CHAPTER 4

Application of smart materials in biosensors for cancer diagnosis

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1. Cancer diagnosis: traditionalism x new perspectives

Cancer can be described as a silent, fast, and uncontrollable disease. In this scenario, early diagnosis may significantly improve the success of treatment and survival rates (Gottlieb et al., 2021; Wishart, 2015). Unfortunately, some types of cancer present nonspecific signs and/or symptoms, such as fatigue, loss of appetite, nonexpected loss of weight, constant nausea, atypical pain, and unexplained laboratory test findings (Murchison et al., 2021; Vasilakis & Forte, 2021), hampering the precise diagnosis and treatment recommendations. In these cases, the expertise of the physician and further investigations are crucial to providing good clinical outcomes.

Lately, some clinics have been considering implementing the rapid diagnosis practice, followed by traditional techniques. In this approach, patients who do not attend specific criteria of cancer's symptoms after a previous examination are redirected to noninvasive image exams, such as computed tomography scan (CT) (Murchison et al., 2021). In a study performed with 681 patients at a University Hospital, 133 received an indication of a cancer diagnosis. In this specific case study, the prevalence of gastro-oesophageal cancer was noticed, but cases of unknown cancer were also reported (Vasilakis & Forte, 2021). Evaluating the use of imaging scans as a diagnostic tool during studies realized between 2016 and 20, a systematic review brings the CT as the most employed technique, followed by ultrasonography, with pulmonary and breast cancer the top list of cases (Fig. 4.1A) (O'Shea et al., 2021). Although an impressive number of diagnoses are based on computer-assisted diagnosis (CAD) and histopathological images, they only reveal the morphology and do not always suggest the aggressiveness of a solid tumor (Fig. 4.1B) (Gurcan et al., 2009; Shao et al., 2020).

The choice of the imaging modality and the time needed for the image to be acquired are relevant aspects to consider in the quality of results. Cases of false positive or false negative are still a concern in image analysis. One example is women who have high breast density and fibroglandular tissue. A fibroglandular tissue and a cancer tissue have similar image properties, and the incorrect diagnosis affects all the treatment procedures

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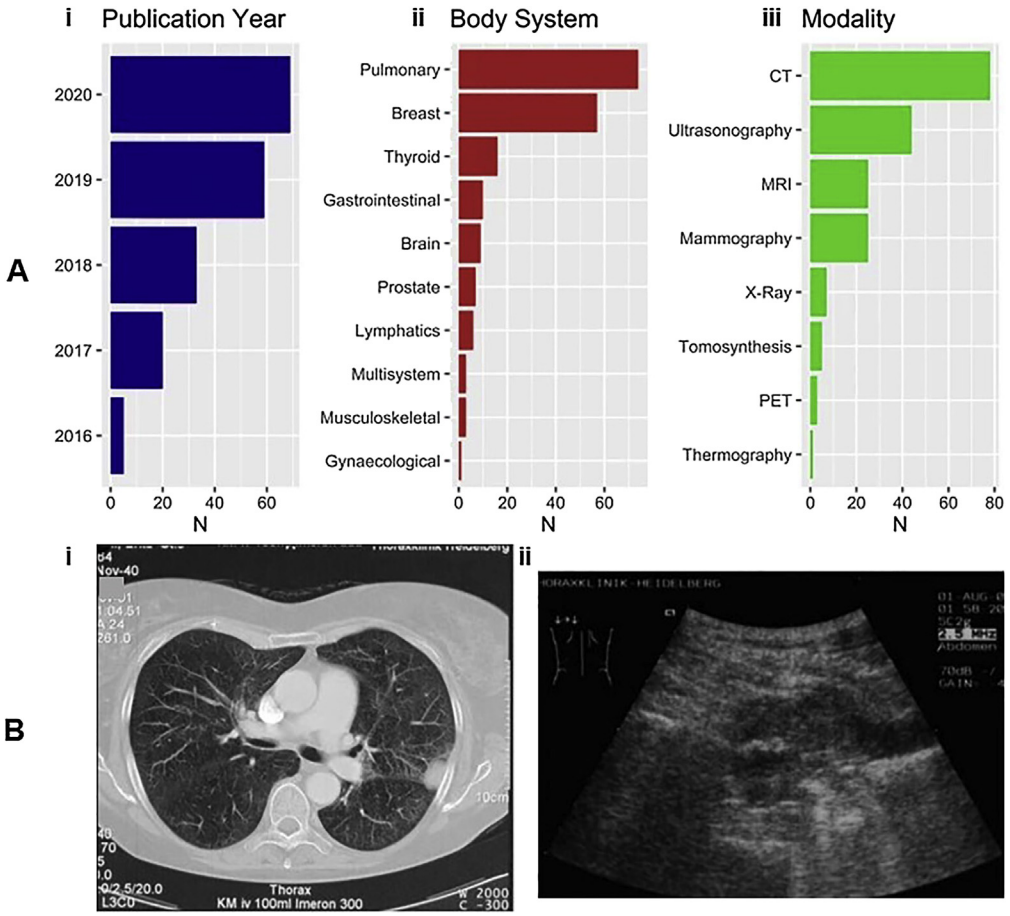

