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Review—Recent Advances Based on a Sensor for Cancer Biomarker Detection

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Cancer is a worldwide disease with a high mortality rate and traditional methods for the diagnosis and monitoring are performed through invasive techniques. Currently, the advance of research in medical and biomedical engineering allowed the use of molecular tools combined with nanotechnology to develop portable sensors specific for major biomarkers to diagnose, monitor, and treatment of several diseases. This sensor can offer a means of homogeneous classification of a disease and risk factor and can extend the basic information about the underlying pathogenesis of the disease. Therefore, they can play a critical role in all stages of the disease. To address all this requirement is important to have a rigorous evaluation, including analytical validation, before incorporated into routine clinical treatment. This review described the current stage in the development of sensors in the study of cancer with an emphasis on surface modification, immobilization of biological agents, and detection approach.
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According to World Health Organization (WHO) Cancer is a large group of diseases that can start in almost any organ or tissue of the body, when abnormal cells grow uncontrollably, and go beyond their usual boundaries to invade adjoining parts of the body, and/or spread to other organs.[1] The latter process is called metastasizing and is a major cause of death from cancer. In 2018, 9.6 million new diagnosed cases and 8.2 million predicted deaths were reported. When discriminating these incidence numbers, we have lung (2.09 million), breast (2.09 million), colorectal (1.8 million), prostate (1.28 million), skin cancer (1.04), and others. The most common types that affect females have percentages referring to the types of cancers such as breast (25.2%), intestine (9.2%), lung (8.7%), cervix (7.9%), and stomach (4.8%).[2] Meanwhile, the most prevalent among men were lung (16.7%), prostate (15.0%), intestine (10.0%), stomach (8.5%) and liver (7.5%). Despite the incidence of new cases occurring in greater numbers in developed countries (North America, Western Europe, and Oceania), 80% of deaths worldwide occur in developing countries.[3]

When a patient has cancer, some biomarkers act as a warning sign, telling doctors that further tests may be needed. These tumor markers can help doctors to detect cancer at an early stage when there is a better chance of treatment and cure. Nowadays there a range of biomarkers used as predictive tools to detect cancer at early stages; for example breast cancer has the human epidermal growth factor receptor 2 (HER2) and the estrogen and progesterone receptors; colorectal cancer has the epidermal growth factor (EGFR), the KRAS gene and the UDP- glucuronosyltransferase1–1 (UGT-1A); leukemia and lymphoma have and the CD20 and CD30 cytokines, the platelet-derived growth factor receptor (PDGFR) and the promyelocytic leukemia protein; lung cancer has the EGFR, KRAS gene and the echinoderm microtubule associated protein-like 4 (EML4); melanoma has the BRAF gene; pancreas cancer has elevated levels of leucine, isoleucine and valine; ovary cancer has the cancer antigen 125 (CA125) and prostate cancer has the prostate specific antigen (PSA), vascular endothelial growth factor (VEGF) and the transmembrane glycoprotein mucin type 1 (MUC1).

A biomarker can be an indicator of a specific biological state and can be used for patient assessment in various clinical settings, including disease risk estimation, screening for hidden primary cancers, distinguishing between benign and malignant cells, or one type of malignancy from another.[4] These prognoses for patients who have been diagnosed with cancer can help manage of developing malignancy and choice the correct strategies to follow up. Nowadays, there are wide varieties of biomarkers, which can include proteins, DNA, RNA, microRNA, antibodies, peptides, and others.[5] Most tumor markers are measured by examining blood or urine (noninvasive). A small dosage of blood or urine is collected to measure the number of these substances (tumor markers) found in the sample. Some tumor markers are measured directly from a tumor sample taken during a biopsy (invasive). These tumor markers provide doctors with information about the tumor and how it might react to different types of treatments.

Here, we will discuss different approaches for the detection of these molecules, demonstrating that biomarkers are moving into conventional practice, but also highlighting the work that is yet to come to make them more clinically useful. This review begins with a state of the art of biomarkers of the common protein biomarkers related to cancer diagnoses. Then, we made a little discussion about the most used materials in electrochemistry to modify surfaces for the matching of biological material. Finally, we describe different approaches using immunosensor, antibodies, DNA, cytosensor, aptasensor, and more recently microRNA for developing devices that present sensitive, robust, simple to operate, and low cost in the field of cancer analysis.

Application of biomarkers.—The definition of a biomarker is "some characteristic that is objectively measured and evaluated as an indicator of biological, pathological processes or pharmacological responses to a therapeutic intervention."[6] It is possible to detect them in several types of models, both in vitro and in vivo tests. In other words, this includes technologies and tools that make it possible to assess the prediction of the cause, progression/regression, and treatment of diseases.[7]

The use of biomarkers as an analytical tool in biomedical applications can provide fast and accurate information on biological parameters that predispose the pathological condition, and can monitor therapeutic responses to treatment related to the level of toxicity of diseases such as cancer. A biomarker can provide complementary information to those related to clinical and pathological studies. They allow the evaluation of the development and optimization of new drugs to increase the efficacy and safety of the treatment.[8] There is a vast catalog of biomarkers reported in the literature for those purposes, which include detection of cardiopathologies, infections, genetic and immunological abnormalities as well as those of cancer.[9]

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Recently, wide ranges of biomarkers have been proposed for cancer detection, but few have been used in the clinical field in the past 30 years. The vast majority of cancer biomarkers were discovered between the mid-1960s and the early 1990s.[10] However, newly discovered biomarkers still need to be validated to have their clinical use approved by regulatory agencies (Fig. 1).

Several biomarkers are already used daily for cancer detection. CA 15–3 is a tumor marker that has been used to monitor the response to breast cancer treatment. After treatment ends, an increase in CA 15–3 levels may detect the return of the disease.[11] However, further studies are needed to determine the best and most reliable use of CA 15–3 in breast cancer. CA 27.29 is another very common tumor marker for breast cancer.[12] Serial exams of CA 27.29 can help the doctor monitor how well the treatment is working. After the end of treatment, tests can help detect the recurrence of the disease. As with the CA 15–3 marker, further studies are being conducted to determine its most appropriate use. Estrogen and progesterone are female hormones. At the time of cancer diagnosis, doctors analyze the tumor tissue removed during the biopsy, to see if the cancer cells in the tissue have receptors for any of these hormones. Women whose cancer is positive for estrogen or progesterone receptors appear to benefit most from hormone therapy.[12] Another protein with clinical evidence for breast cancer is the human epidermal growth factor (HER2). Breast tumors that produce HER2 tend to grow rapidly and appear to spread more frequently to other parts of the body. Doctors can test the breast cancer tissue taken during the biopsy to see if these cancer cells produce HER2.[13] Which may suggest that the treatment of this cancer be with a drug that specifically attacks the breast cancer cells that produce HER2.

Tumor markers for cancer of the gastrointestinal tract are commonly diagnosed worldwide. Alpha-fetoprotein (AFP) is a protein produced only by the baby in its intrauterine life (fetus).[14] Some cancer cells, including liver cancer cells, may return to their previous fetal form and start producing alpha-fetoprotein again. Serial alpha-fetoprotein tests are used to measure how well a treatment for liver cancer is doing. Another generic tumor marker, CA19–9 is found in several types of cancer of the gastrointestinal tract, including pancreatic and stomach tumors.[14] It has been used to monitor the response to treatment in patients with advanced pancreatic cancer. People who have liver cancer have high levels of gamma-glutamyl transferase (GGT) in their blood.[15] Serial GGT tests can help monitor how the treatment is working. After completion, the follow-up with dosages of GGT can detect the return of the disease.

