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Review

Biosensors for Carcinoembryonic Antigen Detection for Future Prospective: A Review

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Abstract: Recently, the increasing number of cancer disease is a challenge for researchers and lots of people died from this cause. Only one solution for this disease is proper diagnosis and detection of the Carcinoembryonic Antigen (CEA) in the human body system which extensively reduces cancer mortality and saves lives for human beings. For that reason, the biologist developing the biosensor detection of CEA is mandatory because they are easy to utilize, handy, and can-do treatment in actual time. The biosensors play a significant role in the early signs of disease. These can be employed for the danger judgment, diagnosis, and projection for the forecast of treatment effectiveness, toxicity, and repetition. This review article systematically bestows a brief overview of the development of biosensors and the identification of CEA and its various types. Numerous approaches to be used for the recognition of cancer biomarkers, such as labelfree approach, electrochemical, mass-based transduction, and optical and analytical performance of several biosensors, explore the unsolved difficulties and future challenges in the development of cancer biomarkers.

***Keywords*:** biosensor, carcinoembryonic antigen (CEA), detection, biomarker, diagnosis, cancer, diagnosis, application, biosensor detection

Abbreviations

CEA: Carcinoembryonic Antigen
POC: Point of Care device
MRI: Magnetic Resonance Imaging
TSGs: Tumor Suppressor Genes
RNA: Ribonucleic Acid
DNA: Deoxyribonucleic Acid
ECB: Electrochemical Biosensors
OB: Optical Biosensors
MSB: Mass Sensitive Biosensors
PCR: Polymerase Chain Reaction

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CMB: Colorimetric Biosensors
ExO: Exonuclease
NMM: N-Menthylmesoporphyrine
GCE: Glassy Carbon Electrode
CVs: Cyclic Voltammograms
KCL: Potassium chloride
PBS: Phosphate Buffer Solution
EIS: Electrochemical Impedance Spectroscopy
ECAB: Electrochemical Aptamer Biosensor
HRCA: Hyperbranched Rolling Circle Amplification
ECB: Electrochemical Biosensor
DPV: Dissimilar Pulse Voltammetry
MMPs: Magnetic Microparticles
SPR: Surface Plasmon Resonance
IgGs: Immunoglobulin
Bio-AuNPs: Biofunctionalized gold nanoparticles (Bio-AuNPs)
SFM: Self-Fabricated Monolayer
MUDA: 11-Mercaptondecanoic Acid
RAM: Rabbit Anti-Mouse
CDs: Carbon Dots
QY: Quantum Yield
AuNPs: Gold nanoparticles
HER2: Epidermal growth factor receptor 2
PSA: Prostate-Specific Antigen
AFP: Alpha-Fetoprotein
SCC: Squamous Cell Carcinoma
CA: Carbohydrate Antigen
HCG: Human Chorionic Gonadotropin
MCA: Mucin-like Carcinoma-associated Antigen
AFP: Alpha-Fetoprotein
EGF: Epidermal Growth Factor
NSE: Neuron Specific Enolase
NY-ESO-1: Esophageal squamous cell carcinoma-1
TAG-72: Tumor-Associated Glycoprotein
ER: Estrogen Receptor
PR: Progesterone Receptor

1. Introduction

In the last decade, cancer is the second most liable reason for loss of the people worldwide. According to the 2004 report cancer killed 7.4 million citizens, mainly from them growing nations this number also increased approximated to achieve 12 million by 2030.[1] All the people were rescued if their cancer was detected prior. Currently, we need to develop new technology for earlier observation and treatment of cancer disease. This is not easy to identify and treat as it usually spreads from the heart of organs and is not distinguished as a foreign body via our immune system. Nevertheless, cancer is the abnormal development of the cells and proliferation into the surrounding tissues and can metastasize to far places and influence some parts of the body.[2,3]

On the other hand, despite the existence of recent technology, the survival rate of cancer patients is still poor which is attributed to the late-stage diagnosis and poor prediction of cancer. Recently, available traditional techniques such as ultrasound, Magnetic Resonance Imaging (MRI), and biopsy are ineffective before the detection of cancer in the primary

stage because these techniques are based on the phenotypic characteristics of the tumor.[4,5]

The investigation of CEA in the human body system plays an important character in pharmaceutical, biochemical, and medical investigations. It is one of the medical tumor markers that can be helped to detect several malignancies.[6,7] At present, various analytical methods have evolved and provide the best qualities and rapid investigation, and a comparatively cost-effective, high-sensitivity, and immunoassay procedure is broadly employed in different areas like diagnosis, observing of diseases, proteomics, and recognition of biological reagents. Moreover, this is also a suitable approach for the identification of CEA.[8-11] However, the CEA tends to be coexisting with other analytes like ascorbic acid and glucose in human body fluids with low concentrations. As a result, developing an extremely sensitive and selective biosensor for the recognition of CEA is in extensive demand. The biosensor market day by day growing from 2000-2030 (Billion USD) for various applications as shown in Figure 1.[12]

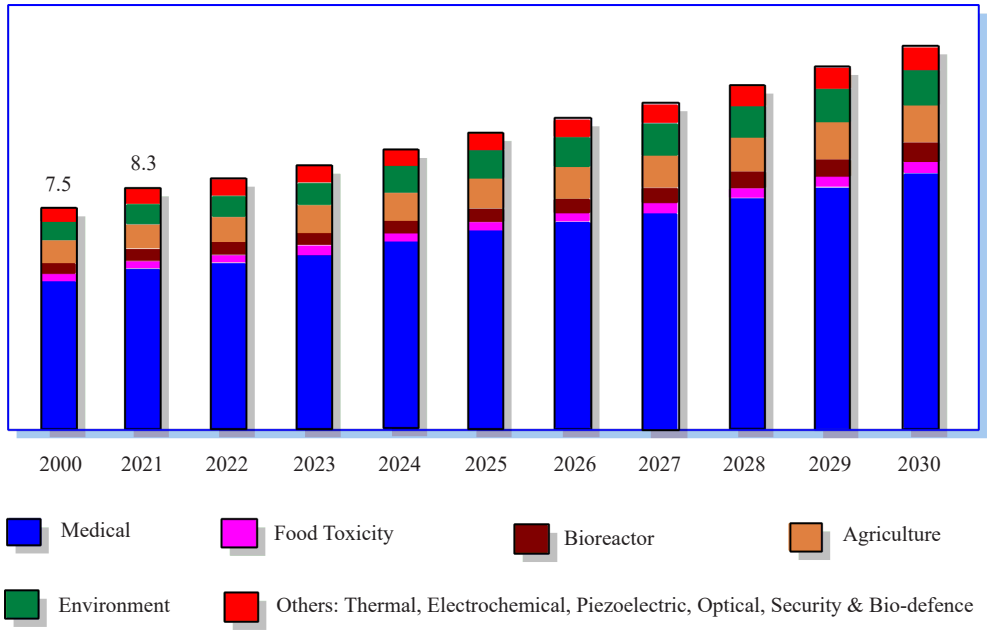

Figure 1. Biosensor market with various applications, 2000-2030 (in USD billion)[12]

Further, the CEA is the mainly investigated tumor marker connected with colon, liver, colorectal, and breast cancers.[13] However, the tumor marker, CEA, is a 200 kDa glycoprotein in the immunoglobulin superfamily and plays a significant role in detecting and screening various cancers.[14]

In general, the percentage of CEA content in biological samples of healthy people is lower than 5 ng/mL while the higher serum CEA level demonstrated the existence of cancer cells. Therefore, the sensitivity and prior detection of CEA are important in the prediction of real carcinoma and clinical tumor detection. Up to now, several methods, as well as enzyme-linked immunosorbent examination,[15] square wave voltammetry,[16] fluorescence,[17] capillary electrophoresis-chemiluminescence,[18] and electrochemiluminescence[19] have been used for the detection of CEA. In contrast with traditional methods, these are to require very costly and complex equipment, an expert operator, and a huge quantity of time for sample pretreatment,[14] while electrochemical, methods reveal the benefit of easy instrumentation and high sensitivity, economical, particular identification and quick retort after applying to identify CEA.[20-22] There are several different types of cancer biomarkers classified into the natures of carbohydrate antigen, embryonic antigen, enzymatic and isoenzyme tumor markers, protein cancer, hormone-related markers, and oncogene-related cancer biomarkers.[12]

This review paper mainly provides information on biosensors used for the recognition of cancer, biomarkers, and types of biosensors. Further, also reported the design of various effective biosensors like electrochemical, micromechanical, colorimetric, Plasmon Resonance (PMR), etc., for the detection of CEA. This review article also

provides information about nanoparticle-based biosensors.

2. Biosensor

In the last two decades, there has been remarkable development and attention in biosensor research and technology.[23] Biosensors are referred to as influential analytical devices and are the potential to use in a broad variety of applications such as medicine, agriculture, environment, and food safety as shown in Figure 2.[24,25]

Figure 2. Biosensor applications in various fields

A biosensor is an independently integrate receptor transducer device and it's able to bestow discriminatory quantitative or semi-quantitative analytical knowledge, employing a biological detection component according to the International Union of Pure and Applied Chemistry (IUPAC) definition.[26] The signal initiated from the interaction between the analyte of attention and the biological detection component is then converted via a transducer to an electrical or optical readout.[27,28] Figure 3 demonstrated a schematic representation of the various parts of the biosensor. The biosensors are more appropriate, consistent, perfect, cost valuable, and simple to employ compared to the further traditional recognition methods owing to their reusability, portability, immediate response, selectivity, and high specificity.[29,30]

3. Biosensor employed for the detection of cancer

A biosensor is described as a bioanalytical device including a molecular detection unit connected by or combined with a physicochemical transducer as shown in Figure 4. A biosensor is a Point of Care device (POC) and exhibits the potential for the identification of clinical samples in the house and hospitals. In this regard, we can develop suitable biosensor technology, particular markers required to be recognized to confirm the specificity of the devices. Biosensor bestows a better platform for biomarker identification and another advantage is easy to use, inexpensive, fast, and strong with providing multi-analyte testing capability.[31]