Figure 4.1 Imaging techniques for the diagnosis of cancer. (A) Distribution of articles from 2016 to 2020 according to the (i) publication year; (ii) body system imaged; (iii) imaging modality employed. (B) (i) CT scan and (ii) US images of lung cancer. *(Reprinted with permission from O'shea, RJ., et al. Systematic review of research design and reporting of imaging studies applying convolutional neural networks for radiological cancer diagnosis.* Eur. Radiol. *© 2021 Springer Nature and from Verkata et al., Ultrasound versus CT in detecting chest wall invasion by tumor.* Chest, 133*(4), 881–886 © 2008 The American College of Chest Physicians.)*

and the chances of the patient (Rainey, 2020). Radiologic examinations also present some level of discomfort to the patient, who could avoid some tests, and the selection of target lesions by the professional is liable to the human subjectivity (Sullivan et al., 2013). Cases of hematological cancer such as leukemia, on the other hand, need more invasive tests and gene expression analysis. But researchers have been inexhaustibly seeking molecular changes that can be addressed to the disease from early stages, avoiding invasive sample collections (Meggendorfer et al., 2020; Rashed et al., 2019).

It is known that the uncontrolled growth of cells with genome alteration can also leave a different mark on the sick organism at a molecular level. Following this

perspective, the race to find new molecular markers that can assist in diagnosing imaging has fueled the hope of new research and the people waiting for a precise diagnosis and an efficient treatment. Thanks to the evolution of omic sciences, a great number of oncometabolites have been discovered. A few researches, for example, suggest that alterations in the respiratory and energetic pathways of cells are related to the growth of tumor cells based on the discrepancy between the biochemistry composition of healthy and cancer cells (Seyfried & Shelton, 2010). Other researchers, however, join efforts to find characteristic compounds (biomarkers) to specific cancers, as the case of fumarate (renal cell carcinoma), succinate (paraganglioma), sarcosine (prostate cancer), glycine (breast cancer), glucose (most cancers), glutamine (myc-dependent cancers), serine (most cancers), asparagine (leukemia), choline (prostate, brain, breast cancer), lactate (most cancers), and polyamines (most cancers) (Wishart, 2015).

Additionally to the metabolites, evidence suggests that the individual's immune system with cancer produces antibodies against tumor-associated antigens, with both (antibody and antigen) being possibly used as disease biomarkers. Such biomolecules can be commonly obtained through blood collection, which is simple, rapid, and presents low invasiveness (Yadav et al., 2019). Cancer biomarkers, in general, are produced by the embryonic tissue or tumor tissue and indicate changes in the biological process related to protein expression. Fortunately, with the growing number of studies and the advance of bioanalytical tools, bioinformatics, and medicine evolution the range of biomarkers has been increasing considerably (Tothill, 2009). Prostate-specific antigen (PSA) is one of a series of examples of a biomarker used to detect cancer. The US Food and Drug Administration (FDA) approved in 1986 the PSA test to measure the protein levels in men's blood to monitor prostate cancer. According to the PSA blood levels, it is possible to estimate if the individual is healthy or with an indication of cancer since the levels of PSA increase substantially due to the disease. Nowadays, men above 45 years old are recommended to perform a screening test, and this practice is helping to reduce prostate cancer mortality (National Institutes of Health, 2021; Patasius et al., 2020; Sadi, 2013). Other proteic cancer biomarkers in clinical use include, for example, HER2 (breast cancer), alfa-fetoprotein (AFP) (nonseminomatous germ cell tumors), CA125 (ovarian cancer), and thyroglobulin (thyroid cancer) (Duffy, 2017; Peiris et al., 2019; Sino Biological, 2021). Although many others are not yet used as screening routinely, monitoring their levels could bring useful information about one's health and encourage further investigations on this field.

Other important classes of cancer biomarkers are the genetic ones. These include, for example, specific mutations in cancer-preventing tumor suppressor genes or cancer-promoting oncogenes. Important single-nucleotide mutants include BRCA1, BRCA2, RAD1, and CYP1A1 in breast cancer, XRCC1, p53, and ATM in the lung, head, and neck cancers, and PGS2 in lung cancer (Khailany et al., 2020). The dysregulated expression of microRNAs (miRNAs) in cancer cells has also been related to

important aspects of the disease, including evading growth suppressors, inducing angiogenesis, resisting cell death, and other biochemical processes, becoming an extensive topic of research and biomarkers identification (Peng & Croce, 2016). Some of the most common cancer-associated miRNAs include miRNA-205 (tumor suppressor in prostate, bladder, breast, and esophageal cancer and oncogenic for ovarian cancer), miRNA-21 (oncogenic for different types of cancer), and miRNA-155 (oncogenic for different types of cancer) (Farazi et al., 2012).

Lastly, cancer-related structures must be mentioned as another important set of biomarkers. These include, for example, circulating cancer cells themselves as well as exosomes. Exosomes are small vesicles secreted by eukaryotic cells that contain proteins, DNA, miRNA, and others, and they are essential for intercellular communication. Such content may play an important role in cancer development, including the regulation of tumor growth and metastasis, for example (Dai et al., 2020). Therefore, these can be used for aiding both in the diagnosis and prognosis of the disease.

This diversity of biomarkers for different types of cancer brings a bright new perspective in disease screenings, diagnosis, prognosis, disease recurrence monitoring, and treatment choices. The use of such biomarkers also enables simpler and more periodic testing, usually being performed in noninvasive body fluids like blood, urine, and saliva—unlike tissue biopsy samples (Jianping Li et al., 2012; Quinchia et al., 2020; Ray et al., 2011). Traditionally, immunoassays (e.g., ELISA) and PCR-based methods can be used to detect specific analytes. However, these are time-consuming procedures that require both specialized personnel and equipment to be performed. The desire for simpler, faster, and in situ diagnosis, especially for aggressive cancers, urges the scientific community to develop alternatives for the early detection of cancer biomarkers. Hence, there is a rise of biosensors that can search for cancer biomarkers in biological fluids in quick and straightforward measurements.