Lung cancer has a high mortality rate worldwide. People with non-small cell lung cancer tend to have high levels of carcinoembryonic antigen (CEA).[16] There is no specific marker for lung cancer, but, in some lung cancer types, CEA can reach high levels in the blood. Another well-known marker for lung cancer is neuron-specific enolase (NSE) and has been used to monitor the effectiveness of the treatment. NSE can help detect whether cancer has spread and whether the disease has returned, even before the person has symptoms or before the changes appear on other tests.[16]

Ovarian cancer mainly affects women who have been through menopause, but it can sometimes affect younger women. CA 125 is a protein produced by ovarian cells and has been widely used in ovarian cancer.[17] Although the test is not sensitive or specific enough to be used for screening, it is of great value at the time of diagnosis when analyzed along with ultrasound and pelvic

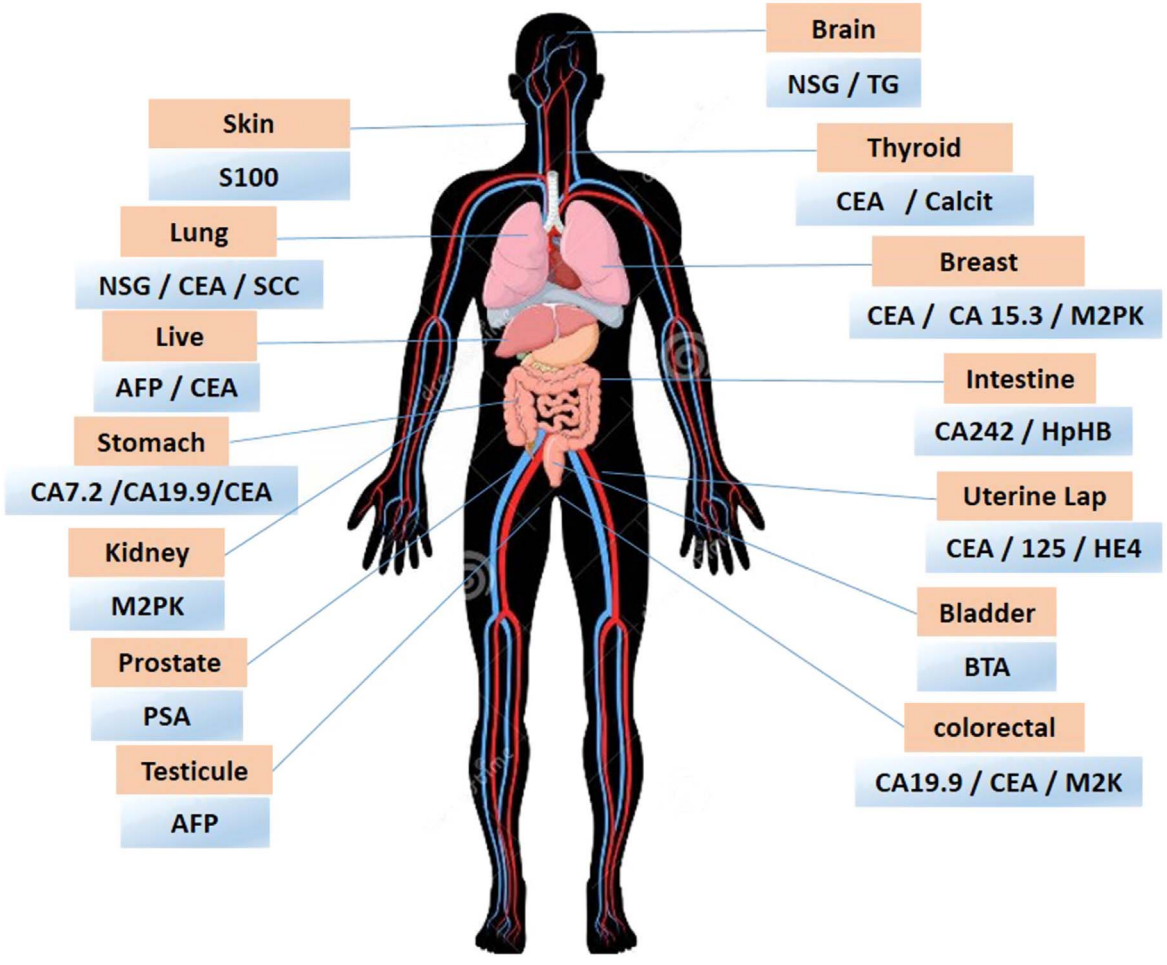

Figure 1. Common biomarkers used in cancer detection.

examination. It is important to remember that a normal or negative CA 125 does not guarantee the absence of cancer. Prostate cancer tumor is the most common cancer in men. PSA (prostate-specific antigen) is a protein produced by the prostate gland and most widely used for clinical diagnosis.[18] During treatment, doctors can use PSA tests to monitor their effectiveness. After the end of treatment, follow-up with PSA dosages can help detect the return of cancer.

Besides the common protein, scientists began understanding the genes that make up the human body and the complex processes that occur in the human cell, one can expect to discover new and better tumor markers. Different neoplasms have been found to have an increased concentration of lipid-associated sialic acid (LASA). At first, it appeared to be a tumor marker, since a study showed that its levels drop after colon cancer surgery.[19] However, LASA levels also decrease after the removal of non-cancerous polyps; therefore, more research is needed, before LASA can be used as a reliable tumor marker. The gene p53 has been studied for several decades as a possible biomarker of a tumor suppressor oncogene. If altered, it can promote the growth of cancer. The gene p53 blocks the growth of tumors. Research is evaluating whether it could be a useful and reliable tumor marker for gastrointestinal tumors.[20] Pancreatic oncofetal antigen (POA) is usually found in the pancreas of fetuses and tumor tissue. Research is being conducted to determine its use as a tumor marker for pancreatic cancer.[21] Ras Mutations has been linked to several gastrointestinal tumors.[22] However, much research must be performed before Ras oncogenes are useful and reliable markers for gastrointestinal tumors. Thymidylate synthase (TS) is an enzyme produced by tumor cells.[23] A recent study showed that doctors will be able to select chemotherapy drugs to treat colorectal cancer based on the amount of TS that the cancer cells produce.[24] Additionally, all tumors need a sufficient amount of blood supply to grow. Many tumors produce large amounts of vascular endothelial growth factor (VEGF), which aids in the growth of blood vessels. Research is being conducted to explore the use of VEGF as a tumor marker in many tumors.[24]

Traditionally, the methods most used to evaluate biomarkers are those that involve the use of immunoassays with antibodies, such as enzyme-linked immunosorbent assay (ELISA), because are reliable test with high sensitivity and specificity.[25] However, the ELISA test requires higher trainer professionals and equipped laboratories with automatic analyzers for immunological assays, which inevitably increase the time of diagnosis and cost. A variety of other techniques, such as radio-immunoassay,[26] fluorescence spectroscopy,[27] mass spectroscopy,[28] and chromatography,[29] are available for detecting cancer biomarkers. These methodologies can provide highly sensitive detection of biomarkers in biological fluids, but there are labor consuming and expensive. Consequently, major efforts have been made to develop a faster, portable, and easy to handle analytical technique highly specific for the diagnosis and monitoring of neoplasms, such as the cancer screening blood tests performed by lateral flow assay in a similar manner as pregnancy test.[4,25,30] Electrochemical strategies can offer robust with precise measurements at low-cost and simple instrumentation.

Electrochemical biosensor.—In this way, biosensors can play an important role in producing earlier and noninvasive diagnoses. The biosensor is an integrated device capable of providing specific quantitative or semi-quantitative information using a biological recognition element in direct contact with a transducer. Biosensors have advantages over traditional clinical methods for detecting cancer monitoring, due to the fast processing of data and the flexibility in its use.[31,32] Besides, biosensors are capable of automatically performing multiple analyses on a single sample, thus reducing the cost of analysis.[33] Diagnostics performed using biosensors can be expanded to diagnose cancer in clinical samples with detection in the early stages of the disease and, simultaneously, more effective care in the prognosis, thus improving medical care in general and benefiting the neediest populations.[34] The development of biosensors can be divided into two large groups, those with direct detection and those that are indirect for cancerous biomarkers.[35] The biosensors for diagnosis and monitoring of cancer commonly used are those for direct detection, where the reaction between the sensor and the target analyte can be directly measured. The indirect way is characterized as those that require a secondary ligand for detecting the target analyte, normally the secondary ligand can be antibodies conjugated with enzymes, DNA sequences, proteins, among others.[36,37] They can be classified based on the type of bio-recognition element (eg, enzyme-antibody, DNA, RNA, proteins), and strategies for transduction of the biological signal can be divided into electrochemistry, optical, gravimetric, or thermal.[38] The desire is obtaining a biological recognition system with high selectivity, accuracy, and at an inexpensive cost.

In the development of biosensors for the diagnosis and monitoring of cancer, the surface component is fundamental for success in research. The challenge is ensuring that immobilized biological molecules remain functional over time. Recently, nanomaterial conjugating with signaling molecules have attracted the attention of several research groups for biomarker sensor, due to its great potential in biomedical applications. Different strategies for improving the sensitivity by the effect of nanoparticle composition, size and shape were studied. However, in this first stage we will focus on the two main materials used for the biomarkers analysis.

