 2). Different ways have been employed for the generation of graphene-based materials/Au NPs to modify

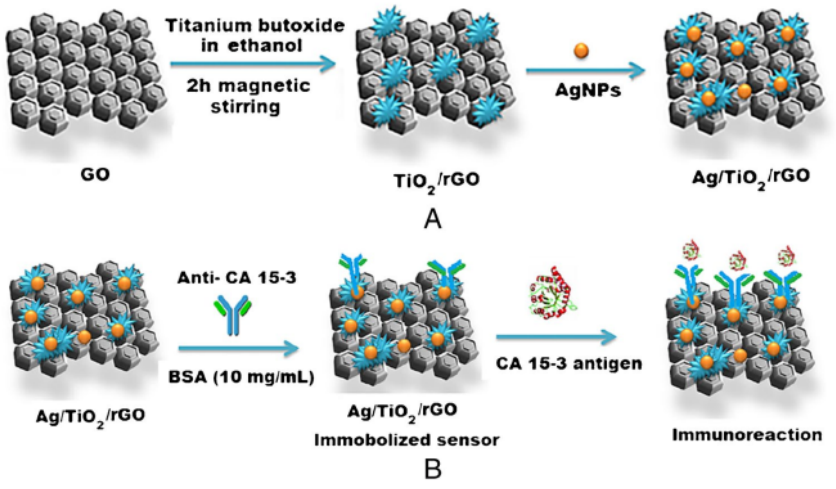

Fig. 1 Reduced graphene oxide (rGO) and TiO2 were coated on the glassy carbon electrode (GCE) (**A**). Then, anti-CA15-3 Abs were immobilized on the modified electrode via chemical groups (**B**) [47]. Copyright 2022, Elsevier. Adapted with permission from ref [47]

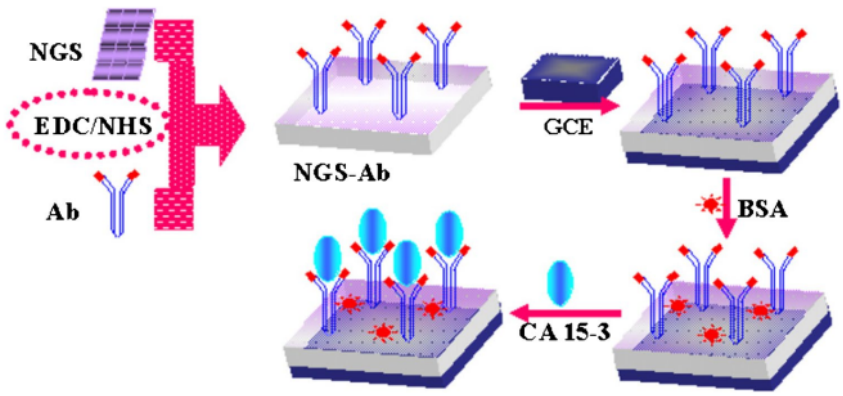

Fig. 2 Glassy carbon electrode (GCE) was modified with anti-CA15-3 NGS/AuNPs and this label-free electrochemical immunosensor detected the CA15-3 [36]. Copyright 2022, Elsevier. Adapted with permission from ref. [36]