2. Biosensors

Biosensors, by definition, are devices capable of analyzing the chemical information from a sample and converting it to a measurable signal. These devices are constituted of two fundamental parts: the bioreceptor, which recognizes the analyte, and the transducer, which converts the biochemical interaction into a useful analytical signal (Bănică, 2012; Hulanicki et al., 1991) (Fig. 4.2). In the sequence, each part will be further detailed, including its implications and potential to aid in cancer diagnosis.

3. Transduction

The transduction component is the part responsible for converting the biochemical information into a physical measurement yielding an analytical signal. Transduction

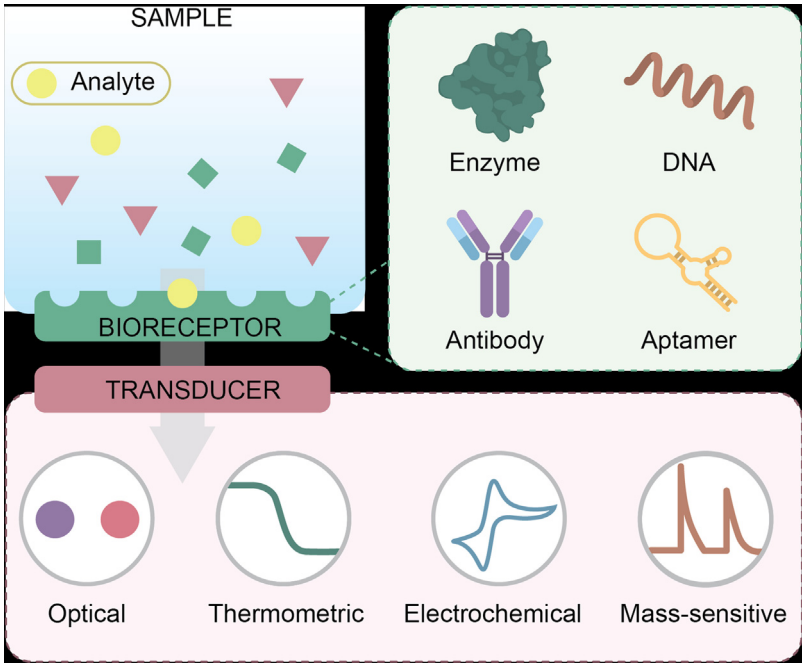

Figure 4.2 Fundamentals of a biosensor. The device comprises a biorecognition layer, responsible for interacting specifically with the analyte, and the transducer, responsible for converting the interaction into a measurable signal.

techniques can be divided into two main categories: physical and chemical transduction. In physical transduction, the signal is based on changes in physical properties when the bioreceptor interacts with the analyte. Changes in the mass, dielectric properties, or electrical resistivity can be measured and correlated to the analyte concentration. In chemical transduction, in turn, the signal relies on the change in the chemical composition, such as the concentration of the analyte or the product generated after the recognition process (such as for enzymes). In cases that the analyte or its product is not detectable, signaling labels can be used.

The operating system of the transducer can be classified as follows:

(a) **Optical**, in which the change of optical property is converted into a useful optical signal such as absorbance, reflectance, luminescence, fluorescence, refractive index, light scattering, etc.

(b) **Electrochemical**, based on the transport, distribution, and electron transfer reactions of the analyte at the solution–electrode interface. It can be subdivided into potentiometric, amperometric, and impedance-based transducers, for example.

(c) **Electrical**, which measures the change in the electrical properties upon the interaction of the analyte with the bioreceptor without any electrochemical event. Changes

in the electrical resistivity and dielectric constant, known respectively as resistive and capacitive transduction, are the most common.

(d) Mass-sensitive, which measures the change in the mass resulting from the accumulation of the analyte at the bioreceptor region.

(e) Magnetic, which assesses changes in magnetic properties or magnetically induced effects.

(f) Thermometric, in which the change of temperature is sensed during the recognition process of the analyte by the receptor.

For cancer diagnosis, optical, electrochemical, and mass-sensitive transducers are most commonly used. Mass-based biosensors have been reported to detect tumor cells (Bakhshpour et al., 2019) and protein biomarkers, for example (Su et al., 2017). However, mass-based transduction can lack sensitivity than other transduction techniques. Also, the detectors are expensive and hard to miniaturize. Optical transductions have gained interest in detecting cancer biomarkers with the development of nanomaterials, which improve the sensitivity of biosensors due to plasmonic interactions with electromagnetic waves. Commonly used optical transduction coupled with nanomaterials for the detection of cancer biomarkers are surface plasmon resonance (SPR), localized SPR (LSPR), and surface-enhanced Raman spectroscopy (SERS). Despite their sensitivity, these optical transductions are complex and challenging to miniaturize. On the other hand, electrochemical devices can be easily miniaturized, allowing portable diagnostic tools, giving fast results, and achieving sensitive detections. However, they can require the use of electrochemical labels, which can be a cumbersome step. More details on each transduction method are described in the Smart Materials section, delineated by the given examples of biosensors for cancer diagnostics.