The first materials used for this purpose was carbon nanotubes (CNTs), due to its unique properties. Based on their structure, CNTs can be classified into two general categories: single wall (SWNTs), which consists of a layer of cylindrical, and multiwall (MWNTs), which contains several concentric sheets of graphene.[39] Ultralightweight, high mechanical resistance, high electrical conductivity, well friendly biofriendly condition, and high thermal conductivity are unique properties for CNT to be widely used in constructing such nanodevices.[40,41] CNTs have been used in various biosensor plataform for preparing the sensing layer of the sensor and for fabricating labels for signal amplification in sandwich-type biosensors. Recently, an impedimetric immunosensor based on a conjugated polypyrrole polymer and CNT was developed for detecting interleukin 6 (IL-6), a prostate cancer biomarker. IL-6 receptor was used as a biorecognition molecule and successfully immobilized by covalent linkage on the modified ITO electrode.[42] The proposed immunosensor was successfully used to quantify the IL-6 biomarker in human serum and it displayed a remarkable response in the real sample analysis with serum samples. Zhang et al. report a metal@protein nanoflower of CNTs-COOH/rGO/Ag@BSA/PEDOT.[43] To create an ultrasensitive electrochemical platform for detecting carcinoembryonic antigen (CEA). Moreover, the ultrasensitive immunoassay detected CEA in real human serum samples, and the results are comparable to those obtained from the commercial ELISA. Another paper describes the development of a novel electrochemical immunosensor for detecting cancer antigen 125 (CA125) based on 3DrGO-MWCNT-PAMAM/AuNPs modified glassy carbon electrode.[44] Three-dimensional reduced graphene oxide-multiwall carbon nanotubes (3DrGO-MWCNTs) were used to improve the electrode conductivity and specific surface area. The reliability of the engineered immunosensor in detecting CA125 was verified by the standard addition recovery method, which was further compared to enzyme-linked immunosorbent assay (ELISA). The developed immunosensor exhibited excellent sensitivity (LOD: 6 μU ml^{-1}) with a wide concentration range (0.0005–75 U ml^{-1}) for detecting CA125. The standard addition recovery method and comparison with the gold standard method (ELISA) verified the performance of the immunosensor in the sensing of CA125 oncomarker in the human serum sample. Newly, PCA3 biomarker has become a promising biomarker for the diagnosis of prostate cancer.[45] Soares et al. developed impedance-based biosensors that are capable of detecting PCA3 down to 0.128 nmol l^{-1}. The immobilization of the ssDNA probe was a builder of a suitable matrix with the layer-by-layer technique, which contained chitosan and carbon nanotubes. Using information visualization methods, they distinguish between cell lines expressing the

endogenous PCA3 long noncoding RNA from cells that did not contain detectable levels of this biomarker. All those methodologies related above employ the sandwich immunoassay approach; in which the sensor surface is modified to capture the protein of interest. The use of multilayer system has provided enhancing of sensitivity compared with the commercial ELISA kit.

Gold nanoparticle (AuNPs) is the second most used material for this subject. Since they can increasing the sensitivity by varying the size, shape and composition of these material, AuNPs has become extraordinary for biosensor, because they have excellent biocompatibility, high conductivity, effective catalysis, high density, and high surface-to-volume ratio.[46] The surface of AuNPs can be tailored by ligand functionalization to selectively bind biomarkers. AuNPs has been modified with biomolecules such as DNAs, proteins, aptamers, antibodies by thiol and amine via Au-S or Au-N bonds without destroying the activity of biomolecules.[47] This simple and inexpensive methods were applied for detection of biomarkers, which brings some advantages over other platforms such as high sensitivity, low-cost, and amenable miniaturization. Recently, Pirzada et al. developed an ultrasensitive electrochemical sensor based on hybrid epitope imprinting and nanomaterial amplification.[48] The AuNPs decorated epitope-mediated hybrid were utilized for the preparation of electrochemical sensors to detect neuron-specific enolase (NSE). The biomarker assay using the standard hybrid MIPs resulted in 2.5-fold higher sensitivity compared to single epitope imprints, whereas the AuNP-hybrid MIPs enhanced the sensitivity level and allowed the recognition of NSE in human serum in a concentration range of 25–4000 pg ml^{-1}. Another paper describes a biosensor using gold nanoparticle/gallium nitride to develop an aptasensor for the epithelial ovarian cancer marker-CA125 detection.[49] The DNA aptamer of CA125 was modified on the surface of the AuNPs via Au-S bonds. The aptamer can bind with the target with high selectivity, and the photoelectron transfer process of the system can be blocked by the protein, which results in the decrease of the photocurrent of the system. The standard addition recovery rates were between 86.01% and 90.09%. This method showed good sensitivity, selectivity, and reliability in detecting CA125 in serum. Furthermore, other nanomaterial will be discuss forward.

Immunosensors.—Immunosensors are designed to detect binding of antigens or antibodies (Abs) due to their exquisite target specificity and affinity.[50] Electrochemical immunosensor is an excellent platform for detecting cancer biomarkers in the early stage of the disease, with rapid results from tumor profiles, high sensitivity, small sample consumption, and noninvasive technique. These characteristics make it outstanding candidates for inclusion in portable (point-of-care) that combines the advantages of biosensor devices, electroanalytical methods, and specific immunorecognition reaction. Table I shows a summary of the common biomarker devolved based on immunosensor.

Recently imuunosensore based on 3D printing was described as an option in the construction of low-cost devices.[57] K. Kadimisetty et al. developed microfluidic arrays for simultaneous measurement of prostate cancer biomarkers, prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and platelet factor-4 (PF-4) using molded or precision cut microfluidic channels. It consists of screen-printed carbon electrodes with gravity flow for sample/reagent delivery and washing. The biomarkers are captured by the antibodies that are on the surface of the sensor and attached to a supercapacitor for a light generation that is detected by a CCD camera. The device showed detection limits of 300–500 fg ml^{-1} for the 3 proteins in undiluted calf serum in 35 min.[58] Another type of immunosensor for detecting tumor biomarkers is the electrochemiluminescence (ECL), where the light emission is initiated by a redox reaction in surface electrode, which has specificity, sensitivity, wide dynamic range, and low background signal.[59] Wang et al. developed an ECL to detect CA15–3. Glassy carbon electrode (GCE) was modified with platinum nickel nanocubes-L-cysteine-luminol nanocomposite (PtNi NCs-L-Cys-luminol) as a signal probe. The CA15–3 antibody was attached to the electrode. When CA15–3 antigen molecules-antibody binding occurred, the ECL signal intensity observably decreased, indicating the quenching detection principle of electrochemiluminescence. The results obtained showed that the sensor presented good catalytic performance and electrical conductivity promoting the decomposition of H_2O_2 to produce various active free radicals, increasing the ECL responses of luminol. The detection limit found was 0.000167 U ml^{-1} (S/N = 3) to CA15–3 with linear response from 0.0005 U ml^{-1} to 500 U ml^{-1}.[60] Hong et al. produced a dual-responsive immunosensor that combines colorimetric recognition and electrochemical response that detect CA125, CEA, and PSA tumor markers. They built biotin-doped polypyrrole (nano-Ppy) immunosensor modified with anti-CA125, anti-CEA, anti-PSA antibodies. Electrochemical detection was conducted using a potentiostat/galvanostat. For colorimetric detection, a TMB substrate was used. With this platform, target antigens can be analyzed without expensive instruments or complex sample preparation steps.[61]

Antibodies.—Antibody-based biosensor is a widespread technique for development of novel tools for cancer diagnosis with a versatile use, because the antibodies can be coupled with several biomaterials and can be used in a range of techniques for optical and electrochemical biosensors and in this section the focus will be in highlight appropriates techniques for specific types of biosensor fabrication.

However, this methodology still has lacks of robust performance (sensitivity and precision). To overcome these issues monoclonal antibodies has been used. Monoclonal antibodies, are a very useful biorecognition element, which is the biological component of the biosensor and it is responsible to generate the analyte specificity for a given target, due to the affinity that they have with respect to the analyte of interest, characterized by their high affinity and specificity, good stability and versatility and low cost compared to other elements of biorecognition.[62] Usually produced in goats, rodents, or rabbits, they exhibit high sensitivity and precision, short response times in connection with antigens. Another class of antibodies, IgYs are being commonly described by several authors in the production of immunosensors. The absence of immunological cross-reactivity between chicken IgY and mammalian IgG, determined by the evolutionary distance, reinforces the advantages of using IgY over IgG as the first antibody in some types of immunological reactions for the diagnosis of biomarkers.[63,64]

Regardless of the immunoglobulin classes used, some parameters must be taken into account when using antibodies, such as their

Table I. Electrochemical immunosensors (EI) for the analysis of cancer biomarkers of breast, prostate, liver, lung, colorectal, and stomach.