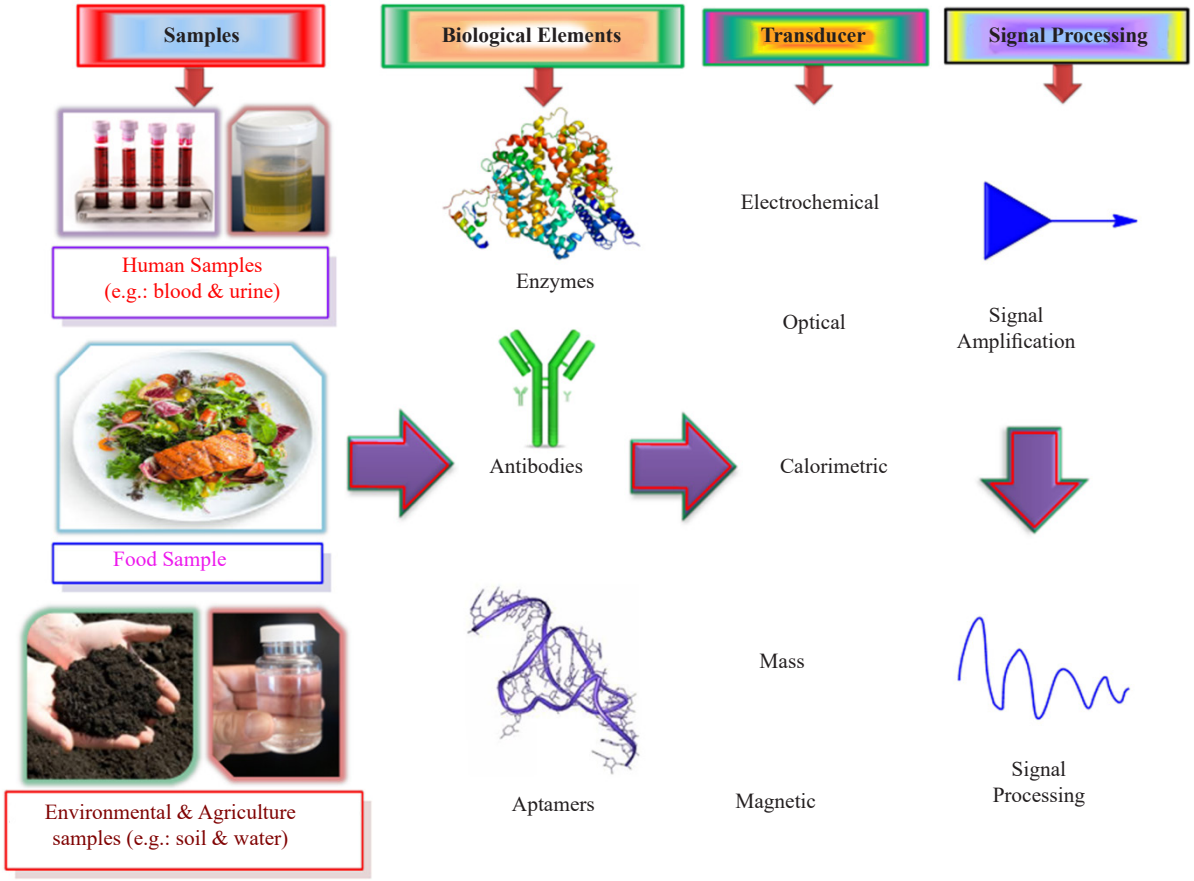

Figure 3. Schematic representations of biosensor parts including biological recognition elements, transducers, and detectors

Recently, cancer is one of the major incurable diseases at this time and lots of researchers have fabricated biosensors for prior identification of CEA antigen confirmation of cancer,[32,33] because the CEA blood test is not possible to identify cancer or as a screening test for prior detection of cancer. Mainly types of cancer do not show the result at the highest level. Serum from separate with colorectal carcinoma frequently has higher levels of CEA than healthy separate (above approximately 2.5 µg/L).[34] The confirmation of the CEA is mainly employed as a tumor marker to observe colorectal carcinoma treatment, to detect repetition after surgical procedure, and for rendering or localizing cancer increase through the confirmation of biological fluid.

Most of cancer is identified via Magnetic Resonance Imaging (MRI), biopsy, and ultrasound techniques which depend on the physical characteristics of the tumor and its existence and create the diagnosis concerning also advanced instruments or invasive.[35] Frequently cancer is related to others in gene orders, for example, mutation, consequently increasing demand for early recognition before the disease growth. Prior identification of cancer, is better for easier and more effective treatment. On the other hand, biosensor provides the best platform for sensing earlier stages of cancer.

According to several researchers, the detection of cancer is possible by biosensors because biosensors can be sensed the irregularities in the chemical and genetic constitution of the human body before the initiation of the disease. Cancer is the uncontrolled and abnormal development of cells due to the accumulation of particular genetic mutations and epigenetic deficiencies.[36] The cancer cells exhibit defiance for apoptosis and the anti-growth defense system of the body. Cancer becomes untreatable after starting the growth and spread to other body organs and systems such as metastasizing.[37,38]

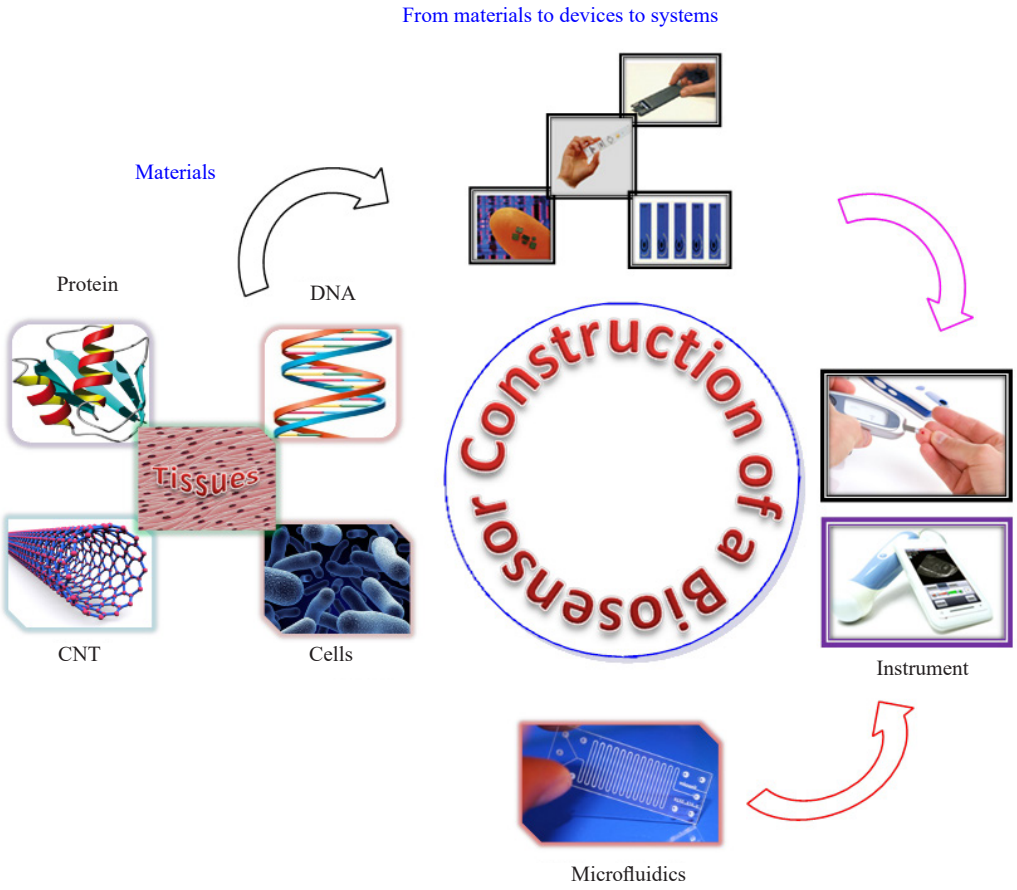

Figure 4. Formation of the biosensor

Further, the two most significant tumorogenesis systems are motivated by oncogenes and decreasing the movement of Tumor Suppressor Genes (TSGs). Owing to the mutation or replication of a regular gene (e.g., proto-oncogene) and the start of an oncogene participates in the main role, such as control of cell expansion, multiplication, and separation for the disease. This type of mutation directs the gene to generate surplus quantities of its gene product and shows to deregulation of cell distribution, cell growth, and tumor formation.[39]

Several oncogenes and growth reason receptors have been observed as favorable cancer biomarkers. The growth of the human epidermal receptor Her-2 is enhanced in 33% of all breast cancers or cancers with increased Her-2 lean-to fabricate and enhance too energetically. Therefore, awareness of the Her-2 gene condition is significant in deciding the promising route of medicine.[40] Trastuzumab (Herceptin) is humanized monoclonal antibody employed in straight therapy for the treatment of breast, and gastroesophageal cancer of the patient with this kind of amplified gene [Her-2] appearance.[41] TSGs are related to the regulation of unsuitable cell growth and multiplication by decreasing or uncertain cell distribution.[39]

The study of Tumor Suppressor Genes (TSGs) in cancer is retinoblastoma protein p5 plays a key role regulation of apoptosis or planned cell death. The mutations in p5 are found in breast, brain, lung, colon, leukemia, and hepatocellular carcinomas. Further, the loss of p5 also plays an important role in chemotherapeutic drug resistance.[42] The biosensor can identify the presence of a mutation in p5 is extremely acceptable and can facilitate confirming prior cancer susceptibility with correct diagnosis and treatment. The Figure 5 demonstrated schematic representation of biosensor detection process.

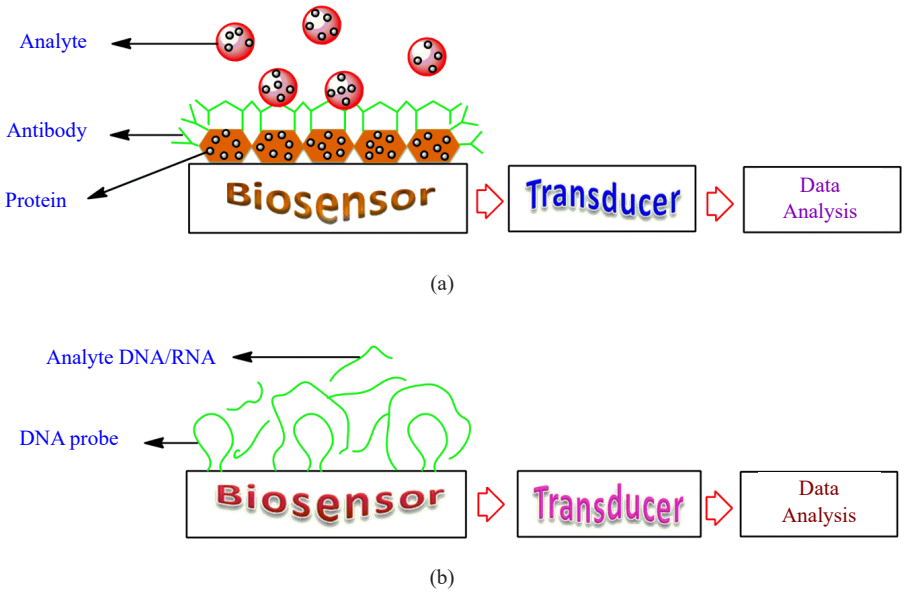

Figure 5. Schematic representation of biosensor detection process[43]

4. Biomarkers

The biomarkers of biological molecules found in blood, other body fluids, and tissues are indications of normal or abnormal growth of the condition of disease.[44] Biomarkers can be employed to observe how the body reacts to cancer treatment as shown in Figure 6. Biomarkers are different types of molecules like RNA, proteins (i.e., antibodies, hormones, oncogene), and DNA (i.e., amplification, mutation, and translocation).[35] They are all related to your health.