4. Bioreceptor

In biosensors, the analyte is recognized explicitly by the bioreceptor, which carries binding sites to interact with the analyte. Due to high-affinity interactions inherent to biochemical processes, biosensors present an improved sensitivity compared to common chemical or physical sensors. The most common biosensing elements are enzymes, antibodies, nucleic acids, and aptamers. Other nontypical biological components that can be used to mediate the reception of the analyte are membrane receptors, microbes, organelles, whole cells, and tissues (Bănică, 2012; Hasan et al., 2014; Brazaca et al., 2016).

5. Enzymes

Enzymes are proteins with a catalytic function in biochemical reactions and have specific binding sites to their substrates. Upon binding, the enzyme catalyzes the chemical reaction of the substrate. Then, the formed product is released from the enzyme, which is

ready for another reaction with a new substrate. The most successful commercial biosensor is the glucose meter, which uses the enzyme glucose oxidase to recognize its substrate glucose and catalyze a measurable oxidation reaction. Enzyme-based biosensors can perform continuous measurement since the enzyme returns to its active state after catalyzing the substrate chemical reaction. However, enzyme activity is susceptible to the chemical environment and is dependent on pH, ionic strength, and temperature as well, which restricts its application conditions (Hasan et al., 2014; Lorigan et al., 2016).

Although enzymes interact with proteins and genetic materials, their interactions are usually generic with no specificity upon cancer biomarkers. Therefore, enzymes are mainly used as a signal amplification strategy rather than a recognition element in cancer diagnostic biosensors. However, generic cancer biomarkers such as hydrogen peroxide (H_2O_2), which is known to be overproduced by tumor cells, can be easily monitored by peroxidase enzyme-based biosensors (Crulhas et al., 2017). Glucose, which is overly consumed by cancer cells, can also be monitored rapidly, simply, and low-costly using electrochemical biosensors based on the enzyme glucose oxidase (Sempionatto et al., 2019).

6. Antibodies

Antibodies are glycoproteins important to the immune system to neutralize and eliminate extraneous molecules and organisms, known as antigens (Lipman et al., 2005). Antibodies and antigens present complementary shapes and chemical reactivities, forming antibody/antigen complexes with extremely high specificity and affinity. Because of the high affinity of the association complex, antibodies have been widely used in the diagnostic field, especially for infectious disease diagnoses, such as AIDS (Guillon et al., 2018), and most recently in COVID-19 (Li, Ji, et al., 2020). Another commercial success is the home pregnancy test, which employs the antibody to recognize human chorionic gonadotropin (hCG), a hormone related to pregnancy.

Despite the highly specific detection using antibodies, the association complex can often be irreversible, which restricts to a one-measurement sensor. However, mechanisms of regeneration of an immunosensor have been reported in the literature. The regeneration of the antibody binding sites consists of overcoming the attractive forces between the antibody and the antigen, which can be achieved by changing the pH out of the antibody isoelectric point (pI) (Loyprasert et al., 2008). The pH modification must be a delicate procedure since extreme pHs cause severe protein denaturation, therefore inactivating the antibodies and reducing the specificity and sensitivity of the biosensor (Goode et al., 2015). Mild detergents or organic solvents can also be used in addition to pH alteration to enhance the regeneration of antibody binding sites (Kandimalla et al., 2004).

With the advent of high-throughput biotechnology, the production of antibodies expands toward in vitro manufacturing over the animal immunization process, decreasing the production cost, increasing the specificity, and providing better batch-to-batch

reproducibility over animal-derived antibodies (Bradbury et al., 2011). Synthetic antibodies include recombinant proteins, consisting of monoclonal antibodies, and nonimmunoglobulin protein scaffolds, such as affibodies, peptide aptamers, affimers, Darpins, Anticalins, and more (Ferrigno, 2016; Škrlec et al., 2015). It is interesting to know the difference between them. Nonimmunoglobulins scaffolds are smaller proteins than antibodies, translating to a higher packing density on the biosensor surface. Moreover, the smaller size contributes to bringing any label (for example, a redox pair for electrochemical transduction) closer to the sensing surface and improving the sensitivity of the biosensor. Nonimmunoglobulins scaffolds have been reported in the literature as bioreceptor to recognize cancer biomarkers, such as affimer (Zhurauski et al., 2018) and affibody specific for human epidermal growth factor receptor 4 (HER4), and emerging biomarker for detection of gastrointestinal stromal tumors (Ilkhani et al., 2016; Ravalli et al., 2015).

In this context, antibodies are mostly suitable for detecting protein cancer biomarkers, and they have already been used to detect carcinoembryonic antigen (CEA) (colorectal cancer), cancer antigen 125 (CA125) (nonspecific), cancer antigen 15−3 (CA15-3) (breast cancer), alpha-fetoprotein (AFP) (nonspecific), PSA (prostate cancer), human epidermal growth factor receptor 2 (HER2) (breast cancer), and insulin-like growth factor 1 (IGF) 1and2 (nonspecific) (Jayanthi et al., 2017).

7. Nucleic acids

When the target is a nucleic acid, the respective sensor receives the name of genosensor. Nucleic acids are strings of nucleotides, which are composed of nucleobases, namely adenine (A), cytosine (C), guanine (G), and thymine (T) for DNA, and uracil (U) replacing thymine for RNA. The nucleobases interact specifically with each other through hydrogen bonds forming the pairs: G-C, A-T, or A-U. The specific pairing allows the association of two complementary nucleic acid strands forming the double-strand complex in a hybridization process (Bănică, 2012). Genosensors utilize the hybridization process to detect complementary sequences. The change of a single nucleobase in one of the strands influences the response to recognizing the target nucleic acid sequence, contributing to the high specificity of hybridization (Brazaca, Bramorski, et al., 2017). Hybridization is a reversible process, allowing the regeneration of the target recognition component and the reusability of the biosensor. The regeneration is accomplished by denaturing the double-strand complex, which can be achieved with temperature variation or in a basic solution, as performed in an electrochemical genosensor for BRCA1 (Shahrokhian & Salimian, 2018).