Type of Cancer	Sample	Methodology	Biomarker	Detection Range or Limit
Breast[51]	Spiked human serum	Linear sweep voltammetry	HER2	4.4 ng ml^{-1}
Prostate[52]	Patient serum	Linear sweep voltammetry	PSA	7.0 pg ml^{-1}
Liver[53]	Human blood	Differential pulse voltammetry	AFP	0.099 ng ml^{-1}
Lung[54]	Patient serum	Square wave voltammetry	NSE	0.26 pg ml^{-1}
Colorectal[55]	Patient serum	Amperometry	CA 19–9	0.0063 U ml^{-1}
Stomach[56]	Spiked serum sample	Amperometry	CA 72–4	0.10 U ml^{-1}

immobilization and orientation in the sensor's surface allowing the antigen-antibody binding to occur with greater precision.[65] Differences in methodologies for efficiently immobilizing antibodies are explored in the literature, such as covalent and affinity bonds, adsorption, and entrapment. Covalent bonds are known to be stable when binding the antibody to the surface of the immunosensor. In this type of bond, the solutions commonly used are 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide (EDC/NHS). As examples of covalent bonds, F. Fathi et al. developed a surface plasmon resonance (SPR) biosensor for cancer stem cell detection using cell surface biomarker; CD133 in acute myeloid leukemia (AML) patients. In this work, they immobilized the CD133 antibodies on the gold sensor surface of the SPR equipment using EDC/NHS solution, the binding of candidate cells to antibodies was monitored in real-time.[66] In another work, Othman et al. developed a fluorescence immunoassay technique based on nitrogen-doped carbon dots (NCDs) for detecting nuclear matrix protein 22 a novel biomarker for bladder cancer. They marked monoclonal antibodies with NCDs using EDC/NHS solution and incubated them with a small amount of NMP22. The immunocomplex on the carboxylated NCDs led to the quenching of the fluorescence intensity.[67] Roberts et al. used graphene nanosheets (GNS) modified with fluorine-doped tin oxide (FTO) to detect urokinase-type plasminogen activator receptor (uPAR), the common biomarker for prostate, non-small cell lung, breast, and colorectal cancer. GNS-FTO was coupled with antibodies (uPAR-Ab) via carbodiimide activation chemistry with EDC/NHS solution to interact with uPAR. The immunosensor showed easy handling and high specificity in detecting the biomarker.[68] Other types of solutions as 1-pyrenebutanoic acid succinimidyl ester (PBSE), phthaloyl chloride and iminithiolane, and glutaraldehyde can also be used to form covalent bonds.[69]

Entrapment immobilization also is been used for construing sensitive immunosensor. In this technique, the antibody is not directly attached to the support surface but entrapped within a polymeric network, which allows only the traverse of substrate and products but retains the biological activity. Amarnath and Sawant created an enriched immobilization matrix of polyaniline, silver nanoparticles, and bovine serum albumin to immobilized capture antibody (monoclonal anti-AFP) and detect α-fetoprotein (AFP). The results showed a linear range from 0.01 to 1 ng ml^{-1} with a detection limit of 4.7 pg ml^{-1} with provided enhanced signal during antigen-detection probe interaction.[70,71]

Furthermore, affinity immobilization also provide a strong interactions involving the Fc regions of antibodies and biomolecules, such as proteins A and G, biotin, streptavidin, and avidin. Li et al. Amplified electrochemical signal using immunoassays. For this, the gold electrode surface was covered with carbon nanotubes dispersed in chitosan functionalized with L-cysteine. Gold nanoparticles containing protein A were added and guided the binding of anti-alpha-fetoprotein (anti-AFP) antibodies. Then, the analyte (AFP) was added to the complex promoting antigen-antibody binding.[69,72] Regardless of the binding method used to immobilize the antibodies, enzyme-linked immunosorbent assay (ELISA) has been widely used for the detection and quantification of tumor biomarkers. The technique presents a simple mode of operation with reliable results in the detection of target analytes. There are four types of ELISA: in the direct method, the analyte to be tested is adsorbed to a solid phase, usually a plate and a primary antibody conjugated to an enzyme (conjugate) and placed directly on the target. When the antibody binds with the analyte, a substrate/chromophore is added and the enzyme catalyzes the reaction causing a visible colorimetric to be measured later by a UV-vis spectrophotometer. The sensitivity of this type of ELISA is the lowest compared to the other.[69,73] In the indirect ELISA, the antigen is adsorbed to the solid phase and the primary antibodies are bound to the specific antigen. Antibodies labeled with the enzyme (conjugate) bind against the primary antibody. (anti-species). Then, substrate/chromophore is added occurring the reaction that will produce the color, later read by UV-vis spectrophotometer. Sandwich ELISA is the most commonly used assay. Capture antibodies are coated on a well plate. Then, the sample of interest that will bind to the capture antibodies was added. Afterward, another antibody linked to the enzyme added to bind to another epitope of the antigen and form the complex. Finally, the substrate that reacts with the enzyme added it, promoting a color change and read by UV-vis spectrophotometer. This type of ELISA has greater specificity and sensitivity compared to other types, as it uses capture antibodies. It is worth mentioning that the intensity of the emitted color will be proportional to the concentration of the detected analyte. Finally, a competitive ELISA method uses a labeled antigen to compete with the target. The labeled antigen binds less when there is a more unlabeled antigen (from the sample). Therefore, the more antigen there is in the sample, the weaker the signal is inversely proportional to the color intensity.[69,73,74]

Different methodologies has focus on amplification and increase the signal obtained in traditional techniques for clinical testing of biomarkers such by using of nanomaterials such as metal oxides, magnetic nanoparticles (NPs), conductive polymers, and carbon-based nanomaterials.[75] La Rica and Stevens, described the modification of ELISA by adding gold nanoparticles (AuNPs) to detect prostate specific antigen (PSA). In the traditional method, the target molecule is anchored to the substrate by capture antibodies and recognized by primary antibodies.[76] A signal is generated by the conversion of the enzyme-substrate into a colored molecule. In their

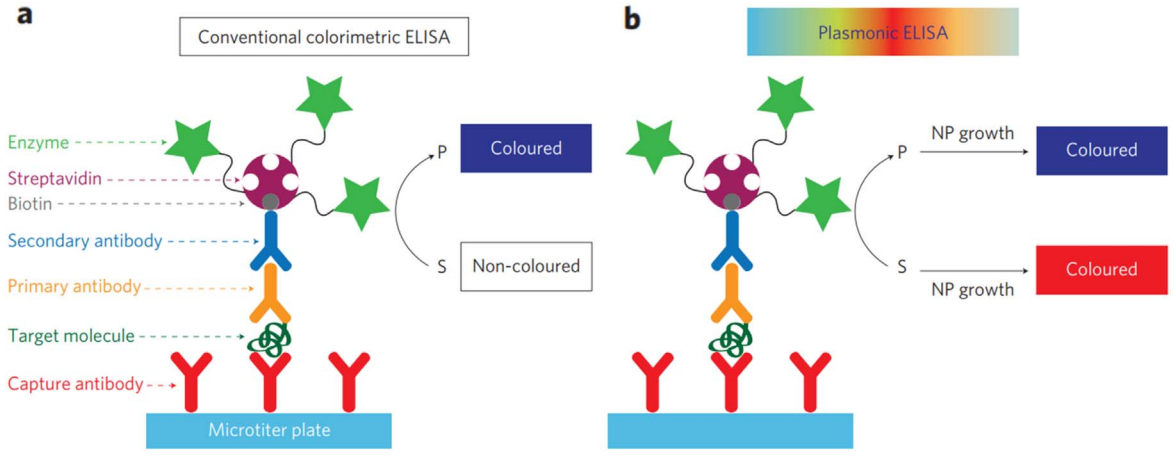

Figure 2. Schematic representation of the sandwich ELISA, (a) conventional colorimetric ELISA showed that the target molecule anchored to the substrate by capture antibodies and recognized by primary antibodies and, (b) modified traditional ELISA sandwich by adding gold nanoparticles. The biocatalytic cycle of the enzyme generates colored nanoparticle solutions of characteristic tonality.[76]

work, they maintained the same structure as the ELISA sandwich until biotinylated goat anti-rabbit IgG (secondary antibody). The streptavidin-conjugated catalase enzyme was added followed by the hydrogen peroxide solution. The gold solution (0.2 mM) will be added to the wells and the formation of the nanoparticles was observed. If the sample used contains the analyte of interest, the formation of the sandwich in the ELISA will cause the substrate (hydrogen peroxide) to be consumed quickly by the enzyme catalase, not reducing gold to AuNPs efficiently, resulting in nanoparticles with different shapes and a large number of aggregates, with blue color. In contrast, if the desired sample does not contain the analyte of interest, after the washing step it will be removed and there will be no ELISA sandwich formation. In this way, hydrogen peroxide added to the wells will reduce the gold solution added last, generating AuNPs with spherical shapes, of proportional and non-aggregated sizes, resulting in a red color (Fig. 2). PSA can be detected with the naked eye in the ultra-low concentration of 1×10^{-18} g ml^{-1}.