surfaces and improve electrode surface area and electrical conductivity. These ways contain the electrodeposition of Au NSs onto the graphene-modified surfaces [57], simultaneous formation of reduced graphene oxide and Au NSs through electrodeposition [58], and layer-by-layer assembly of Au NPs and reduced graphene oxide [59]. Hasanzadeh and his colleagues [43] have synthesized graphene-based materials/Au NPs as gold nanosphere (Au NSs)/GQD to construct a new immunosensor. In this study, CA15-3 proteins have been linked easily on a modified electrode to recognize by Abs. But there is a very important defect about graphene, and that is when graphene is combined with other materials, the advantages of the generated composites are lower than theoretical predictions [60]. Converting the 2D material to 3D structures is the best way to solve this problem and it is very crucial to develop and characterize graphene materials with three-dimensional (3D) structures such as hydrogels, macroporous, and aerogels films [61]. Among these structures, graphene aerogels (GA) exhibit good environmental and chemical stability, suitable electrical conductivity, and a larger specific area [62]. β-Cyclodextrin (β-CD), a toroidal cyclic and shape oligosaccharide, can improve the bioavailability of drugs. This material has been developed as a novel approach to immobilization of antibodies on the immunosensor surface, because it easily binds with antibodies through amino functional groups [63]. The stability of the β-CD is not very good, but β-cyclodextrin polymer (Pβ-CD) that contains β-CD units has high stability. Jia et al. [34] have shown the combination of Pβ-CD and GA on the GCE electrode prepared an excellent surface for immobilizing anti-CA15-3 Abs and this selective, stable, and reproducible biosensor could detect the biomarker with 0.03 mU/ml LOD. Polypyrrole (PPy) nanowire as a common nanomaterial of conducting polymers is a very effective substrate for designing biosensors, because of its small cross dimensions, suitable biocompatibility, good ordered polymer chain structure with high aspect ratio, and excellent electrical features [64]. Different recognition molecules are deposited onto the PPy surface modified through entrapment methods or via adsorption [65]. When the PPy nanowire has linked with poly(1,5 diaminonaphthalene) [P(1,5DAN)] via amine groups, it could exhibit a promising composite for the electrochemical biosensor [44]. Nguyen et al. [44] have succeeded in the electrochemical deposition of P(1,5DAN) as the outer layer and PPy NWs as the inner film and finally coating anti-CA15-3 Ab on this modified screen-printed electrode (SPE). This technique can be useful for the fabrication of microdevices and even reproducible mass production. Fe3O4 nanocomposites have been highly utilized in bioanalysis and biomedical domains containing magnetic resonance imaging and drug and delivery hyperthermia, because of the nontoxic and biocompatible properties of Fe3O4 NPs [35, 66]. Fe3O4 NPs can also employ themselves as self-sacrificial labels to secrete iron ions, which are further adopted to amplify and create the measuring signals [67]. In a study, Li et al. [35] have used polyethylene glycol (PEG) to decrease nonspecific protein adsorption and employed it as an antifouling substrate. This material was coated onto an Au electrode surface via the formation of Au–S and then anti-CA15-3 was immobilized via carboxyl groups and finally Fe3O4 NPs were linked to the modified electrode. In this immunosensor, Fe3O4 NPs produce electrochemical signal for sensing of target marker.

Platinum (Pt) is a crucial particle in direct methanol fuel cells (DMFCs). However, the scarce resources of noble metal Pt severely limit the widespread commercialization of DMFCs. So, decreasing the consumption of Pt and increasing the operation of the catalyst are important problems [68]. Among the bimetallic nanomaterials, Pt-based nanocrystals, especially alloying various transition metals with Pt, have expanded remarkable research fields due to their highly increased catalytic activity. For instance, Pt–Co, Pt-Fe, Pt-Cu, Pt-Ni, and Pt–Pd. Pt-based bimetallic nanocrystals can remarkably enhance the catalytic operation of Pt via synergistic effects electronic effects or strain effects [69–71]. Ge and his colleagues [72] have fabricated the PtCo ND–based

label-free biosensor and demonstrated a competitive LOD of 0.0114 U ml^{1} and a dynamic linear range (0.1e200 U ml^{1}) together with high reproducibility, suitable specificity, and excellent stability. The immunosensor was successfully employed for the precise and sensitive measurement of the CA15-3 biomarker in the patients' serum samples. So, this method has caused a possible avenue for the prospective applicability of such biosensors in clinical diagnostics approaches.

Optical immunosensors and CA15-3 detection

Optical biosensors have been introduced as sensor techniques, and in these types of analytical systems, optical methods are used for the transduction of a biochemical interaction into appropriate detectable signals [73]. The bimolecular interaction on the sensor surface regulates the light characteristics of the transducer, and the biosensing signals can be evaluated via the change in various optical methods including refractive index luminescence, absorption, or, fluorescence, among others [74]. These types of biosensors show real-time, highly specific, rapid, high-frequency monitoring, and without any high costs or needing more time for measurement. Also, optical sensing systems have appropriate applications in food safety, diagnosis, environmental monitoring, drug development, and biomedical research such as CA15-3 detection that are summarized in Table 3 [75].

Fluorescent immunosensors

A fluorescent biosensor is a new approach that semi-quantitatively or quantitatively converts information about the presence of a specific biomarker to a measurable optical signal [84]. In recent decades, the evaluation of a single fluorescent molecule has come true, greatly enhancing novel, specific, and sensitive sensing systems. Fluorescent biosensors can create a suitable way to reveal small analyte signals and quantify and exhibit different molecule activities in living cells with excellent spatial and temporal resolution [85]. Fluorescent immunoassay tools have effective roles such as operational simplicity, great sensitivity, low detection cost, and convenience, and subsequently making it the preferred way among the available devices and the focus of considerable attention [86].