DNA and RNA are easily designed and produced at low costs for a variety of applications, making them a versatile bioreceptor. Genosensors are commonly applied in clinical diagnosis to detect genetic anomalies and pathogen microorganisms (Canniatti Brazaca et al., 2021). However, the small amount of genetic material in samples limits

the application and it usually needs to precede an amplification step. Genosensors are especially suitable for detecting oligonucleotide-type cancer biomarkers, such as microRNA and cancer susceptibility genes.

8. Aptamers

Aptamers are nucleic acid molecules mainly engineered through the SELEX process (Systematic Evolution of Ligands by Exponential enrichment) designed to associate with the desired target molecule (Song et al., 2008). Shortly, the SELEX process enables the synthesis of a biorecognition component to interact with small molecules and full microorganisms previously hard to be recognized by antibodies (Binning et al., 2012).

The single-strand sequence folds into various structures and its conformational shape functions as a pocket to bind the target molecule through affinity reactions rather than hybridization. Therefore, aptamer-based sensors are often compared to immunosensors rather than genosensors. The production of aptamers is significantly easier and cheaper than the process for antibodies (Binning et al., 2012). Also, aptamers are smaller molecules than antibodies, which translates to a more densely functionalized sensor, increasing the number of binding sites for the target molecule. Although its characteristics are promising, the aptamer is still a recent technology. Thus, there is a limited knowledge of its structural and biochemical mechanism (Binning et al., 2012; Song et al., 2008).

In the literature, there are aptamer sequences developed and described for several cancer biomarkers such as CEA (nonspecific), AFP (nonspecific), CA125 (nonspecific), PSA (prostate cancer), HER2 (breast cancer), Michigan Cancer Foundation-7 (MCF7) (breast cancer), Mucin 1 (breast cancer), vascular endothelial growth factor 165 (VEGF165) (nonspecific), protein tyrosine kinase 7 (PTK7) (nonspecific), interleukin-6 (nonspecific), cancer exosomes, and mi-RNAs, which can be used for the construction of biosensors for aiding in cancer diagnosis (Negahdary, 2020).

9. Application of smart materials in biosensors for cancer diagnosis

Improving the performance of biosensors is a constant goal in analytical chemistry, allowing the achievement of lower limits of detection (LODs), higher sensitivities, reduced biofouling, improved stability, and rapid analysis. Among the numerous strategies applied, the use of smart materials stands out due to their versatility, compatibility with biomolecules, and improved performance. Smart materials are characterized by responding to one or more *stimuli* reversibly, which can be chemical, biological, electrical, optical, thermal, magnetic, or mechanical (Erdem et al., 2021).

Currently, the most used smart materials in the development of biosensors are hydrogels and nanomaterials such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), graphene, and quantum dots (QDs). Other promising smart materials for biosensing

applications include photonic crystals and molecularly imprinted polymers (MIPs) (Guo et al., 2020). While polymeric-based materials present great flexibility, lightweight, mass-producibility, and transparency, their mechanical strength and stability are low. Carbon-based nanomaterials, however, are electrically and thermally conductive besides being mechanically strong and flexible, and biocompatible (Erdem et al., 2021).

Following, different smart materials and their properties will be presented and discussed along with their application in biosensors for cancer diagnosis. It is important to notice that smart materials have interesting characteristics not only for the development of biosensors but also for drug delivery, tissue engineering, tumor-on-chip, and many others—detailed information can be found in different chapters of this book.

10. Gold nanoparticles

One of the most versatile smart materials for constructing biosensors is AuNPs. These tiny particles of gold with diameters typically ranging from 1 to 100 nm present unique properties for biosensing, such as large surface-to-volume ratio, excellent biocompatibility, and optical properties related to their size, shape, surface chemistry, and aggregation state (Yeh et al., 2012). Furthermore, AuNPs can catalyze numerous redox reactions and present low charge transfer resistance, being extremely useful for the construction of electrochemical biosensors. These nanomaterials also present a tunable and expressive SPR peak (500−550 nm) (Jain et al., 2006), which is extremely sensitive to the nanoparticle's microenvironment and can quench fluorescence, making AuNPs also a valuable tool for optical devices (Yeh et al., 2012).

Furthermore, AuNPs have attracted significant attention from the scientific community due to their simple synthesis, which is most commonly performed by chemical reduction. The use of different reduction (sodium citrate, sodium borohydride, and others) and stabilizing agents (polymers, citrate, alkanethiols, and others) can be used to produce nanoparticles with the desired characteristics (Brazaca, Ribovski, et al., 2017; Yeh et al., 2012). AuNPs surfaces are easily modified, improving their stability, monodispersity, and biocompatibility. Functional groups, biomolecules, or different nanomaterials can also be attached to the AuNPs, opening space to countless applications and special relevance in different research areas (Chen et al., 2017).