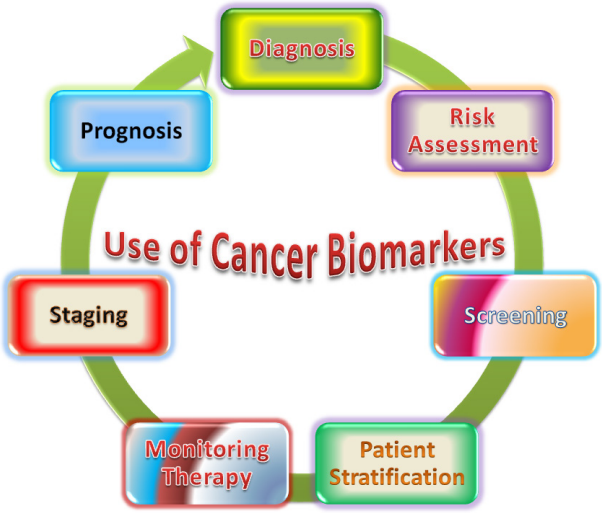

Figure 6. Utilization of cancer biomarkers

However, these biomarkers are usually identified in a body fluid such as cerebral fluid, urine, and blood as well as appearing in cancer cells. The generally utilized biomarkers for cancer detection are depicted in Table 1.

Table 1. Commonly used biomarker for cancer recognition

S. No.	Types of cancer	Biomarker
1.	Breast	BRCA1, BRCA2, CA 27.29, CA 15-3, CA 125, CEA, ING-1, NY-BR-1, ER/PR, HER2/NEU[35]
2.	Esophageal	SCC[45]
3.	Ovarian	CA 19-9, CA 15-3, CASA CA 125, CA 549, HCG, p53, MCA, MOV-1, CEA, TAG72[46]
4.	Liver	AFP, CEA
5.	Colon	CEA, EGF, p53[47]
6.	Lungs	NY-ESO-1, CEA, CA 19-9, NSE, SCC[48]
7.	Prostate	PSA[49]
8.	Melanoma	Tyrosinase, NY-ESO-1[50]

5. The various types of biosensors use for the detection of cancer

Mainly biosensors are divided into 5 types, optical biosensors, electrochemical biosensors, calorimetry biosensors, and mass-based biosensors as depicted in Figure 7.[51,52] Further, there are numerous kinds of biosensors related to the types of biological materials and sensor devices used.[53]

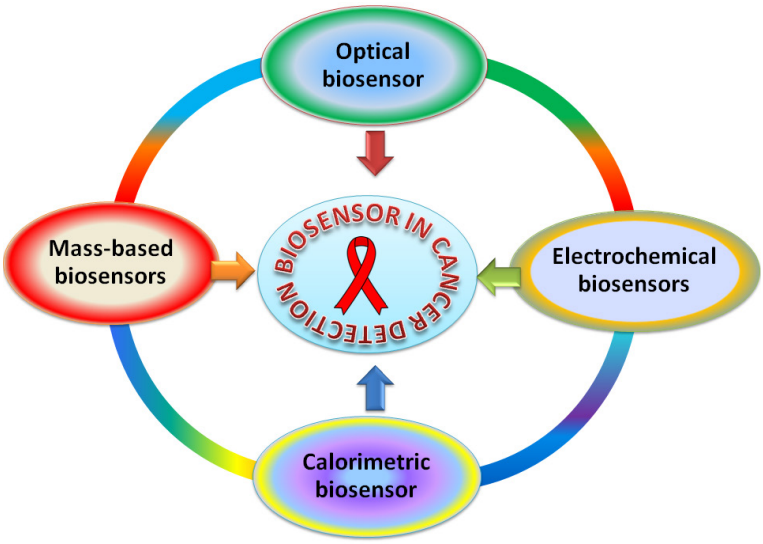

Figure 7. Biosensors used in cancer detection

5.1 *Electrochemical Biosensors (ECB)*

Electrochemical Biosensors (ECB) are mainly used biosensors owing to their low cost, portability, small size, and ease of employment which is controlled in a doctor's office or home. The glucose biosensor is mainly extensively employed by ECB. This is based on the screen, printed amperometric disposable electrode as shown in Figure 8. These types of biosensors have been utilized globally for glucose examination at home. On the other hand, i-STAT is a

handheld medical analyzer combined with numerous ECB on a particular chip and utilized for numerous metabolites and electrolytes in medical specimens.

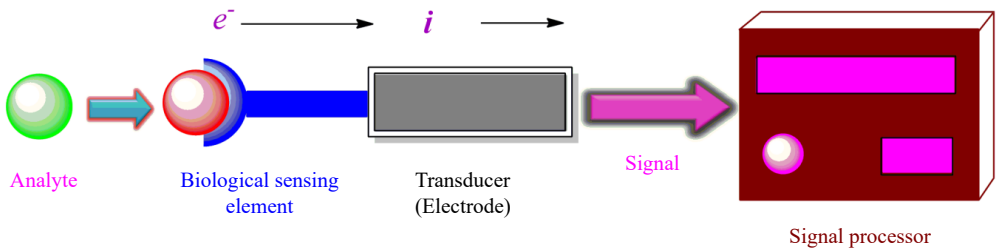

Figure 8. Schematic representations of electrochemical biosensors[54]

The electrochemical sensor used for earlier cancer detection is based on antibodies with large sensitivity. The various varieties of the electrochemical transducer employed contain amperometric impedimetric/conductivity and potentiometric, apparatus. The amperometric and potentiometric biosensors are generally Electrochemical Biosensors (ECB).[54] The ion-selective electrode detects the electrical response and occurs molecular recognition after using a potentiometric sensor. These biosensors exhibited a higher ability for the analysis of cancer-like breast tumor cells (e.g., MDA/MB231 marker hPRL-3).[55] Further, the ECB detected the mutations such as Breast cancer type1 (BRCA1) and BRCA2 and also plays a significant role in the finding of damaged DNA plus carcinogens that reason the damage. Antibodies also joined with an electrochemical transducer in immune sensors, are employed, to identify cancer.[56] ECB is the greatest technique of identification and prohibits cancer and various varieties of tumors.

5.2 *Optical Biosensor (OB)*

Optical Biosensors (OB) count the difference in wavelengths of light and they can be used as fluorescent, colorimetric, luminescent, or interferometric transducers. After changing the wavelength, the response to the perception of the analyte is transferred via an optical transducer and provides digital analysis.[54] This type of sensor is used for the optical transducer biosensor with photonic crystals as shown in Figure 9. The sensor is developed to confine very small quantities or light areas and allow measuring at high sensitivity, and demonstrate the outcomes of the light spread to a high electromagnetic field. This method identifies the molecules and cells that detach or attach to the surface ready of the crystal during the calculation of the light reflected in the crystal. The performance of these biosensors has been improved to change the observation in apoptosis and growth of breast tumor cells with contact to cytotoxicity. Therefore, there are playing important roles in observing the continuation of disease.[56]

Further, laser-induced fluorescence is a new OB for observing and analysis of throat cancer. The patient devours the biosensor, the laser beam is handled by the apparatus, and on the surface of the esophagus, a particular wavelength of light is released. The esophagus walls reflect the light of an extremely particular wavelength, and the difference in the imagination of different wavelengths is affected by the existence or deficiency of cancer cells or normal cells. More than 200 patients have used this type of biosensor technique for testing the cancer cells contrasted to the usual technique. Surgical biopsies and pain combined recovery are prohibited via this biosensor.[56]

5.3 *Mass-Sensitive Biosensors (MSB)*

Mass biosensors are divided into two types, acoustic and piezoelectric biosensors both, depending on the mass changes as depicted in Figure 10. The piezoelectric sensors are working on the mass changes of a quartz crystal after applying the potential energy and the frequencies produced by mass alterations can be transformed into signals. For example, immunosensors biosensors, and microcantilevers are based on piezoelectric techniques and help identify tumor biomarkers. Further, more appearance of human p53 genes indicates mutations in various kinds of tumors have been described with piezoelectric biosensors attached with Polymerase Chain Reaction (PCR) amplification.[54]

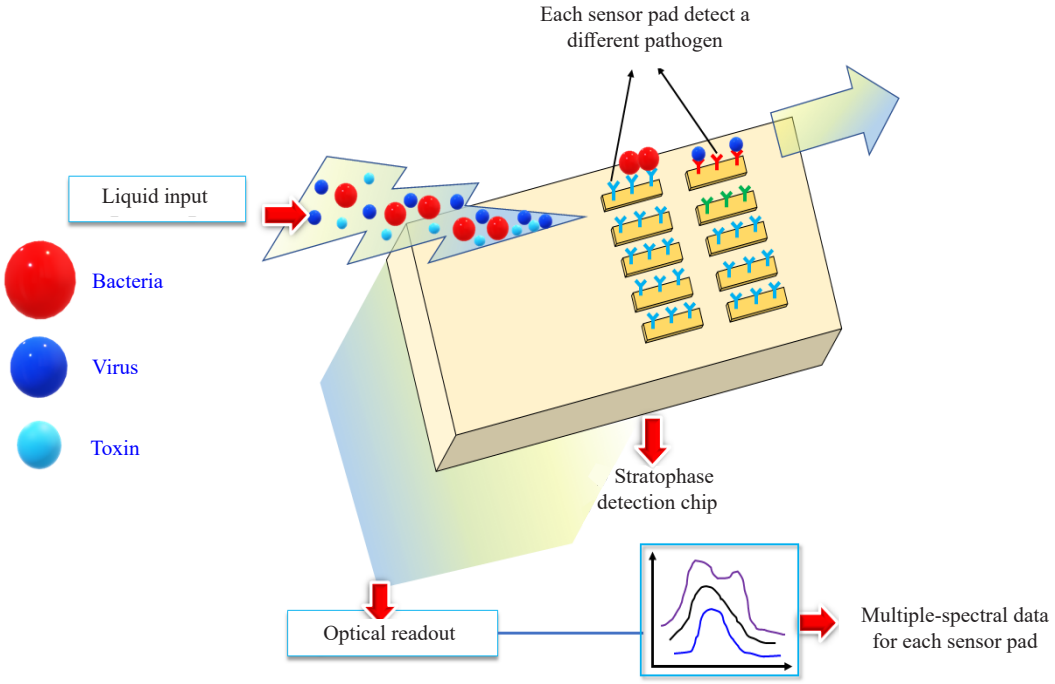

Figure 9. Schematic representation of optical biosensor[57]