Cadmium NCs (Cd NCs) and nickel (Ni NCs) are low-cost metal nanoclusters and are highly studied due to their low cost, high fluorescence, high solubility, and easy synthesis. With a single excitation wavelength and two emission wavelengths, Cd and Ni NCs can offer an appropriate choice for the simultaneous monitoring of cancer markers [87]. Along with Cd and Ni NCs, molecularly imprinted polymers (MIPs) have been approved to be a potent approach for the synthesis of synthetic particular polymeric receptors [88]. MIPs have been extremely used in many fields, containing chromatographic separation [89], drug controlled release [90], chemo-/biosensors [91], solid-phase extraction, and catalytic reactions, and have excellent features such as high adsorption capacity, suitable practicability, good physical and chemical stability, high selectivity, and easy preparation. Bahari et al. [78] have constructed a developed fluorescent immunosensor based on: (I) Ni/Cd NCs as a luminophores, (II) magnetic molecular imprinted polymers (MMIPs) containing CA15-3 and CA125 Abs as a detector of CA15-3 antigens with 50 µl/ml LOD. In fluorescence immunosensors, as well as electrochemical immunosensors, stability and reproducibility are the main parameters of immunosensors during target analysis in practical applications, and previous studies showed that parameters by FL-MMIPs sensor.

MoS2 nanosheets (NSs) showed great properties in creating fluorescent biosensors due to their high surface–to–volume ratios, high efficient quenching ability, biocompatibility, and high capacity for loading specific molecules [92]. MoS2 NSs with wide UV–vis adsorption spectra can quench diverse fluorophores with various

Table 3 Optical immunosensors for CA15-3 detection

Type of optical system	Platform	LOD	Reference
Fluorescent	CdS/QDs	0.002 KU/l	[76]
FRET	AuNP-labeled PAMAM dendrimer/aptamer	0.9 µM/ml	[77]
Fluorescent	NiNCs/CdNCs/MMIP	50 µU/ml	[78]
FRET	AuNPs, PAMAM dendrimer/carbon dots	300cells/ml	[79]
SPR	Au/ZnO	4U/ml	[80]
SPR	Au/ZnO	0.025U/ml	[81]
LSPR	SBSM-LSPR	0.87U/ml	[82]
Colorimetric	RCIA/AuNPs/magnetic beads	50U/ml	[83]

NCs nanoclusters, *MMIPs* magnetic molecularly imprinted polymers, *FRET* fluorescence resonance energy transfer, *SPR* surface plasmon resonance, *RCIA* reverse colorimetric immunoassay

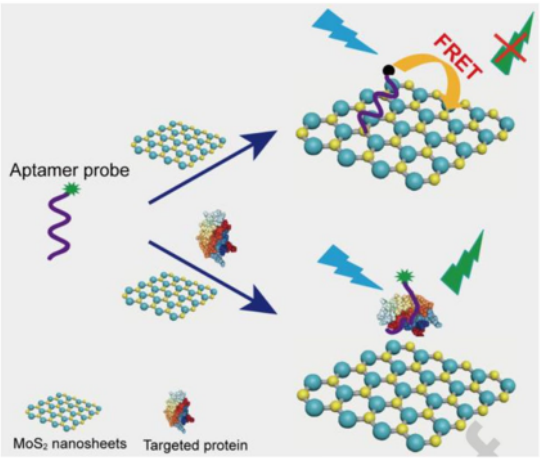

Fig. 3 Aptamer-based fluorescent biosensor that including specific DNA probe against CA15-3 proteins [95]. Copyright 2022, Elsevier. Adapted with permission from ref. [95]

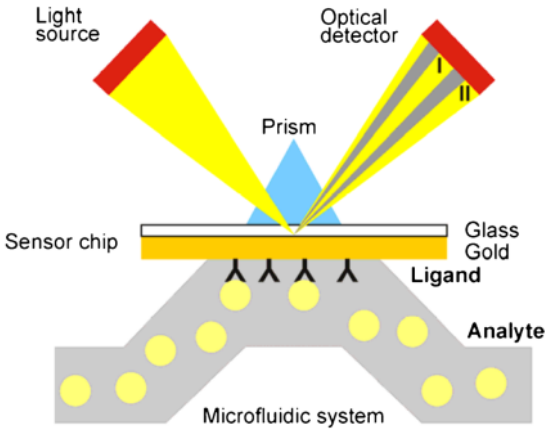

Fig. 4 In SPR biosensor function, the molecule linked to the sensor chip is termed ligand, and the other one analyte [96]

emission wavelengths including MoS2 quantum dots, carbon dots [93], and CdTe quantum dots [94]. The aptamer-based fluorescent biosensor, including a specific DNA probe against CA15-3 proteins, was fabricated by Zhao et al. [95]. Like other types of biosensors, this mentioned biosensor is cost-effective and has high sensitivity, excellent selectivity, good stability, and easy construction (Fig. 3). However, some limitations such as cracking and pulverization of the MoS2 are the main problems that decrease its applications in energy storage devices. But it is also important to note that one of the main disadvantages of fluorescent immunosensor is that not all compounds are fluorescent, and this reason has decreased the variety of study fields.