Another work using nanoparticles was describe by Li et al.[77] The traditional ELISA method was modified with manganese dioxide nanoparticles (MnO_2 NPs) for the determination of alpha-fetoprotein (AFP), a biomarker in some types of tumors, such as the ovary, pancreas, and stomach. MnO_2 NPs were used as an artificial enzyme to cause the conversion of TMB (popular chromogenic substrate) to give a colored product. Compared with biological enzymes, MnO_2 NPs are more stable at room temperature with high catalytic activity, resulting in a better limit of detection and performance compared to the traditional ELISA using peroxidase enzyme. Xia et al. demonstrated an alternative method using artificial enzymes in traditional ELISA to detect biomarkers. In this work, peroxidase mimics that was produced by depositing Ir atoms (iridium) as ultrathin skins (a few atomic layers) on Pd nanocubes (palladium) for PSA detection. The results showed a LOD of 0.67 pg ml^{-1}, which was approximately 110-fold lower than that of the conventional HRP-based ELISA using the same set of antibodies and the same procedure.[78]

DNAs.—Urine and blood samples can contain cell-free circulating DNA, serving as biological samples in the construction of immunosensors.[79] These fluids contain information about circulating tumor cells (CTC), cancer and immune interactions, microvesicles, and tumorigenesis. The analysis of DNA sequences play a fundamental role in rapid detection of genetic mutations, offering the possibility of performing reliable diagnosis even before any symptoms of a disease appear. Recent advances in literature brought new opportunities. DNA biosensors, are rapidly being developed towards the assay of rapid, simple and economical testing of genetic and cancer. Unlike methodologies that used enzyme or antibodies, nucleic acid recognition layers can be readily synthesized and regenerated for multiple use. DNA sensors can be made by immobilizing single stranded (ss) DNA probes on different electrodes using electroactive indicators to measure the hybridization between DNA probes and their complementary DNA strands.[79,80] Two main methodologies have been used to construct this device with a well-defined probe orientation and accessible to the target for hybridization. Self-assembled monolayers have been used to attach the thiolated DNA probe onto to a gold surface by functional alkanethiol reaction. In another way, biotinylate DNA is attached through biotin-avidin interaction on the electrode surface. Newly, other methodologies have been tested.

S. N. Topkaya et al. developed an electrochemical DNA biosensor for detection câncer biomarker GSTP1 hypermethylation using DNA oligonucleotides and PCR samples.[81] They demonstrated the combination with a single-use carbon graphite working electrode and differential pulse voltammetry to detect glutathione S-transferase P1 (GSTP1) gene, a specific biomarker of prostate cancer. The electrochemical DNA biosensor converted hybridization events to analytical signals. First, the DNA probe solution prepared in PBS was placed in a pretreated graphite electrode (PGE). The modified PGE was dipped in the target solution prepared in PBS to form the probe-target hybridization. The real samples obtained from PCR amplification were added to the PGE followed by the readings. The oxidation signals of guanine bases were measured by voltammetry and electrochemical impedance spectroscopy was used to detect DNA hybridization. 2.92 pmol of the target sequence in a 100 μl reaction volume.

Cervix uteri cancer is mainly caused by human papillomavirus (HPV). The main biomarkers for this type of cancer are HPV16 and HPV18.[82] Bartosik et al. developed an electrochemical-chip-based assay, in which target DNA HPV is captured via magnetic bead-modified DNA probes. HPV-16 and HPV-18 target and probe sequences were designed from NCBI database.[82] Detection was performed by hybridization step between target HPV and biotinylated capture probe (CP) modified magnetic beads coated with streptavidin and between target HPV and detection probe. The steps were made on a carbon working electrode and monitored by chronoamperometrically. The results showed that sensitive detection in HPV DNA atomoles, good reproducibility, and discrimination between HPV-16 and HPV-18. Several types of biomarkers are used for breast cancer. In the work of A. Benvide et al. developed an electrochemical biosensor formed by glassy carbon electrode (GCE) modified with gold nanoparticles and graphene oxide to detect BRCA1 5382 insC mutation.[83] The biosensor used single stranded DNA (ssDNA) as a probe. Cyclic voltammetry and electrochemical impedance spectroscopy techniques were used to measure the electrochemical response for synthesis and DNA hybridization, presenting LOD of 1.0×10^{-20} M.

DNA sequences are not only used as cancer biomarkers, but also can enhancing the signal and performance of immunosensors. For example, F. Wei et al. use a sixteen electrochemical chip coated with a DNA dendrimer and polypyrrole (DDPpy) film with capture antibodies to measure the levels of two salivary protein markers (IL-8 and IL-1b) for oral cancer.[84] DNA dendrimer was constructed of short DNA sequences in nanometric size that can be introduced polymer matrix. In this work, dendrimer DNA was added to the surface of the working electrode by a simple electrical polymerization process to improve its performance, followed by the addition of antibodies and protein markers IL-8 and IL-1b in a buffer. The LOD of salivary protein can reach 100–200 fg ml^{-1} with 90% sensitivity and specificity. The use of DNA in the ECL technique is also reported in the literature. D. Lin and his collaborators describe the use of glassy carbon electrodes modified with layer-by-layer of carbon nanotubes, CdS quantum dots (QDs), and capture antibody for detection of α-fetoprotein (AFP). The formation of an immunocomplex on the electrode modified with bio-bar-coded (G-quadruplex DNA + hemin) AuNP conjugated with antibodies allowed the detection of the biomarker with good stability, presenting the linear range of 0.01 pg ml^{-1} to 1 ng ml^{-1} and a detection limit of 1.0 fg ml^{-1}.[85] Gao designed a 3D DNA nanosphere to develop a photoelectrochemical (PEC) biosensing platform. The (PEC) biosensor was prepared using a piece of bulked indium tin oxide (ITO) glass doped with gold nanoparticles, zinc selenide, and quantum dots (ZnSe QDs) solution to form ITO/Au NPs/ZnSe QDs. Finally, 3D DNA nanosphere was placed on the surface to modify the biosensor. The 3D DNA nanostructure was self-assembled by base complementary pairing in a few minutes and a rolling circle amplification (RCA) reaction. Carcinoembryonic antigen (CEA) was added and competed to capture DNA on the surface of the biosensor by releasing 3D DNA nanospheres and expanding the signal obtained. The results of the experiment were indistinct linear range from 1.0 fg ml to 10 ng ml and LOD of 0.12 fg ml for CEA.[86]

Cytosensors.—Over the past decade there was an increasing development of a range of electrochemical cytosensors to detect cancer cells or cancer cells byproducts inside the tumor microenvironment,[87–89] and the improvement of nano-fabrication and biotechnology generated new insights to monitor living cells in a more precise manner and in real-time by using electrochemical biosensors.[90–92] Basically, the definition of cytosensor is sensing

platform with capability to monitor cells in a non-invasive way,[93–95] and it must be developed using a combination of a biorecognition element, such as aptamers and/or antibodies and a signals transducer to convert the biological signal in a detectable signal.[96–98]

Cytosensors transducers can be used in a range of bioassays,[95] for example, optical,[99] fluorescence,[100,101] flow cytometry,[102] surface-enhanced Raman scattering,[103] colorimetric assays,[104] and electrochemical.[105] The most widely applied methods for cancer cell detection rely on flow cytometry or fluorescence techniques, which have several drawbacks and disadvantages such as the need of high cost equipment and reagents, high-level technical skills and are time consuming experiments.[106,107] For example, cytosensors can be used to predict tumor malignancy and metastatic sites by detection and quantification of CTCs, in addition there are electrochemical-based cytosensors for electrochemical for cancer cells; acoustic wave based; field effect transistor based, gravimetric based, field affect transistor based and others.[108–110]

In this way, the electrochemical cytosensors arise as a new pathway to develop reliable, cheaper, more sensitive and easy to access tools for cancer cell detection and monitoring using electrochemical outputs such as current, impedance and capacitance.[110] The use of a cell-based electrochemical biosensor can enhance the detection of ions, enzyme, proteins, cytokines and other biomarkers in a more fashioned way due to possibility of miniaturization, easy operation, fast outcome data, high selectivity, real-time an non-destructive assays, providing point-of-care devices for early cancer detection and diagnosis, cancer monitoring and treatment.[111–113] Here we will focus on electrochemical biosensors based on aptamers and microRNA strategies for cancer cell detection and monitoring.