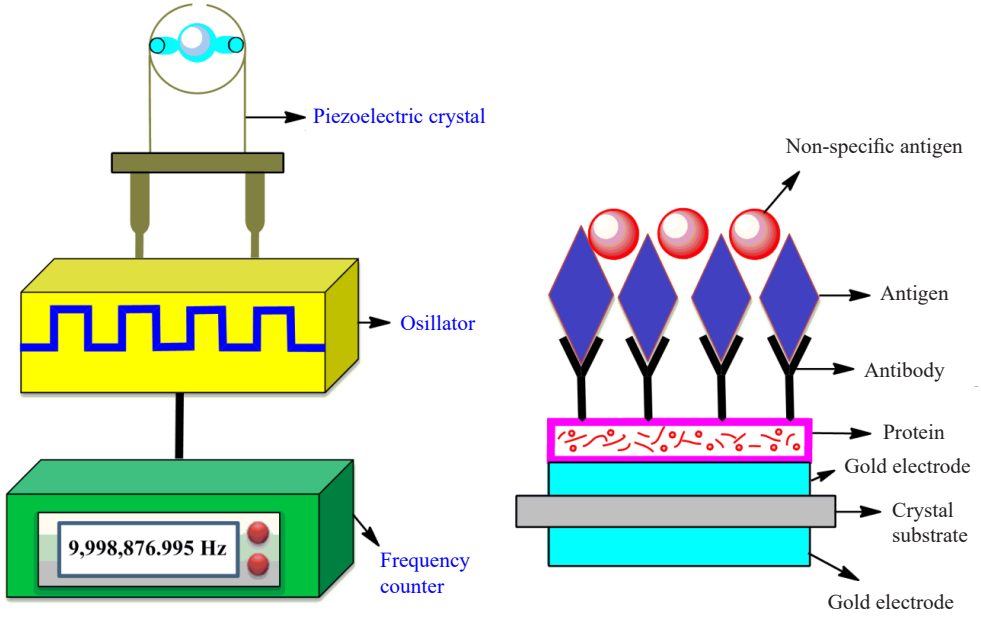

Figure 10. Piezoelectric biosensor demonstrated antibody-antigen binding[58]

5.4 *Colorimetric Biosensors (CMB)*

The colorimetric biosensors determine exothermic reactions and are not employed extensively in the diagnosis of cancer cells (Figure 11). Owing to the temperature changes and generated heat during the enzymatic reaction and provides information about the appropriate molecules by calculating the enthalpy of the reaction. This gives the required

data for determining the quantity of the molecules. There are less broadly employed in the identification of cancer, but there are some helpful properties for the diagnosis of cancer, such as sarcoma identification of two various kinds of cells: Burkitt's and acute leukemia cells.[59]

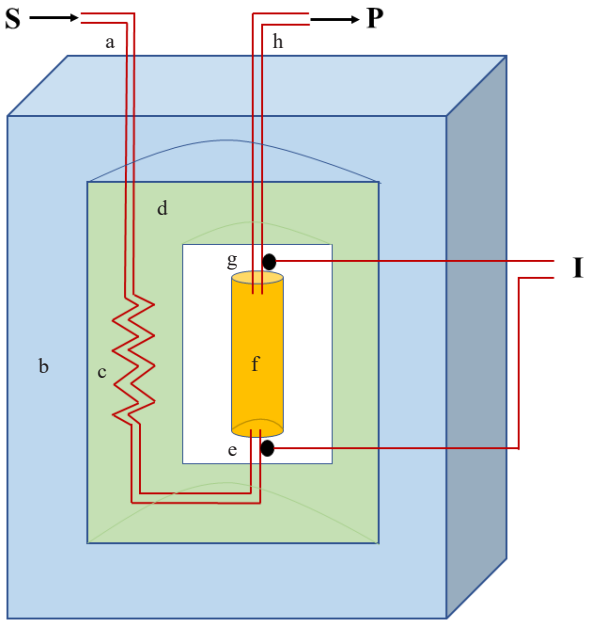

Figure 11. Calorimetric biosensors for diagnosis of cancer cells[60]

6. Biosensor detection of Carcinoembryonic Antigen (CEA)

The detection of carcinoembryonic antigens was investigated by various researchers. Omidi et al.,[61] fabricated Micromechanical (MM) biosensors to detect Carcinoembryonic Antigen (CEA), protein biomarkers related to different cancers like lung, colorectal, pancreatic, breast, and bladder cancer. The sensing process is based on the changes in surface stress influence of antigen-antibody interaction on the MM surfaces. MM contains a membrane suspended via 4 piezoelectric sensing elements. The results demonstrated that the isotropic uniaxial surface of stress on the membrane in every sensing element enhances the sensitivity. The experiment exhibited that the MMs, surfaces stress sensitivity to a 2 (mJ/m) and allow detect CEA concentration as low as 500 $pg \cdot mL^{-1}$. This shows that the self-sensing MM process is helpful for the pathological test.

The first time He et al.[62] developed a smart DNA walker biosensor for label-free identification CEA based on the new cascade amplification approach of Exonuclease (ExO) II-assisted targeting reclaiming amplification (ERA) and DNA hiker as shown in Figure 1. The initial step of ERA amplification produced the walker DNA and at the same time independent movement of the walker DNA on the silica microsphere modified substrate, and 2^{nd} step of amplification generates an ultrasensitive fluorescent signal with the assistance of N-menthylmesoporphyrine IX (NMM) as depicted in Figure 12. The DNA device used as a biosensor demonstrates an extensive quantitative variety, high sensitivity, suitable specificity, and eschewing elaborated reporter factors and thermal cycling.

The identification limits of the DNA biosensor are low such as 1.2 $pg \cdot mL^{-1}$ for CEA identification which is ascribed to the cascade amplification approach of the ERA and DNA walker. For that reason, the developed walker biosensor has been effectively used for the identification of CEA in human serum with suitable results and it reveals huge capability in clinical analysis.

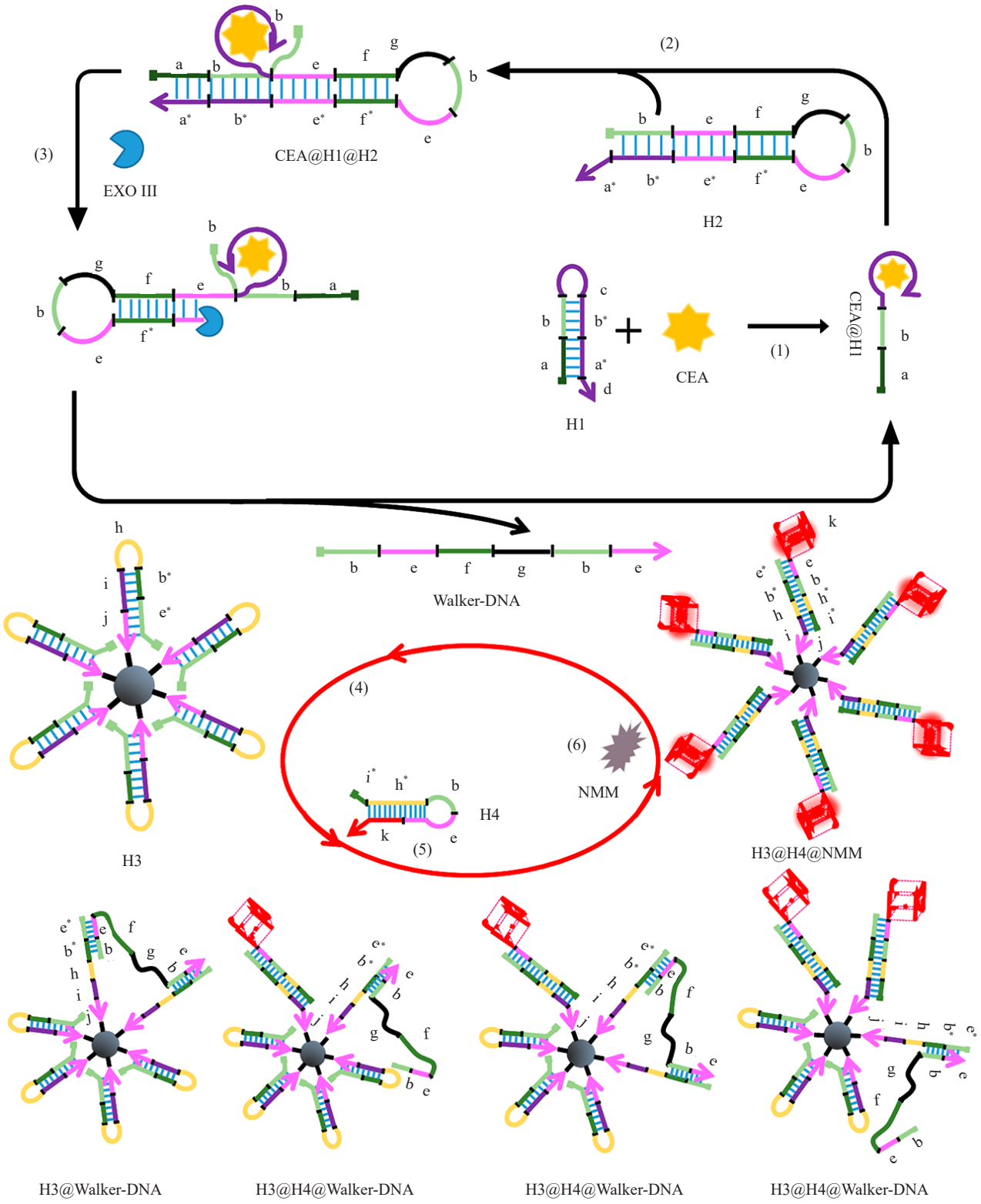

Figure 12. Schematic representation identification of CEA related to the ERA and DNA walker cascade amplification approach, Copyright 2020 ACS[62]

6.1 *Electrochemical biosensor*

Li et al.[63] prepared the porous structure, thiol graphene-thiol chitosan-gold nanoparticles (ThGP-ThCTS-AuNPs) nanocomposites film by electrochemical deposition method on Glassy Carbon Electrode (GCE). This demonstrated better biocompatibility and enhanced conductivity, to make a free label for the identification of the CEA investigated as depicted in Figure 13.