Various types of surface plasmon resonance (SPR) immunosensors

Surface plasmon resonance (SPR) is one of the most crucial and highly employed sensing systems. SPR biosensors are label-free optical tools as worked-based on the interaction between a molecule coated on the chip of the sensor and the interacting molecular partner in a solution. So, SPR sensors are important tools for biomolecular research in general and biomolecular interaction analysis [96] (Fig. 4). In recent years, SPR biosensors have been widely used for the detection of chemical and biological substances related to food safety and security, medical diagnostics, and environmental monitoring [97]. SPR sensors offer rapid evaluation of low concentrations of target molecules in real-time and detect biomolecules directly without labeling. A gold film is used as a sensing substrate for the sensor chip, owing to its excellent operation for the excitation of the SPR response. Also, it can be easily functionalized using thiol groups in self-assembled monolayers and is immobilized simply [81]. Au/ZnO nanocomposites have been explored in many studies because of some great optical and electrical features of ZnO nanoparticles, such as a high transparency, wide band gap, and low resistivity [98, 99]. Liang et al. [80] have prepared an SPR immunosensor based on thin-film ZnO/Au and have used it for evaluating the amounts of CA15-3 in human saliva with high sensitivity and without concentrating the samples. The localized surface plasmon resonance (LSPR) procedure has promising applications in the study of DNA-protein interactions, proteins, vesicles, and toxins [100]. SPR excitation needs incident light that is entirely inside reflected, while LSPR is that the nano-plasmonic resonance condition is satisfied in a transmitted light geometry or simply reflected typical to equally microscopy and spectroscopy applications [101]. Fan and his colleagues [82] have presented a smartphone biosensor system with the multi-testing unit (SBSM) based on LSPR and SBSM and this immunosensor has a role in recording simultaneously nine sensor units to achieve the detection of CA15-3 Ags with 0.87 U/ml LOD. A necessary point to note is that there are few studies on measuring CA 15–3 by SPR methods, and this problem has made it impossible for us to talk about more applications of SPR in CA 15–3 detection.

Colorimetric assay

Colorimetric immunoassay has attracted excellent attention in different studies such as food safety analysis, environmental monitoring, and biomedical diagnosis, because of some advantages, such as simplicity practicality, and

low cost [102]. The main point for improving colorimetric immunoassay is to transform the detection event into color change. Enzymes including alkaline phosphatase (ALP) and horseradish peroxidase (HRP) have excellent feature values in the colorimetric immunoassays owing to their great properties [103]. Catalase (CAT) has exhibited great operation in biosensing owing to its two important functions such as being much less expensive and more efficient than other popular alternatives including ALP and HRP. Gao et al. [83] have constructed a reverse colorimetric immunoassay (RCIA) system based on gold nanoparticles and magnetic beads and also two specific Abs. In this RCIA, anti-biomarker-conjugated magnetic beads have been utilized as a colorimetric developer and functional gold nanoparticles as enzymatic bioreactors. Finally, this novel colorimetric immunoassay could detect CA 15–3 with 50 ng/ml LOD. Compared with the conventional colorimetric biosensor, the RCIA technique does not need sophisticated instruments and is well appropriate in both biodefense and clinical application for high-throughput biomedical sensing via controlling the target antibody. Like the SPR methods, an important point to note is that there are few pieces of research on measuring CA 15–3 by colorimetric methods and this defect has challenged the efficiency of this immunoassay for detecting CA 15–3.

Conclusion

Analytical detection approaches founded upon whole cell-based assays are crucial in fundamental studies of biomolecular recognition and early-stage drug development. CA 15–3 has much better clinical specificity than other biomarkers and is the most highly utilized serum marker in breast cancer. Patients with primary breast cancer or metastatic breast cancer exhibit enhanced CA15-3 to more than 30 units/ml [77]. Early analysis of the cancer antigens CA 15–3 is one of the main ways for the early diagnosis of breast cancer and can increase treatment effects. As mentioned in this review article, various types of immunosensors are suitable and reliable tools for detecting CA 15–3. These techniques can overcome important limitations of routine devices such as low specificity and sensitivity and complexity of methods. In this study, we reviewed the efficiency of graphene-based, other nanomaterial-based electrochemical immunosensors, and different optical devices such as fluorescent, SPR, LSPR, and colorimetric immunosensors for CA15-3 Ags monitoring. According to previous review articles, there are several studies on the use of biosensors methods to detect cancer biomarkers. But this review has some features that make it unique: (I) all research examined contain immunosensors based on specific Abs; (II) various nanomaterials are produced for enhancement of CA15-3 specific immunosensors; (III) this article is the first paper summarizing the studies performed to detect CA15-3 by optical and electrochemical immunosensors. There are two major shortcomings in the detection of CA 15–3 marker with immunosensors that should be further considered in future studies, including (I) the use of different nanomaterials to further increase the sensitivity of electrochemical biosensors, (II) CA 15–3 measurement in various patient samples, and (III) extensive studies in the field of optical biosensors.

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Declarations

Conflict of interest The authors declare no competing interests.

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