Electrochemical cytosensors.—The eletrochemical-based cytosensors is a type of biosensor that convert the biological response between the biorecognition element and living cells into electrochemical output for quantitative analysis of the cell status[114] (Fig. 3). The most used methodology for electrochemical cytosensors is the sandwich assay [refs] which is a simple technique where the cell or biomarker of interest is comprised between a layer of biorecognition elements and the great advantages of this technique are the possibility of multiplexing sensing, signal amplification and high specificity signal of cancer cell detection.[115,116]

As well to the biorecognition element, the signal transducer plays a key role in the development of cytosensors and there are several types of nanomaterials and/or nanostructure that can be used in combination with aptamers, enzymes and miRNA to enhance and amplify the detectable signal such as carbon nanotubes, metal nanoparticles, graphene, nanofibers and quantum dots.[121–123] Moreover, the most used electrochemical techniques for cancer cell sensing are: electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), square wave voltammetry (SWV), and square wave stripping voltammetry (SWSV).[110]

Electrochemical impedance spectroscopy cytosensors.—The EIS technique is a method used to analyze the electrical resistance of a system in a high sensitive, low cost, fast, label-free and non-destructive way. The EIS can detect small quantities of biomolecules in contact with the electrodes and due to its high sensitivity response; the EIS is suitable to monitor target-ligand kinetics being a substitute technique to enzyme-based cytosensors.[96] Usually the main design of an EIS cytosensor is to immobilize the biological sensing element in the surface of electrode, followed by a blocking of the unmodified surface to avoid unspecific binding into the bare electrode that could lead to false positive or negative results.[110]

For example, H. Shen et al. developed a label-free EIS cytosensor to detect MCF-7 based on EpCAM marker, which is overexpressed marker in the membranes of cancer cells based on a complementary strand release assay upon the binding of target cell.[124] In paralel, B. Seven et al. developed a cytosensor for MCF7 detection based on HER2 biomarker and it was able to verify correlation between the number of cells capture by the cytosensor and the charge transfer resistance even for only 100 cells ml^{-1}.[125]

In its turn, S. Tang demonstrating the capability of using an EIS biosensor to detect as low as 10 cell ml^{-1} in a precise, fast and reliable way without destroying the cells by using glassy carbon electrode combined with a matrix made of mannose to create a matrix for breast cancer cell capturing. In this way, the EIS sensor shows the ability to detect cells even in a low number that can be a suitable platform to be used when precious cells are the focus of detection, for example, primary cells derivate from human biopsies for clinical aplications or CTCs detection.[126]

Voltammetry cytosensors.—Typically, a voltammetric sensor measures the current signal as function of a potential, which can be applied in several ways such as steps, linear sweep and pulses. In this way, one of the most used technique for cytosensing is the DPV. The DPV operates by applying small potential pulses under a constant signal amplitude on a linear potential sweep and its usage is widely for sensors because it can achieve low limit of detection with high linear range and sensitivity because it sample the current twice, 1st before the pulse application and the 2nd at the end of the pulse application.[127] For example, D. Ou et al. achieved a limit of detection of 6 cell ml^{-1} by using a cytosensor based on tetrahedral DNA structures immobilized in gold electrodes to detect breast cancer cells.[128] In addition, Y. H. Tang et al. developed a Pt/Ag nanocomposites for MCF-7 cells detection by signal amplification using antibodies labeled with Pt/Ag and reach a limit of detection of 3 cell ml^{-1} and, moreover, the sensor was also able to detect CTCs in complex serum samples.[129] N. Liu et al. produced a reusable cytosensor to detect MCF-7 cells with a limit of detection of 20 cells ml^{-1} by combining glassy carbon electrode and aptamers with capability to reuse the sensing platform for up to 12 assays after biosensor regeneration. DPV-based cytosensors can be used in complex samples such as serum, whole blood in a high-throughput way.[130]

In addition to DPV, the SWV and the SWSV are two widely used techniques for cytosensing. The SWV detects a symmetric square wave shape of potential, which is applied, to the working electrode and the output signal is a similar peak to DPV, however the SWV is a faster assay, which reduces any minor chance of damaging the cells during the measurements.[96] For example, Q. Sheng et al. developed a circle amplification DNA/RNA cytosensor to detect MCF-7 cells based on the EpCAM detection by SYL3C aptamer and the limit of detection of this cytosensor was 12 cells ml^{-1} with an impressive linear range from 20 to 5×10^5 cells ml^{-1}.[131]

SWSV technique is very similar to SWV, the only difference is one more step in the SWSV, in which an anodic or cathodic potential are applied to the working electrode and the principle of work it is an ion accumulation step on top of the electrode surface followed by a stripping of the anions/cations.[96] The stripping step is the detection assay that will provide a current peak related to the ions concentration in the electrode surface. The SWSV needs a metallic-based material like quantum dots (cadmium, lead) or metal nanoparticles and the metal components should be close to the electrode surface and must be coupled with the biological recognition element of the cytosensor.[96] The idea of the accumulation step in the SWSV is to improve the sensitivity of the sensor by signal amplification, the drawbacks of SWSV is the possibility of metal-ion uptake by cells, unable to reuse the sensor surface due to effects of stripping the ions. However, the advantage of using SWSV is the ability to multiplex the sensing surfaces and detect more than one marker at the same time. For example, T. Li et al. developed an SWSW cytosensor with CdS to dectect MCF-7 by incubating the cells in a gold electrode modified with aptamers for MUC-1, after cells capturing by aptamer, an antibody for carcioembryonic antigen labeled with CdS was used and it was possible to detect 3.3 x 10^2 cells ml^{-1}.[132]

Cytosensors are a novel type of biosensor that is start to be used more widely on cancer cell research and with the advance of biochemistry, surfaces modification, nanomaterials development

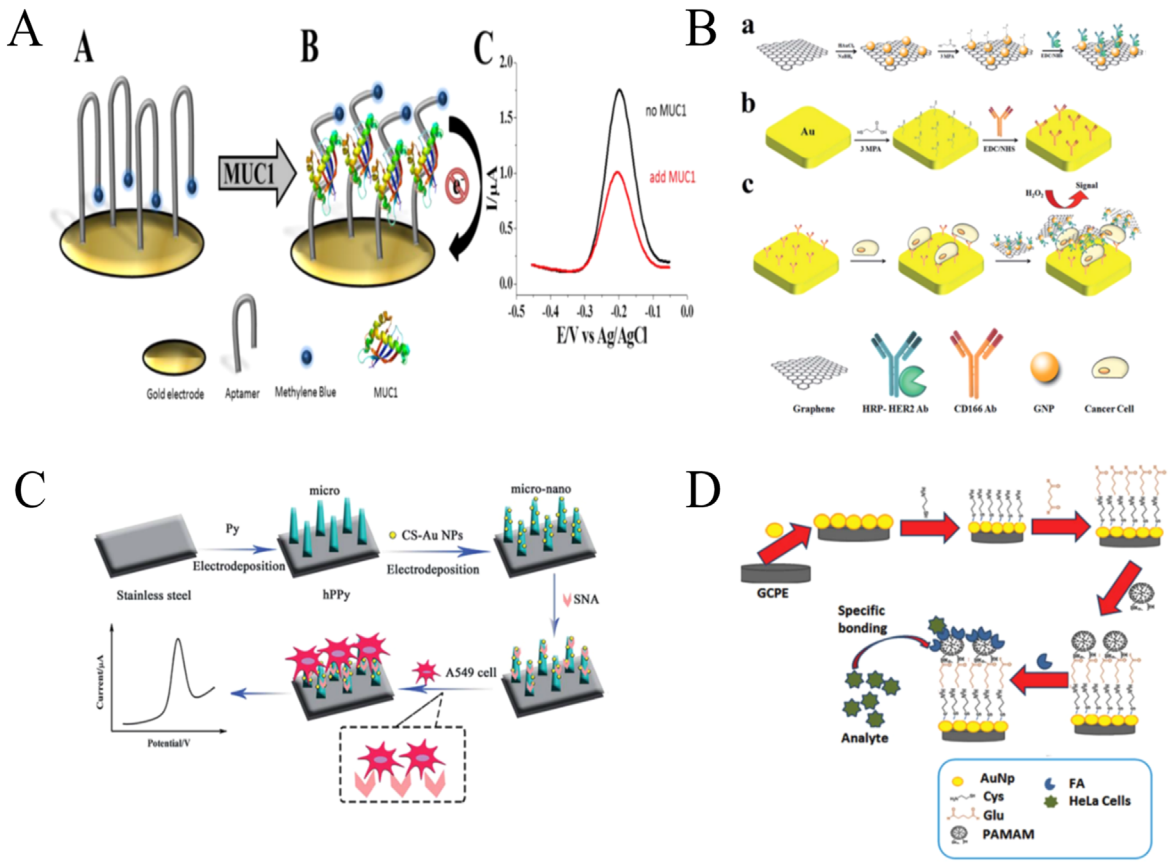

Figure 3. Schematic of electrochemical based cytosensor for cancer detection, (A) Signal-off biosensor to detect MUC1 from prostate cancer cells,[117] (B) Au/AuNp/Ab-HRP cytosensor to detect prostate cancer cells,[118] (C) CS-Au/hPPy cytosensor to detect sialic acid release by cancer cells[119] (D) GCPE/AuNp/Cys/Gly/PAMAM/FA cytosensor for lung cancer detection.[120]

and biotechnology, the cytosensors are in the spotlight for *in situ* and real-time measurements and the application of electrochemical techniques open new pathways that can be exploited for cancer cell research.

Aptasensors.—The aptamers are a class of biorecognition element in biosensor development. Usually aptamers are short sequences of ssDNA or RNA (40–100 nucleotides); their first descriptions are dated at beginning of 90s decade by several groups.[133–135] Since their discovery, they are on the rise in the development of new sensors since its discovery as a next generation of biological component that will replace antibodies in biosensor fabrication and development.