Currently, many strategies report the use of AuNPs for aiding in cancer diagnosis. Among the ones reported, colorimetric proposals stand out due to their simplicity and commonly dispensing specialized instrumentation. An important example of this approach was reported by Xiao et al. They have used AuNP-decorated Bi_2Se_3 nanosheets for the simple detection of carcinoembryonic antigen, a biomarker for colorectal cancer (Xiao et al., 2017) (Fig. 4.3A). In this case, Bi_2Se_3 nanosheets were chosen as a synergist with AuNPs due to their low redox potential, serving as an electron donor (Cao et al., 2015), and their topological insulating properties, making electron transfer

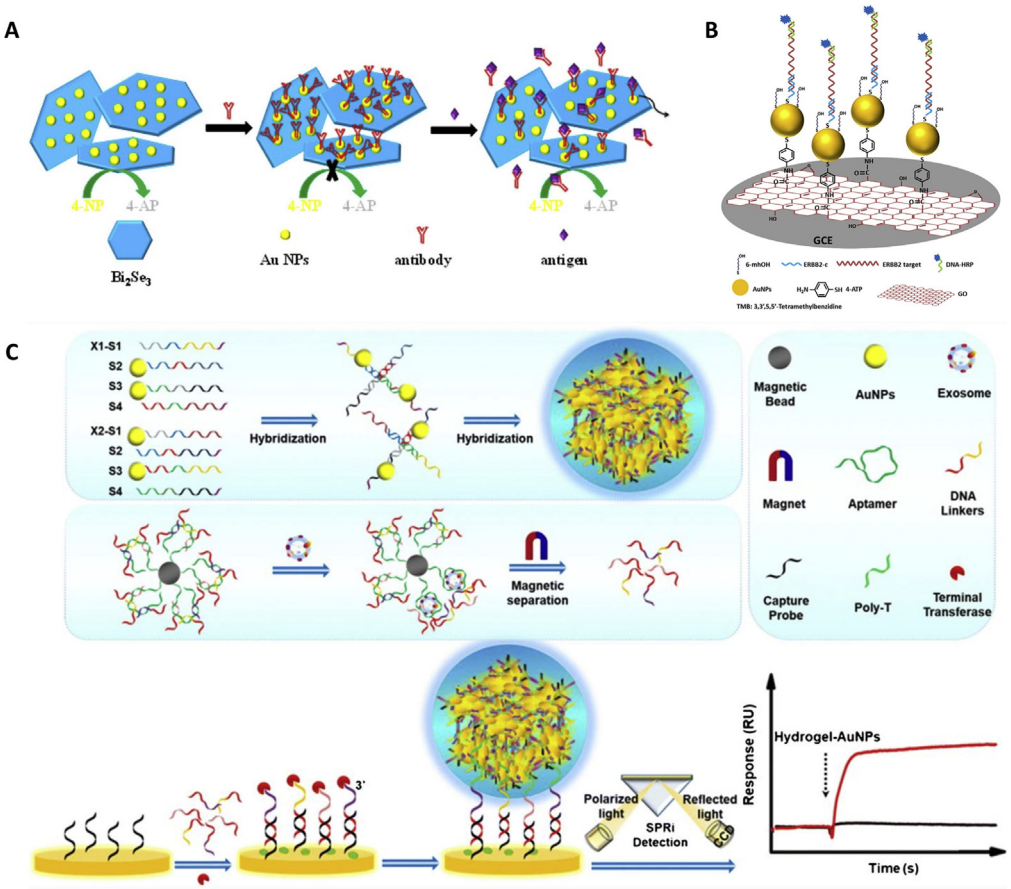

Figure 4.3 AuNPs-based devices for the diagnosis of cancer. (A) AuNP-decorated Bi_2Se_3 nanosheets modified with specific anticarcinoembryonic antibodies can catalyze the reaction of 4-NP to 4-AP in the presence of the analyte, but not in its absence. (B) AuNPs used for anchoring specific ssDNA for the detection of genetic cancer biomarkers ERBB2c and CD24c. (C) The use of a complex of hydrogel, AuNPs, and DNA to improve the detection of prostate cancer-derived exosomes using SPR. *(Reprinted with permission from Xiao et al., Colorimetric biosensor for detection of cancer biomarker by Au nanoparticle-decorated Bi_2Se_3 nanosheets.* ACS Appl. Mat. Interfaces, *9(8), 6931−6940, © 2017 American Chemical Society, Saeed et al. DNA biosensors based on AuNPs-modified graphene oxide for the detection of breast cancer biomarkers for early diagnosis.* Bioelectrochemistry, 118, *91−99, © 2017 Elsevier BV and Chen et al. (2020). Surface plasmon resonance biosensor using hydrogel-AuNP supramolecular spheres for determination of prostate cancer-derived exosomes.* Microchimica Acta, 187, *590, © 2020 Springer Nature.)*

occur between the surface of the material and the catalyst (Hong et al., 2012; Zhang et al., 2009; Zhang et al., 2010). Due to such properties, the catalytic activity of AuNPs is considerably increased. Furthermore, the nanosheet provides a great superficial area for the deposition of AuNPs without aggregation. The combined AuNPs-nanosheets

showed great catalytic activity for reducing 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) by $NaBH_4$, displaying a color change visible to the naked eye, from bright yellow to colorless. When antibodies specific to the biomarker are absorbed in the surface of the nanocomposite, in its turn, its catalytic activity is hampered and no visible color change is observed. However, if the analyte is present, the antibodies are released from the surface, reestablishing the catalytic properties and causing a visible color change correlated with the biomarker concentration. The detection performance of the developed technique was compared to ELISA, the gold standard method, in both positive and negative controls. Furthermore, using AuNPs is simpler and more rapid than the traditional technique, presenting significant advantages for improving the diagnosis of colorectal cancer.