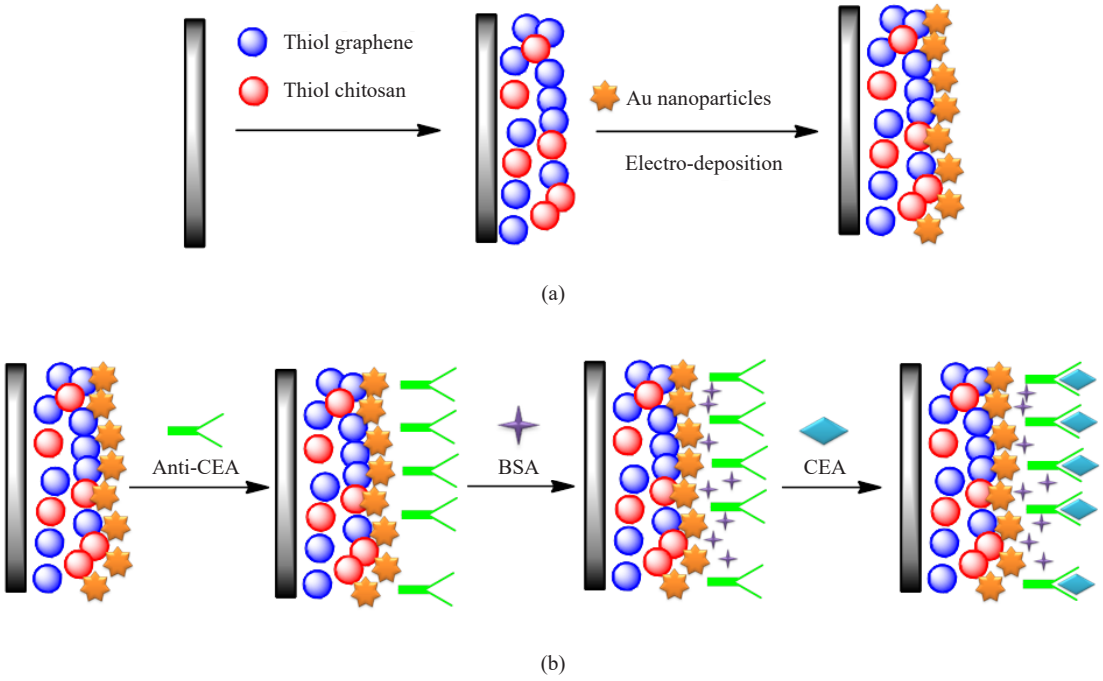

Figure 13. Schematic representation preparation of immusensor: (a) nanocomposites film, and (b) CEA immunosensor[63]

The modified Cyclic Voltammograms (CVs) electrode via bio-macromolecule evaluated in 0.1 M Phosphate Buffer Solution (PBS) and 5.0 mM [Fe(CN)6]4-*l*3 and 0.1 m potassium chloride (KCl). Following, the modification of THGP-ThCTS-AuNPs composite film shows a high peak current compared to bare GCE. This is indicated that nanocomposite film promoted electron transfer. However, the peak current reduced noticeably after the immunosensor was incubated in the anti-CEA and was associated with a further decline due to obstructing non-specific sites via BSA. On the other, the peak temperature further decreases after immobilizing of EEA antigen onto the surface of the immunosensor, this confirmed that the macromolecules obstructed the tunnel for the movement of electrons.

Further, the Electrochemical Impedance Spectroscopy (EIS) investigation was employed to observe that the impedance alternations of the modification route corroborate the sensing interface that has been assembled. Figure 14 demonstrated a half circle and a direct line of EIS plot.

The length of the half-circle identical to the electron movement resistance is examined. The bare GCE shows a tiny circle and approximately direct line area at high frequencies. The modified film demonstrated that smaller semicircle compared to the bare GCE. and favor to be a direct line, this confirmed the nanocomposite film exhibited better conductivity characteristics that can be appropriate for electron movement. While the prepared electrode was modified with anti-CEA, the half-circle became larger. After BSA absorption on the surface and the incubation in CEA, the half circles were gradually bigger, and the interfacial resistance was enhanced. This is attributed to the protein on the surface obstructing the layer, and restricted electron movement.

As a result, the prepared immunosensor was used for the detection of CEA under the most favorable conditions and the immunosensor demonstrated a better amperometric response to CEA in two scales (0.3-8.0 - 8.0-100 $ngmL^{-1}$) with

an identification limit of 0.03 ngmL^{-1} as well as the immunosensor exhibited the advantages of high sensitivity, good selectivity, and stability for the determination of CEA.

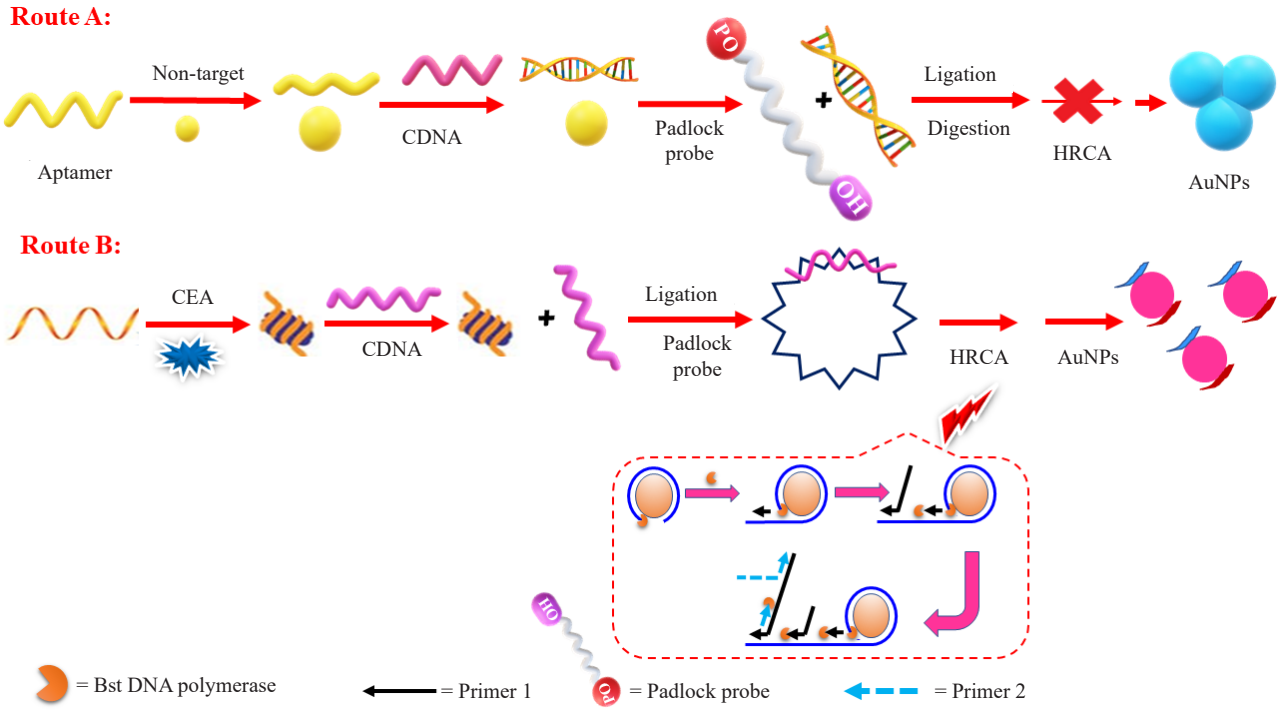

Figure 14. Working route of HRCA-based colorimetric biosensor of (A) and (B) routes[64]

Shu et al.[65] designed a new signal amplification of AuNPs Electrochemical Aptamer Biosensor (ECAB) for the identification of a CEA as depicted in Figure 15. The Electrochemical Biosensor (ECB) was prepared by sandwiching the CEA and Au electrode after modification of thiol-terminated CEA aptamer-1 (Apt-1), AuNPs thiol-terminated CEA aptamer-2 (Apt-2) and 6-ferrocenyl hexanethiol (Fc). The amperometric observation of Fc investigated by Dissimilar Pulse Voltammetry (DPV) on the ECB was employed to measure the concentration of CEA.

6.2 *Colorimetric biosensor*

During this investigation, Liang et al.[64] prepared Hyperbranched Rolling Circle Amplification (HRCA) based on high sensitivity and specificity calorimetry biosensors for the detection of CEA. Figure 14 revealed the working principle of the calorimetry biosensor. Route A, indicates the lack of the object (CEA) and aptamer hybridizes with C-DNA to form ds-DNA. The C-DNA doesn't hybridize with the padlock to start HRCA. On the other hand, padlock proves HRCA primer's presence in solution. The amount of the primers is not high sufficient to inhibit salt influence AuNP aggregation, and the route A solution color is blue. Therefore, free C-DNA can circulate an HRCA reaction formation of a huge number of single-stranded DNA (ss-DNA). Because ss-DNA can be simply absorbed onto AuNPs and prohibit salt-influenced AuNPs aggregation. On the other hand, the color of the solution changes (red) in the system as shown in Route B. The absorption intensity ratio (A_{520}/A_{660}) demonstrated a linear correlation with the concentration of the object on the scale of 5 pM-0.5 nM, the identification limit is lower than 2 pM.

The novel selective and sensitive calorimetry biosensor system for CEA via the inclusion of the rapidness and comfort of a calorimetry test with isothermal and exponential amplification of HRCA technology has evolved. This homogeneous test network not only reduces thermal cycling, but, as well as obtained enhanced test properties, for example (i) wide linear response range, and (ii) low diagnosis limit and high specificity. For that reason, the developed

biosensor exhibits potential application in the bio-identification of diseases, biochemical investigation, and clinical and environmental applications.