Aptamers generation are done by a process called systematic evolution of ligands by exponential enrichment (SELEX), which is an in vitro development of aptamers of interest with high affinity to target molecule.[136] In the SELEX process, the first step is to generate a large and random library of oligonucleotides with equimolar concentration of pyridine and purine bases to ensure same probability of occurrence of each oligonucleotide is the same; the next step is the incubation of this library in under specific conditions with the target of interest followed by elution. Normally, the target is immobilized on a substrate so in this way, the proteins that bind to it are not removed during washing and it can be amplified by PCR after the elution, and after several PCR amplification the ssDNA or RNA that specific binds to target of interest is obtained. The amplification step is the most vital procedure during SELEX and there are several steps to prevent the formation of dsDNA such as the use of asymmetrical PCR, and during every PCR cycle, the pool of specific oligonucleotides is obtained.[135,136] Finally, after the selection of specific sequences, they need to be tested in order to calculate the binding constant (Kd) to find the sequence the best perform, which is introduced into plasmids, cloned and sequenced. After sequencing, the aptamer is ready to be produced in a high throughput way by chemical synthesis.[134,136]

The aptamer-based biosensor is a promising tool and have advantages when compared to antibody-based platform. The small size allows it to penetrate easier a tissue and it can also target markers that are the size of antibodies without causing steric hindrance; the aptamers can form different secondary structures that enhance the number of receptor conformations.[137] The SELEX process is an in vitro process without the need of animals making it to be cheaper and more ethical and with less variation from batch to batch synthesis and low to absent immune response or toxicity. Aptamers can be used for in vitro and *in vivo* sensing and the possibility to detect a wide range of targets (from ions to whole cells).[138]

Aptamers demonstrate high potential to be used in clinical application for early cancer diagnostics, cancer monitoring and treatment because it can target a range of biomarker of interest such as: enzyme, regulatory proteins, growth factors, antibodies, organic molecules, amino acids, peptides, nucleotides and even whole cells.[138,139] In addition to the wide target range, the aptamers have also the versatility to be chemically modified with chemical tags (fluorescence probes, quencher, electrochemical indicators, enzymes and nanoparticles) and integrated in any platform for biosensing applications such as, fluorescent, colorimetric, optical and electrochemical.[140,141]

In this way, different cancer cell and its byproducts can be detected by aptamer-basesd electrochemical biosensors; for example, blood cancer cells; breast; prostate; liver and cervical cancer cells and CTCs.[142–144] For the development of novel aptasensors two main types of sensing strategies are commonly used, the use of

labels covalent linked to the aptamer (metal nanoparticles, enzymes, redox pairs) or label-free detection systems (Fig. 4).

In addition to the main type of aptasensor developed, the readout of aptasensor can also be classified into two major groups, first the signal-off which is a technique that measures the decay off the electrochemical signal upon binding of your target of interest.[145] The second is the signal-on technique that detects a signal gain after binding of the target to the aptamer.[146] For example, H. Liu et al. developed an aptamer-based biosensor to detect blood related cancer cells by using gold nanoparticles combined with multiwall carbon nanotube and QDs aptamers and achieve a limit of detection of 50 cells ml^{-1} by using anodic stripping voltammetry.[148]

Aptamer for breast cancer detection were developed by mainly targeting MUC-1 and HER-2 for early cancer diagnostic, X. Zhu et al. developed a HRP-labeled aptamer for MUC-1 detection and with signal amplifications steps it was able to reach a limit of detection of 100 cells ml^{-1} with a linear range up to 1×10^7 cells.[149] On the other hand, Y. Zhu developed an aptasensor to detect the HER2 protein in a sandwich combination of antibodies and aptamers, the antibody against HER2 was immobilized in the top of AuNPs modified glass carbon electrode and the aptamer was casted in the top of the AuNPs. The aptamer had SK-BR-3 cancer cell as a target and the detection strategy was based on signal amplification with a limit of detection of 26 cell ml^{-1}.[150]

Futhermore to breast cancer, the cervical cancer has high incidence in women with an increasing mortality rate in developing countries,[151] in this way, there are several studies targeting an early and accurate detection of HeLa cancer cells and its unique cell markers that might act as biomarkers for aptamers development.[152] For example, Feng et al. developed a label-free aptasensor based on EIS technique to detect HeLa cancer cells in a graphene-based sensor combined with aptamers with a limit of detection of 794 cell ml^{-1}.[153] Another work from Wang et al., demonstrate the ability to detect HeLa cells by combination of gold electrode with graphene and aptamers with signal amplification by using a ferrocene tag into the graphene achieving a limit of detection of 10 cells ml^{-1}.[154]

Electrochemical aptasensors for liver cancer cells was also developed, the liver plays vital roles in human body and an early stage detection of liver cancer is important to avoid the disruption of its function. The TLS11a aptamer is being widely used to detect HepG2 cells, for example, Chen et al.[155] developed a sandwich-type aptasenor based on hybridization chain reaction to target HepG2 and by using DPV, and a linear range of detection from 10^2 from 10^7 cells ml^{-1} was achieved. In parallel, Sun et al. developed an aptasensor by combine AuNPs and glass carbon electrode with a sandwich-based electrochemical sensor with a limit of detection of 15 cell ml^{-1}.[156]

Detecting proteins that have its expression change during cancer establishment is important for early diagnosis, cancer monitoring and response to treatment. Prostate cancer, which is the second most common type affecting men, had the prostate-specific antigen (PSA) as a popular marker for its detection, however over the last decade the clinical data demonstrate lack of accuracy when only PSA was taken in account for early prostate cancer detection. In this way, B. P. Crulhas et al. developed a biosensor to detect a PSA, VEGF and MUC1 from different types of prostate cells (RWPE-1, LNCaP and PC3) in order to provide new insights of protein expression between normal and cancer cells with differences on androgen dependence.[145] The aptasensor was based on a signal-off strategy and aptamer was covalent bound to gold electrodes, the aptamer were modified with a methylene blue as a redox pair. The sensing strategy was a signal-off sensing by using SWV and it was possible to detect proteins even in lower concentration as 1 ng ml^{-1} with a linear range up to 100 ng ml^{-1}. Targeting byproducts from cancer cells is an interesting topic for researcher in biosensor field because it can help in the early diagnostic and can provide new insights to understand cancer progression during treatment and cancer monitoring.[145]

Also, detection of CTCs in blood is a new trend in aptamer-based biosensors. Detection CTCs might provide a better understanding of metastasis cycles and development, and aptamers have the ability to overcome the major drawback of working with CTCs that is the low abundance of the cells in blood. For example, Zhang et al. developed an aptasensor to detect the A549 cells in blood by using an iodide-selective electrode with porous graphene oxide combined with the AS1411 aptamer. The AS1411 aptamer covalent binds to the CTCs in blood samples and a limit of detection of 10 cells/100 μl was achieved.[157]

The application of aptamers in electrochemical biosensor for cancer detection are in great development and with the possibility to use novel biomaterials and transducers it is possible to overcome the majority of the drawbacks from the sensing platform, such as low sensitivity, low selectivity and stability under complex media (whole blood, plasma and other type of biological samples). In this way,

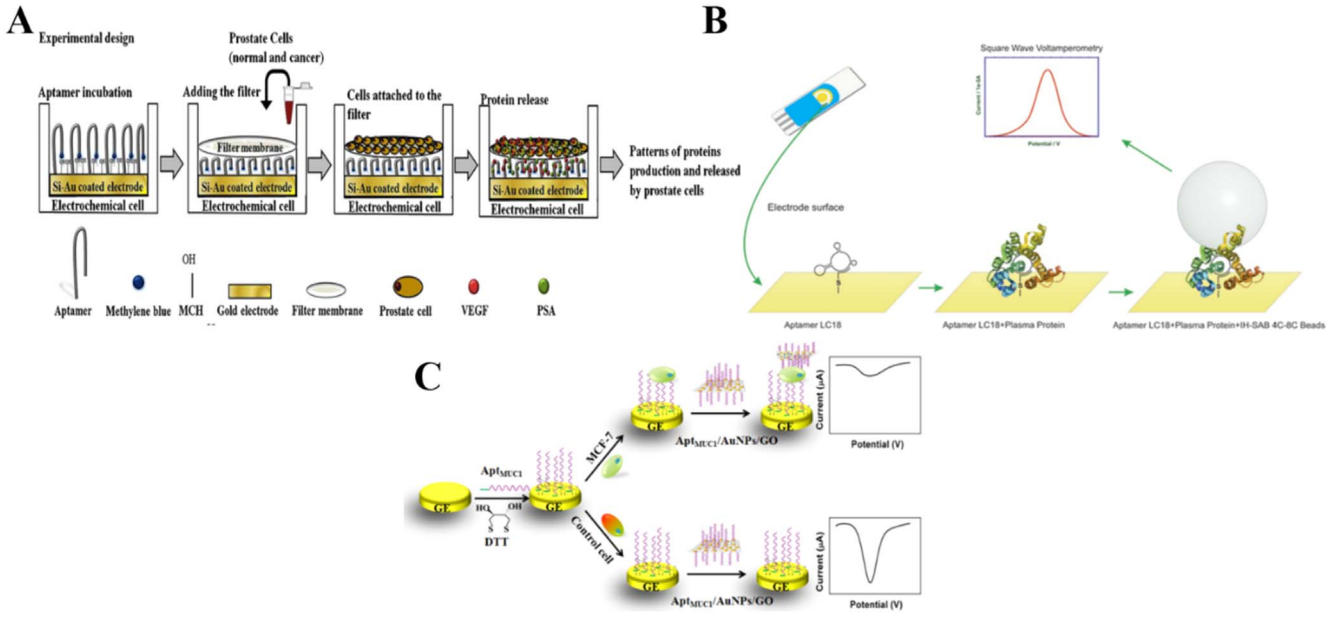

Figure 4. Schematic of electrochemical-based aptasensors for cancer detection, (A) Signal-off biosensor to detect PSA and VEGF from postate cancer cells,[145] (B) Signal-on aptasensor for plasma protein detection,[146] (C) Label-free aptasensor to detect MCF-7 cells.[147]

aptamer-based biosensors emerge as a powerful tool for early cancer diagnosis and disease progression.