As previously mentioned, electrochemical approaches can also greatly benefit the use of AuNPs for improving its analytical performance, including features such as increased electron transfer capabilities and the achievement of simpler and higher density bioreceptors loadings. An example of this was reported by Saeed et al., who developed genosensors based on AuNPs-modified graphene oxide (GO) for the detection of the breast cancer biomarkers ERBB2c and CD24c (Saeed et al., 2017) (Fig. 4.3B). In this case, AuNPs-modified GO was first immobilized at the surface of a glassy carbon electrode (GCE), serving as an anchoring point for specific thiolated capture sequences for detecting the desired genetic mutations. After target hybridization, a report sequence labeled with HRP was allowed to interact with the biosensor, and the generated signal was monitored by chronoamperometry. The use of the combination of such smart materials generated a clear signal enhancement, allowing the achievement of LODs in subnanomolar ranges, which is adequate for the analysis of clinical samples and the early screening of breast cancer. Therefore, the platform may serve as a rapid (1 h) and sensitive method for aiding in diagnosing the disease, only requiring simple and compact instrumentation.

A different example of the use of AuNPs for aiding in cancer diagnosis was recently published by Chen et al. The authors, in this case, developed an SPR biosensor using hydrogel-AuNPs supramolecular spheres for the detection of prostate cancer-derived exosomes (Chen et al., 2020) (Fig. 4.3C). The sensor design principle is based on three separate lines: (1) magnetic beads were modified with specific aptamers—DNA linker complexes. DNA linkers were released and purified using magnetic separation when the sample containing the desired exosomes interacted with the aptamers present in the modified magnetic beads. (2) The purified DNA linkers were allowed to hybridize with a capture sequence present in a modified surface and the enzyme terminal transferase catalyzed the formation of poly-A tails. (3) Such tails were used to hybridize with a complex composed of hydrogel, DNA sequences, and AuNPs, used for signal amplification. The presence of such complexes at concentrations proportional to the ones presented by the prostate cancer—derived exosomes in the original sample was detected using SPR with great sensitivity due to the amplification of the mass cumulative hydrogel and the localized SPR effect of the AuNPs. The use of clinical serum samples from volunteers

allowed a clear differentiation between healthy and diseased subjects, showing great specificity and validating the developed technique. Although being extremely complex, the method was able to illustrate some of the capabilities of enhancing the analytical features of biosensors for cancer diagnosis using smart materials.

11. Graphene

Graphene is the thinnest material known to men and the building block for graphitic materials, consisting of a one-atom-thick sheet with planar sp^2-bonded carbon in a honeycomb shape. Single layers of graphene display many fascinating properties that can be explored for improving the analytical performance of biosensors, including high surface area (theoretically 2630 m^2/g) (Shao et al., 2010), excellent thermal (4840−5300 W/mL at room temperature) (Balandin et al., 2008) and electric conductivity (up to 10^8 mS/cm) (Bahadır & Sezgintürk, 2016), and high mechanical strength (tensile strength of 130 GPa) (Lee et al., 2008). Graphene also exhibits a broad electrochemical window (2.5 V in 0.1 M of PBS) (Shao et al., 2010), a low charge transfer resistance, and it is transparent, being adequate for electrical, electrochemical, and optical biosensors (Pumera, 2011). The nanomaterial is also low cost and presents a low environmental impact.

Due to challenges for the mass production of graphene, valuable graphene derivatives such as graphene oxide (GO) and reduced graphene oxide (rGO) have attracted significant attention from the scientific community. GO presents several oxygen-containing functional groups (carboxyl, hydroxyl, carbonyl, and epoxide) in a disrupted sp^2 structure. Depending on the degree of oxidation, GO shows a semiconductive or insulating behavior, decreased surface area, and increased hydrophilicity, forming stable solutions in a wide range of concentrations. The chemical, thermal, or photo-thermal reduction of GO, in turn, generates rGO. This process removes part of the oxygen-containing functional groups and partially restores the sp^2 structure, increasing its electric conductivity, surface area, and hydrophobicity while lowering its dispersibility (Nanografi, 2021). As rGO still presents abundant structural defects and functional groups, it is extremely promising for biosensing applications (Shao et al., 2010).

To be applied in biosensing, graphene or its derivatives are commonly modified. The covalent or noncovalent immobilization of biomolecules, for example, can be performed to provide specificity to the desired biomarker. While the direct adsorption of bioreceptors is a simple and straightforward strategy, it is prone to degradation over time. The covalent immobilization using available functional groups, on the other hand, generates more stable complexes but also disrupts graphene structure, affecting its electrical and mechanical properties (Jin et al., 2020).