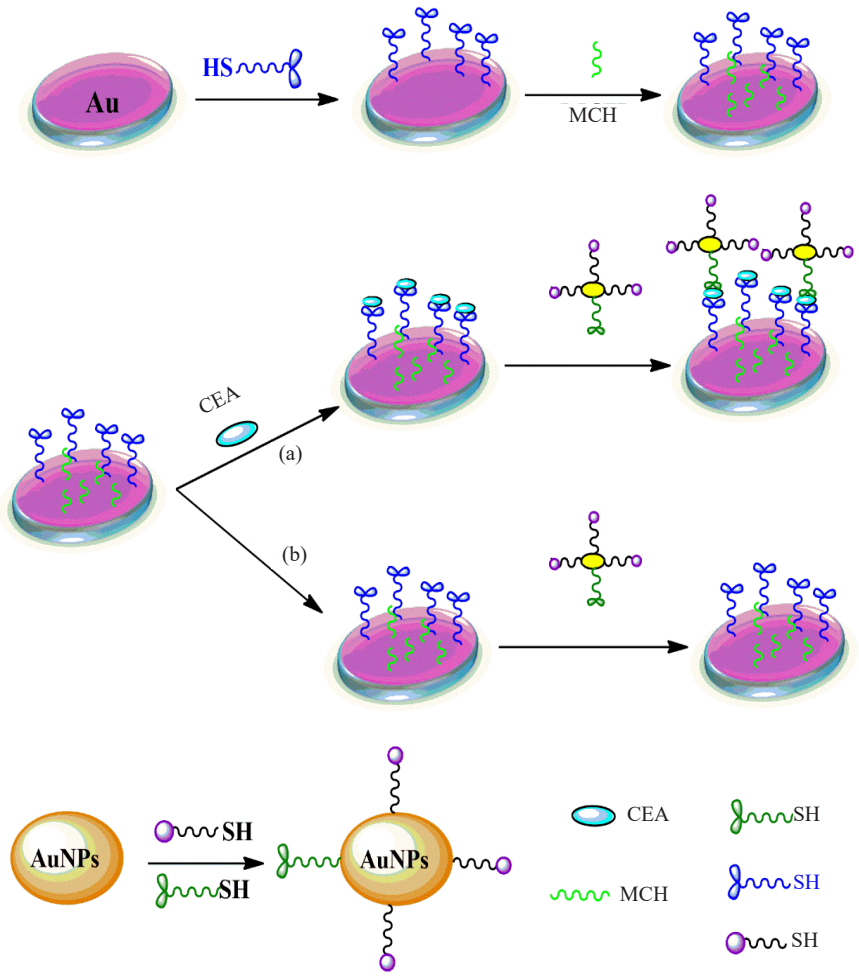

Figure 15. Schematic representation of AuNPs signals amplification electrochemical aptamer biosensor identification of CEA [65]

Further, Liu et al.[66] also prepared an extremely sensitive calorimetry enzyme immunoassay for the identification of CEA depicted in Figure 16. In this technology, AuNPs are used as carriers of anti-CEA antibody branded with biotin and distributed as an affinity label for streptavidin-horseradish peroxidase (streptavidin-HRP) binding. Thus, Magnetic Microparticles (MMPs) were employed as assisting substrates for anti-CEA confine antibodies. According to this approach, the 12 HRP molecules were coated onto every AuNP. During sandwich-kind immunoreactions, the AuNP-anti-CEA-HRP complex was conducted in the presence of MMPs. The result demonstrated that the HRP molecules detained at the surface of the sandwich immune complexes catalyze the substrate and produced an optical signal. The spectrophotometric investigation corroborated efficient signal amplification; these signals were linearly based on CEA concentration (0.05 to 50 $ngmL^{-1}$) in a plot with a 48 $pgmL^{-1}$ identification limit. The AuNP-anti-CEA-HRP complex was filled by the higher quantity of HRP amplification enzyme, as a result, they recommend immunoassays based on the AuNP complex revealed enhanced sensitivity as contrasted with a typical CEA ELISA kit. This characteristic, plus its suitable magnetic detachment, and shorten assay time made it a capable substitute for the traditional CEA ELISHA technique.

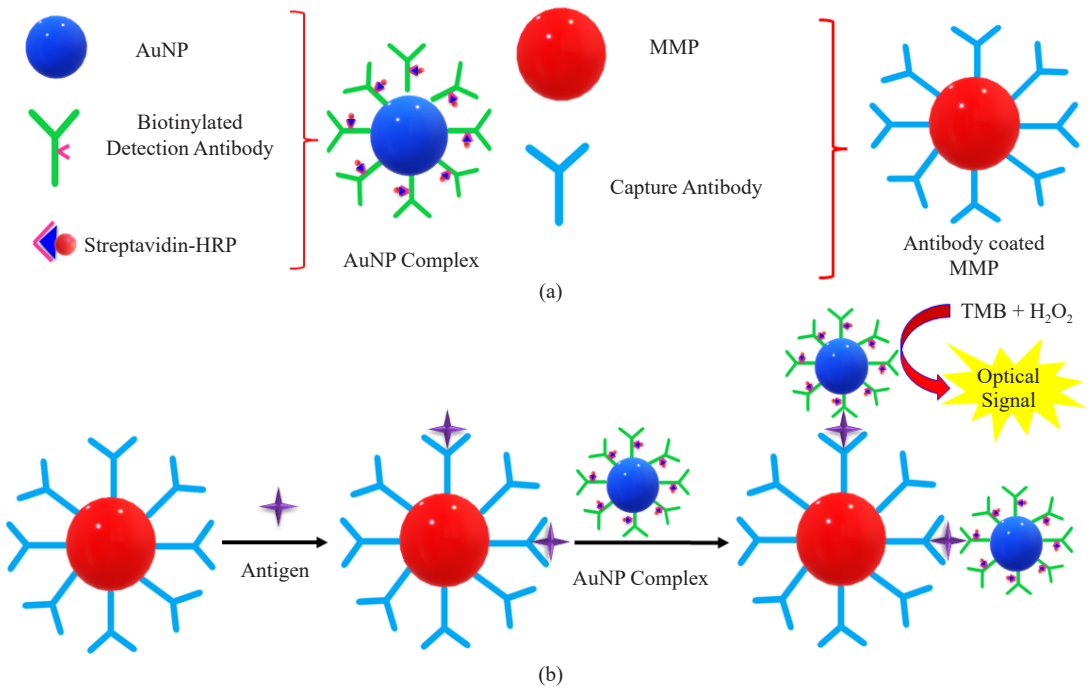

Figure 16. Development of antibody-coated MMP based (a) AuNP-anti-CEA-HRP complex, and (b) AuNP complex based on colorimetric enzyme immunoassay route[66]

6.3 *Other biosensors for the detection of CEA*

The Surface Plasmon Resonance (SPR) employed as a CEA immunoassay has been developed, and it has provided highly sensitive labeled-free identification for the diagnosis of CEA in human serum. This development is based on the analytical circumstances studied by Su et al.[67] The effect of the various analytical circumstances as well as immobilization techniques for anti-CEA antibody and constitution of sensor surface on the selective and sensitive diagnosis of CEA. The results confirmed that the anti-CEA antibody was immobilized by protein A or protein G for that reason the resonance signal increased upon injection of human serum owing to the interaction with immunoglobulin (IgGs) in serum. On the other hand, the covalent immobilization of anti-CEA antibodies can be significantly decreased. Further, kinetics analysis and employing an off and the third antibody for the sandwich assay permit sensing spike CEA in human serum with lower concentration (25 ng/mL).

Springer et al.[68] developed the biofunctionalized gold nanoparticles (Bio-AuNPs) based surface SPR biosensor to an identification lower levels of CEA in humans (blood plasma). Bio-AuNPs contain AuNPs functionalized both e.g., (i) streptavidin, to bestow high affinity of the biotinylated secondary antibody employed in the 2nd stage of the CEA sandwich assay, and (ii) bovine serum albumin, to reduce the distracting interaction of the Bio-AuNPs with blood plasma system specimens. This method makes it possible for the SPR biosensor to detect CEA in blood plasma at a low concentration (1 ng/mL) with nanogram/milliliter (ng/mL) approximately. Furthermore, the limit of identification obtained employing this method is good via a reason of more than 1,000 than the limits of identification described, therefore, far for CEA in blood plasma employing SPR biosensors.

Further, the SPR-based biosensor was designed for the diagnosis of CEA with a highly sensitive surface by Altintas et al.[69] The various designs of the immunoassay were first explored on the surface of the Au sensor chip. The Self-Fabricated Monolayer (SFM) was prepared on the Au gold chip employing 11-Mercaptondecanoic Acid (MUDA) before the immobilization of the antibodies was performed. The direct confine and sandwich assay enhance the sensor signal CEA antigen was incubated with the identification/confine antibody before it was inserted into the sensor chip surface (Figure 17) and measured actual time.

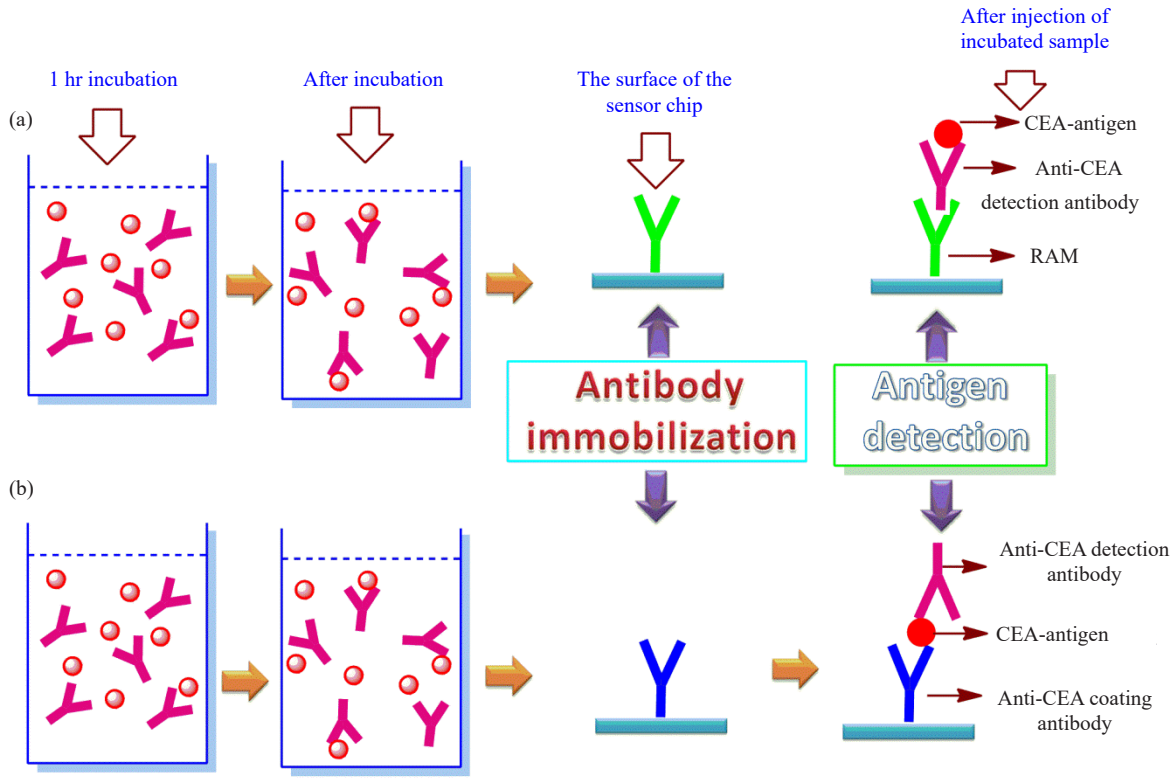

Figure 17. Schematic demonstration of (a) homogenous RAM-capture, and (b) sandwich assay[69]

The identification limit of 3 ng/mL CEA was achieved with the dynamic identification scale from 3 ng/mL to 400 ng/mL with association coefficients of 1.00-0.99 for the sandwich and Rabbit Anti-Mouse (RAM) confine assay. SPR immunosensor employing the sandwich analysis system demonstrated the high sensitivity and reproducibility for CEA recognition and it is a potential process for cancer biomarker investigation.