MicrRNA biosensors.—MiRNAs are described as small non-coding RNA widely conserved through evolution (15–25 nucleotide. Nowadays, the miRNA is at great focus by cancer research field in a similar manner as aptamers, over the past year the number of papers that focused miRNA as cancer biomarker was around 700 in 2019. The mainly focus is because its biochemical properties and abundant concentration in biological fluids which allows a simple detection without complicate steps for sample treatment.[158,159]

The miRNA expression level can be strongly related with several diseases such as diabetes, cancer and neurodegenerative disorders, more specific, recent studies demonstrated the development of several cancers are related to miRNAs.[159,160] The relationship between miRNA and cancer was first described by Calin et al., which found a deletion at the chromosome 13q14 that is related to a tumor suppressor gene involved in leukemia, and this specific region encodes two miRNAs (miR-15a and miR-16–1), and deeper investigations demonstrate the involvement of the miRNAs in the leukemia pathogenesis.[161] In addition to that, Constinean et al. demonstrate the overexpression of miR—155 in B cells can induce lymphoma pre-B leukemia. In addition, to leukemia related miRNAs, a range of miRNAs have deregulated expression in cancer for example, the let-7 family has a miRNA that regulate a RAS family of oncogene, the miR-106b-25 has a major role in gastric cancer, miR-155 is overexpressed in Hodgkin lymphoma, miR-143 and miR145 are downregulated in colon cancer.[162] Finally, the miR-21 is overexpressed in range of tumors (glioblastoma, cholangiocarcinoma, and myeloma and breast cancer.[163]

The traditional methods for miRNAs detection rely mostly on real-time PCR (RT-qPCR), Northern Blot and deep sequencing, which are complex, expensive techniques that requires specialized laboratory equipment and work force, so there is a need to develop novel analytical methods for fast, specific and sensitive identification of miRNAs present in cell, tissue and complex fluid (serum and plasma) samples.[164,165]

For this reason, the electrochemical methods for miRNAs detection emerged as a promising tool because it can overcome the disadvantages of traditional techniques. Electrochemical biosensors can be miniaturized, multiplexed, have low-cost and, can be produced easily in large scale.[166] Moreover, the versatility of sensing elements that can be used, such as novel nanomaterials (nanoparticles, carbon nanotubes, graphene), organic and bioorganic polymers, eletroactive molecules enhances the use of electrochemical sensors for miRNAs detection. Mohammad et al. reviewed several amplifications techniques for miRNA analysis and Micheal et al. demonstrate a range of oligonucleotide combinations for the development of electrochemical biosensors.[167,168]

The basic design of an electrochemical biosensor for miRNA usually uses a complementary DNA probe to the miRNA of interest and the hybridization with the miRNA promote direct redox current response that will be detect by a transducer which is the most of the time is a gold electrode, but AuNPs, AgNPs, CD-QDs, PB-QDs can be used as inorganic probes.[169] Ferrocene, methylene blue and thionine are some example of organic probes used for miRNA detection. More specific, the sensing platform for miRNA detection is usually a signal-ON or signal-OFF type, where the current response after hybridization alters the basic state of the sensor and this change can be calculate as function of miRNA presence and concentration.[169,170] For example, Jou et al. developed a signal-OFF sensor using methylene blue as redox pair and achieved a limit of detection around 3.57 fM for miRNA-155 for blood cancer detection.[171] Yammouri et al. used a methylene blue labeled DNA probe conjugate chemically in surface based on pencil carbon and it was able to detect up to 1 fM of miRNA related to cancer.[172]

In addition to ON/OFF probe detection, another technique that can be used for miRNA biosensors is the elimination of the labeled probe. This technique consist in releasing the labeled probes after the hybridization with the target. The most common method is the use of a cleaving agent such as, endonucleases, duplex-specific nucleases or calcium ions that will remove the hybridized molecules eliminating the probe from the sensing surface. For this type of application, usually the biosensor are based on carbon materials, such as graphene, or carbon nanotubes because in this surface the ssDNA probe has more affinity than the hybridized dsDNA/RNA complex.[169,170]

Gao demonstrate the detection of miRNAs using nanoparticles in a indium tin oxide electrode with dna probe immobilized at the surface, upon hybridization the isoniazid-capped nanoparticles catalyze the oxidation and amplify the signal, enhancing the detection of miRNAs.[173] Azimzadeh et al. developed a biosensor based on graphene oxide and gold nano-rod against miRNA 155 in plasma for early breast cancer detection and achieved a linear detection of miRNA with a limit of detection of 0.6 fM.[174] It is important to note that this biosensor was able to detect early breast cancer without any additional sample preparation such as RNA extraction and amplification. In this way, they demonstrated how the electrochemical biosensors against miRNA could overcome the traditional techniques for early cancer detection.

Zeng et al. was one of the first to introduce an ultrasensitive electrochemical biosensor to detect multiple markers of pancreatic carcinoma, in his work a screen-printed gold electrode to improve the detection and it was able to detect miRNA21, miRNA155, miRNA196a and miRNA210 without the need of any amplification step.[175] In addition, Hu et al. developed a biosensor based on graphene-QDs and horseradish peroxidase to detect miRNAin real samples with a detection limit of 0.14 fM and a linear range from 1fM to 100 pM.[176] Zhang et al. in its turn developed a free-immobilization electrochemical biosensor based on capture probes and recycle assisted target by using a duple-specific nuclease to detect circulating miRNA21 in plasma with a detection limit of 0.2 fM and linear range from 0.2 fm to 1 nM.[177]

Several researchers demonstrate the expression of a miRNA in tumors and in hematologic diseases have differences in miRNA expression when comparing neoplastic and normal tissues, indicating that neoplastic tissue can be distinguished from healthy tissue by the expression of 20–30 different miRNAs can be high predictive for cancer detection and monitoring.[174,175] MiRNAs play a major role in tumor progression and metastasis, for example miR-139 and miR10-b are related to hepatocellular and breast cancer metastasis, in this way the ability to miniaturize and multiplex the electrochemical biosensors become an interesting platform to target miRNAs for early cancer diagnostic and cancer monitoring.[175]

Conclusions and perspective.—In this review, we pursued to give an overview of different approaches in the development of biosensor to detect cancer biomarkers such as immunosensors, cytosensor and aptasensors and each type of sensor has its own advantages and drawbacks. However the electrochemical biosensing is a promising field and with the evolution of biotechnology and nanofabrication the electrochemical biosensors are paving its way toward to be the most reliable, specific and sensible sensor capable to multiplex several assays in a easy to use device and due to the low cost when the scale up the production.

These devices hold enormous potential for early cancer detection and treatment. Many of the above-mentioned methodologies have complexity-manufacturing process. There are so many steps to be overcome that many devices are valid only laboratory benches. The best scenario is to find methodologies that present ease surface modification with nonspecific bind and higher binding efficiency in biomedical application. Much progress has been made in amplifying the signal using nanostructured and hybrid materials, allowing the ultra-sensitive detection of biomarkers with those properties.

Over the past decades, CNTs have obtained excellent results, bringing excellent bio interface, high stability, and sensitivity. However, the functionalization and modification of CNTs remain

the greatest barrier to obtain more reliable electrodes for application in complex media, maintaining high selectivity and sensitivity.

The use of nanostructures has constituted an excellent strategy because all advantages discussed above. But, the major challenge that particles are still not synthesized in the same way every time, causing a large variation in the signal, limiting reproducibility and sensitivity. The mass production becomes limited because multi-step manifacturing. Despite a long way to go, nanosensors have great potential to be used as a point of care devices due to their low cost and high sensitivity. Therefore, there is a need to produce simple and more reliable methodology that could lead to more accurate clinical analysis.

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