Lin et al.[70] developed the polymer dots FRET-based biosensor for the investigation of particular proteins as depicted in Figure 18. Initially, poly(9,9-dioctylfluorenyl-2,7-diyl) dots (PFO dots) and Au-NPs both are modified with partial-aptamer complementary ss-DNA. The CEA aptamer connects the PFO dots with Au-NPs simultaneously via the hybridization of ss-DNA and the formation of a sandwich-type structure. The fluorescence resonance energy moves among the PFO dots and AuNPs were noticed and a low fluorescence signal was observed in this condition.

In the concept of green technology Miao et al.[71] developed label-free fluorimetric biosensor identification of CEA employing Carbon Dots (CDs) obtained from tomato juice as depicted in Figure 19. Another benefit of the total synthesis procedure is environment friendly and CDs exhibited various advantages, including fluorescent Quantum Yield (QY), non-toxicity, tremendous photostability, and sufficient stability. The various carboxylic groups' presence on the CD's surface was used for the quantification of CEA. First, the CDs surface is absorbed by the CEA-aptamer, this is an attribute to the π - π stacking interaction among CEA-aptamer and π-rich CDs encourage the fluorescence quenching of CDs. After that, the incorporation of CEA into this composition improved the fluorescent signal of CDs by untwisting CEA-aptamer from the surface of CDs owing to the high binding affinity among CEA and CEA-aptamer.

This study demonstrated acceptable stability and detection, linear range from 1 ng/mL-0.5 mg/mL with a 0.3 ng/mL detection limit. The lower recognition limit presented the assay with sufficient sensitivity to correctly analyze the CEA level in human serum.

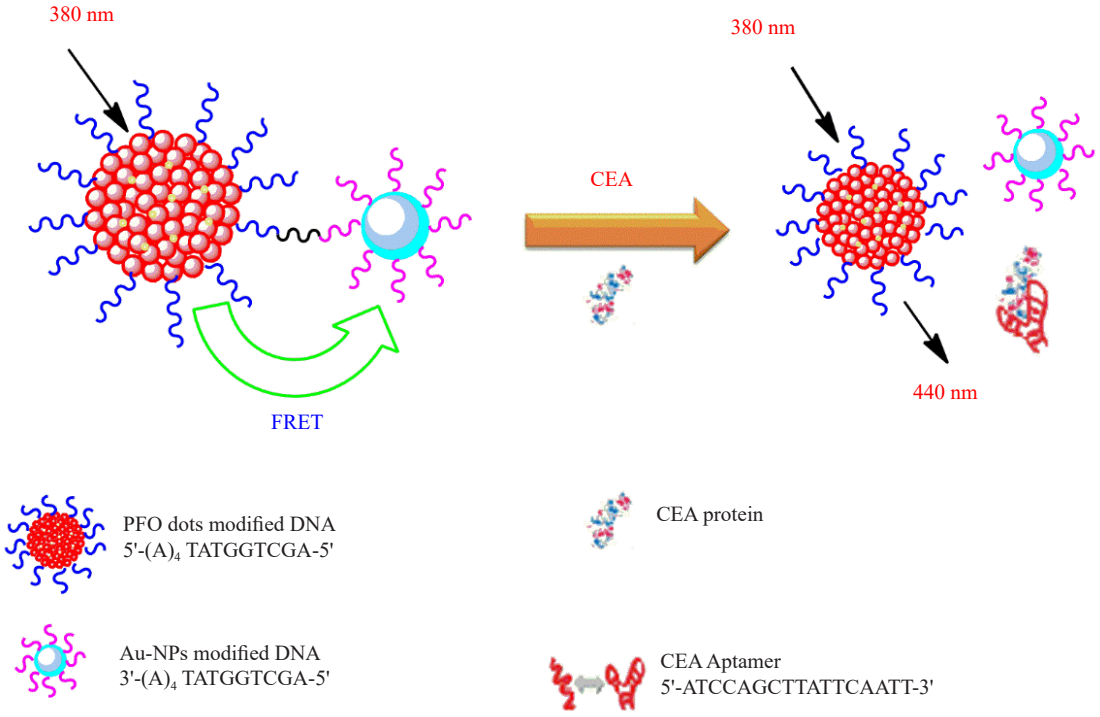

Figure 18. Schematic representation of FRET among PFO dots and Au-NPs for CEA detection[70]

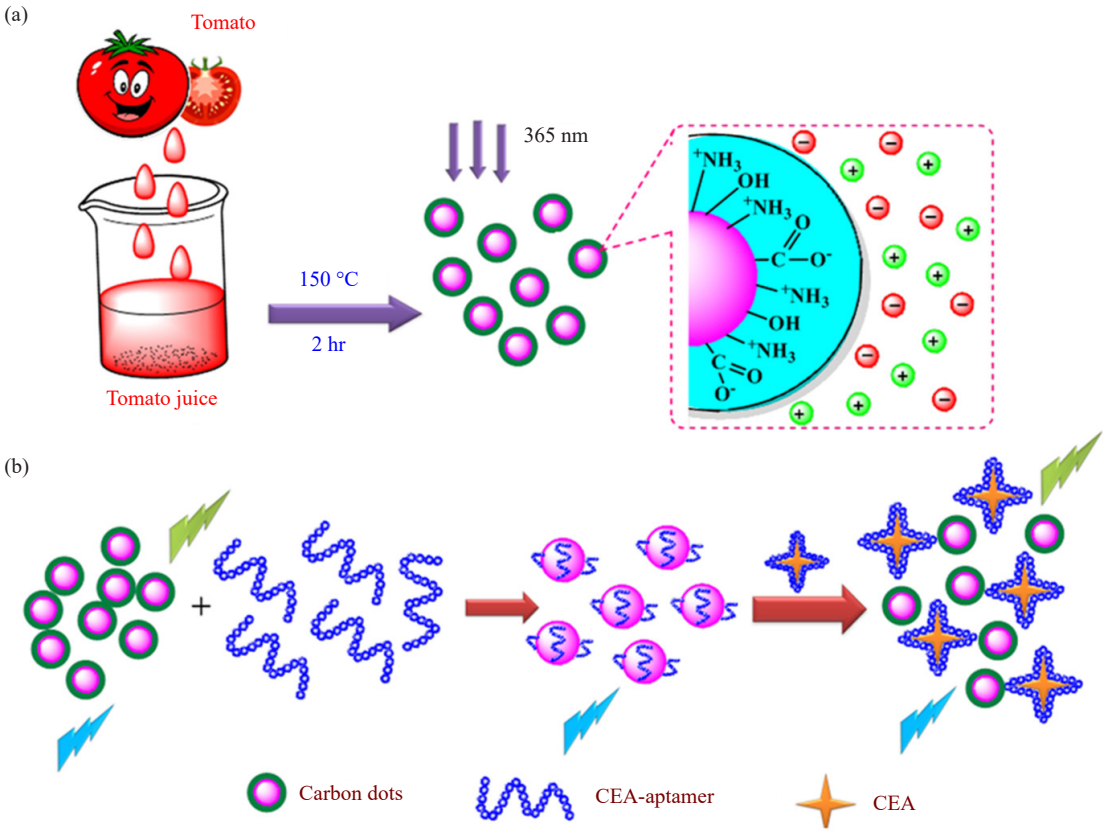

Figure 19. synthetic routes of (a) carbon dots (CDs), and (b) assay CEA via CDs[71]

7. Future prospective development of biosensor

In the future, many researchers will focus on the development of new biosensors and techniques for the detection of Carcinoembryonic Antigen (CEA) and bestow rapid, reliable, and sensitive detection systems with lower cost, reduced size and weight of biosensors and increase system utility. However, the handheld and simply portable and fashionable biosensor is required for early-stage detection of biomarkers and is also focused on their long-term stability. Currently, there is a big demand for a well-organized biosensor for fast diagnosis of cellular variation to detect at its initial stage. Recently, nanoparticles enhanced the interest in biosensor construction against the disease like silver nanoparticles (Ag, NPs), and metal oxide nanoparticles.

8. Conclusion

In this review, we have given information regarding the recent progress in the development of different types of biosensors using various approaches to detect cancer. The developed biosensors exhibit characteristic properties of cost-effective, better, sensitive, portable, easy to operate, and simple to construct properties. Several biosensors can identify the biomarker in a broad range and demonstrate better compatibility with physiological transforms in cancer. Further, also reported nanoparticles-based biosensors for the detection of CEA biomarkers. The controlled shape and size of nanoparticles have garnered increasing interest as promising materials for ultrasensitive detection of chemical and biological molecules. Gold nanoparticles, with their admirable electrocatalytic activity, high electroconductivity, and good biocompatibility, have been widely utilized in the development of biosensors.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Perfezou, M.; Turner, A.; Merkoçi, A. *Chem. Soc. Rev*. **2001,** *41*, 2606-2622.
[2] Sharma, S.; Kelly, T. K.; Jones, P. A. *Carcinogenesis*. **2010,** *31*, 27-36.
[3] Valastyan, S.; Weinberg, R. A. *Cell*. **2011,** *147*, 275-292.
[4] Altintas, Z.; Uludag, Y.; Gurbuz, Y.; Tothill, I. E. *Talanta*. **2011,** *86*, 377-382.
[5] Altintas, Z.; Tothill, I. *Sen. Actuators B Chem*. **2013,** *188*, 988-998.
[6] Bohunicky, B.; Mousa, S. A. *Nanotechnol. Sci. Appl*. **2011,** *4*, 1-10.
[7] Catuogno, S.; Esposito, C. L.; Quintavalle, C.; Cerchia, L.; Condorelli, G.; De Franciscis, V. *Cancers*. **2011,** *3*, 1877-1898.
[8] Rasooly, A.; Jacobson, J. *Biosens. Bioelectron*. **2006,** *21*, 1851-1858.
[9] Turner, A. P. *Science*. **2000,** *290*, 1315-1317.
[10] Li, J.; Li, S.; Yang, C. F. *Electroanalysis.* **2012,** *24*, 2213-2229.
[11] Jayanthi, V. S. A.; Das, A. B.; Saxena, U. *Biosens. Bioelectron*. **2017,** *91*, 15-23.
[12] *Biosensors Market Size, Share & Trends Analysis Report By Application (Medical, Agriculture) By Technology (Thermal, Electrochemical, Optical) And Segment Forecasts, 2012-2020*; Report ID: 978-1-68038-321-8, 2013.
[13] Guo, C.; Su, F.; Song, Y.; Hu, B.; Wang, M.; He, L.; Zhang, Z. *ACS Appl. Mater. Interfaces*. **2017,** *9*, 41188-41199.
[14] Hasanzadeh, M.; Shadjou, N.; Lin, Y.; de la Guardia, M. *TrAC Trends in Analyt. Chem*. **2017,** *86*, 185-205.

[15] Dong, J.; Sun, G.; Zhu, H. *Tumor Biol*. **2016,** *37*, 3257-3264.
[16] Pandey, B.; Demchenko, A. V.; Stine, K. J. *Mikrochim. Acta*. **2012,** *179*, 71-81.
[17] Chen, Z.; Liang, R.; Guo, X.; Liang, J.; Deng, Q.; Li, M.; Wu, Y. *Biosen. Bioelectron*. **2017,** *91*, 60-65.
[18] Liu, Y.; Yue, H.; Tian, W.; Chen, Y.; Li, F. *Analyt. Lett*. **2009,** *42*, 45-57.
[19] Pang, X.; Li, J.; Zhao, Y.; Wu, D.; Zhang, Y.; Du, B.; Wei, Q. *ACS Appl. Mater. Interfaces*. **2015,** *7*, 19260-19267.
[20] Turner, A. P. *Science*. **2000,** *290*, 1315-1317.
[21] Li, J.; Li, S.; Yang, C. F. *Electroanalysis*. **2012,** *24*, 2213-2229.
[22] Feng, T.; Qiao, X.; Wang, H.; Sun, Z.; Hong, C. *Biosens. Bioelecton*. **2016,** *79*, 48-54.
[23] Grieshaber, D.; MacKenzie, R.; Voeroes, J.; Reimhult, E. *Sensors*. **2008,** *8*, 1400-1458.
[24] Lindholm-Sethson, B.; Nyström, J.; Geladi, P.; Koeppe, R.; Nelson, A. *Anal. Bioanal. Chem*. **2003,** *377*, 478-485.
[25] Rasooly, A.; Jacobson, J. *Biosen. Bioelectron*. **2006,** *21*, 1851-1858.
[26] Thévenot, D. R.; Toth, K.; Durst, R. A.; Wilson, G. S. *Pure Appl. Chem*. **2001,** *71*, 2333-2348.
[27] Holford, T. R.; Davis, F.; Higson, S. P. *Biosen. Bioelectron*. **2012,** *34*, 12-24.
[28] Turner, A. P. *Chem. Soc. Rev*. **2013,** *42*, 3184-3196.
[29] Jain, P.; Chakma, B.; Patra, S.; Goswami, P. *Biomed. Res. Int*. **2014,** *2014*, 852645.
[30] Luka, G.; Ahmadi, A.; Najjaran, H.; Alocilja, E.; DeRosa, M.; Wolthers, K.; Hoorfar, M. *Sensors*. **2015,** *15*, 30011-30031.
[31] Tothill, I. E. *Seminars in Cell & Developmental Biology*; Academic Press, 2009; pp 5-62.
[32] Jayanthi, V. S. A.; Das, A. B.; Saxena, U. *Biosen. Bioelectron*. **2017,** *91*, 15-23.
[33] Metkar, S. K.; Girigoswami, K. *Biocatal. Agric. Biotechnol*. **2019,** *17*, 271-283.
[34] Ghosh, I.; Bhattacharjee, D.; Das, A. K.; Chakrabarti, G.; Dasgupta, A.; Dey, S. K. *Indian J. Clin. Biochem*. **2013,** *28*, 24-29.
[35] Bohunicky, B.; Mousa, S. A. *Nanotechnol. Sci.Appl*. **2011,** *4*, 1-10.
[36] Sharma, S.; Kelly, T. K.; Jones, P. A. *Carcinogenesis*. **2010,** *31*, 27-36.
[37] Elmore, S. Apoptosis: a review of programmed cell death. *Toxicol. Pathol*. **2007,** *35*, 495-516.
[38] Valastyan, S.; Weinberg, R. A. *Cell*. **2011,** *147*, 275-292.
[39] Lee, E. Y.; Muller, W. J. *Cold Spring Harb. Perspect. Biol*. **2010,** *2*, a003236.
[40] Loo, C.; Lowery, A.; Halas, N.; West, J.; Drezek, R. *Nano Lett*. **2005,** *5*, 709-711.
[41] Claret, F. X.; Vu, T. T. *Front. Oncol*. **2012,** *2*, 62.
[42] Yoshida, K.; Miki, Y. *Cancer Sci*. **2004,** *95*, 866-871.
[43] Ahmed Kh, S. *Biosensors, Am. J. Biomed. Eng*. **2016,** *6*, 170-179.
[44] Henry, N. L.; Hayes, D. F. Cancer biomarkers. *Mol Oncol*. **2012,** *6*, 140-146.
[45] Tan, C.; Qian, X.; Guan, Z.; Yang, B., Ge, Y.; Wang, F.; Cai, J. *Springerplus*. **2016,** *5*, 1-7.
[46] Coticchia, C. M.; Yang, J.; Moses, M. A. *J. Nat. Compr. Cancer Netw*. **2008,** *6*, 795-802.
[47] Lech, G.; Słotwiński, R.; Słodkowski, M.; Krasnodębski, I. W. *World J. Gastroenterol*. **2016,** *22*, 1745.
[48] Zamay, T.; Zamay, G.; Kolovskaya, O.; Zukov, R.; Petrova, M.; Gargaun, A.; Berezovski, M.; Kichkailo, A. *Cancers*. **2017,** *9*, 155.
[49] Smith, D. S.; Humphrey, P. A.; Catalona, W. J. *Cancer: Int. Int. J. Am. Cancer Soc*. **1997,** *80*, 1852-1856.
[50] Aung, P. P.; Liu, Y. C.; Ballester, L. Y.; Robbins, P. F.; Rosenberg, S. A.; Lee, C. C. R. *Human Pathol*. **2014,** *45*, 259-267.
[51] Sabr, A. K. *Global J. Eng*. **2016,** *16*, 41-51.
[52] Monosík, R.; Stredansky, M.; Sturdík, E. *Acta Chim. Slov*. **2012,** *5*, 109.
[53] Luong, J. H.; Male, K. B. *Biotechnol. Adv*. **2008,** *26*, 492-500.
[54] Birch, B. *The Future of Biosensors*. 1996.
[55] Sin, M. L.; Mach, K. E.; Wong, P. K.; Liao, J. C. *Expert Rev. Mol. Diagn*. **2014,** *14*, 225-244.
[56] Mohanty, S. P.; Kougianos, E. *Ieee Potentials*. **2006,** *25*, 35-40.
[57] Bhatta, D., Michel, A. A., Villalba, M. M., Emmerson, G. D., Sparrow, I. J. G., Perkins, E. A., Cartwright, G. A. *Biosen. Bioelectron*. **2011,** *30*, 78-86.
[58] Shruthi, G. S.; Amitha, C. V.; Mathew, B. B. *J. Instrument. Technol*. **2014,** *2*, 26-39.
[59] Medley, C. D.; Smith, J. E.; Tang, Z.; Wu, Y.; Bamrungsap, S.; Tan, W. *Anal. Chem*. **2008,** *80*, 1067-1072.
[60] Tiquia-Arashiro, S. M. *Thermophilic Carboxydotrophs and their Applications in Biotechnology*; Springer Cham, 2014; pp 29-101.
[61] Omidi, M.; Choolaei, M.; Haghiralsadat, F.; Azhdari, M.; Moghadam, N. D.; Yazdian, F. In *A Label-free Detection of Carcinoembryonic Antigen (CEA) using Micromechanical Biosensors,* Proceedings of the International

Conference on Biomedical Electronics and Devices (BIOSTEC 2014) - BIODEVICES 2014, ESEO, Angers, Loire Valley, France, 2014; pp 134-139.
[62] He, M. Q.; Wang, K.; Wang, W. J.; Yu, Y. L.; Wang, J. H. *Anal. Chem*. **2017,** *89*, 9292-9298.
[63] Li, G.; Xue, Q.; Feng, J.; Sui, W. *Electroanalysis*. **2015,** *27*, 1245-1252.
[64] Liang, K.; Zhai, S.; Zhang, Z.; Fu, X.; Shao, J.; Lin, Z.; Chen, G. N. *Analyst*. **2014,** *139*, 4330-4334.
[65] Shu, H.; Wen, W.; Xiong, H.; Zhang, X.; Wang, S. *Electrochem. Commun*. **2013,** *37*, 15-19.
[66] Liu, M.; Jia, C.; Jin, Q.; Lou, X.; Yao, S.; Xiang, J.; Zhao, J. *Talanta*. **2010,** *81*, 1625-1629.
[67] Su, F.; Xu, C. M.; Murayama, K.; Shinohara, Y.; Nishimura, S. I. *Sensors*. **2008,** *8*, 4282-4295.
[68] Springer, T.; Homola, J. *Anal. Bioanal. Chem*. **2012,** *404*, 2869-2875.
[69] Altintas, Z.; Uludag, Y.; Gurbuz, Y.; Tothill, I. E. *Talanta*. **2011,** *86*, 377-383.
[70] Lin, Z.; Zhang, G.; Yang, W.; Qiu, B.; Chen, G. *Chem. Commun*. **2012,** *48*, 9918-9920.
[71] Miao, H.; Wang, L.; Zhuo, Y.; Zhou, Z.; Yang, X. *Biosen. Bioelectron*. **2016,** *86*, 83-89.