Diverse important examples can be found in the literature regarding the application of this smart material in cancer diagnosis to improve the analytical performance of biosensors. Mao and colleagues, for example, have reported a label-free electrochemical

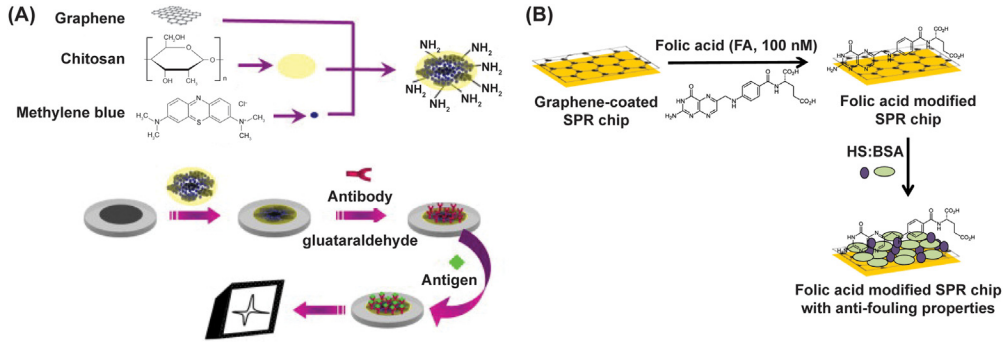

Figure 4.4 Graphene-based devices for the diagnosis of cancer. (A) Electrodes modified with graphene, chitosan, and methylene blue are used to efficiently immobilization specific anti-PSA antibodies, allowing the sensitive electrochemical detection of the target. (B) Folic acid was immobilized in graphene-coated SPR chips, with HS and BSA being used for the generation of an antifouling surface—an important feature for the detection of FAP in real samples. *(Reprinted with permission from Mao et al. Label-free electrochemical immunosensor based on graphene/methylene blue nanocomposite.* Analytical Biochemistry, 422*, 1, 22–27, © 2012 Elsevier BV and He et al. Label-free femtomolar cancer biomarker detection in human serum using graphene-coated surface plasmon resonance chips.* 2D Materials in Biosensors and Bioelectronics, 89*(Part 1), 606–611, © 2017 Elsevier BV.)*

immunosensor for the detection of PSA (Mao et al., 2012) (Fig. 4.4A). The authors have modified the surface of a glassy carbon electrode (GCE) with a nanocomposite film of graphene sheets (GS), chitosan, and methylene blue (MB). Then, glutaraldehyde was used as a cross-linker for the covalent immobilization of specific anti-PSA antibodies through available $-NH_2$ groups. To block nonspecific binding sites and provide greater specificity, bovine serum albumin (BSA) was used. The antigen–antibody binding was then precisely quantified using amperometry, which provided a linear response in PSA concentrations, including clinical relevant ranges (in the order of ng/mL). A low LOD of 13 pg/mL was achieved, and it is mainly attributed to the large specific area of GS—which contributes to its ability to absorb a great number of MBs and to increase the amount of antibody on the surface of the electrodes, and the high electroactivity of the GS–MB–chitosan nanocomposite. The biosensors were tested in serum samples, showing great recovery rates and great specificity to PSA. The simple instrumentation required by the devices combined with its rapid response is essential for increasing the accessibility of PSA testing, especially in regions with low resources.

Graphene and its derivatives can also be applied to achieve better performance for improving genetic identification by its enhanced electronic and optical properties. Shahrokhian and coworkers, for example, have applied rGO for the ultrasensitive electrochemical detection of the genetic breast cancer biomarker BRCA1 (Shahrokhian & Salimian, 2018). In this case, an electrochemical procedure was applied to reduce GO and for the polymerization of pyrrole-3-carboxylic acid (P3CA) monomers into

GCEs. Following, the cross-linkers EDC and NHS were applied as cross-linkers to react with the carboxyl groups in the polymeric surface and an amino-labeled specific BRCA1 capture DNA sequence was immobilized. Lastly, nonspecific binding was reduced by blocking with ethanolamine. The use of differential pulse voltammetry (DPV), a simple and rapid electrochemical detection technique, along with the modified electrode, allowed the achievement of an ultralow LOD of 3 fM and the discrimination between complementary, noncomplementary, and mismatch DNA sequences. The developed device presented other important features for aiding in the clinical diagnosis of breast cancer, including its good performance in human serum, rapid results (1 h), and its simple reusability ($n = 4$), eliminating the need for constant complex assembly of the genosensor. Similar to the previous example, the great performance of the developed device was assigned to the large surface area of rGO, hence the increased electron transfer kinetics and the increased potential of capture DNA loading into the electropolymerized surface.

While graphene presents interesting properties for electrical and electrochemical biosensors, including its large surface area, fast electron transfer, fast response time, and reduced surface contamination, it also presents detecting relevant properties for optical devices, including high spatial resolution, wide and deep detection range, high sensitivity, and the possibility of analyzing unlabeled samples (Zongwen Li, Ji, et al., 2019). Recently, He and colleagues reported a label-free femtomolar detection of folic acid protein (FAP) (or folate receptors), a biomarker for the early detection of cancer, using graphene-based SPR chips (He et al., 2017) (Fig. 4.4B). In this case, folic acid was integrated through πstacking on graphene-coated SPR chips. A mixture of concentrated human serum and BSA was applied to create a highly antifouling interface, allowing adequate response even in complex biological samples. The combination of the nanomaterial with an antifouling surface allowed the reaching of a femtomolar LOD, a promising feature for clinical analysis of FAP biomarkers. The long-term storage (20 days) and possible regeneration of the sensor surface are also important features for its application in real scenarios, representing an important alternative for aiding in early cancer diagnosis.

12. Carbon nanotubes

Carbon nanotubes (CNTs) are hollow cylindrical structures with one or more concentric walls composed of hexagonal lattices of carbon atoms. While its diameter can be as small as a nanometer, its length can reach several centimeters (Tîlmaciu & Morris, 2015). These nanomaterials can be categorized in single-walled CNTs (SWCNTs), which can be thought of as a single graphene sheet rolled up, or multiwalled CNTs (MWCNTs), formed by rolling up multiple graphene sheets. SWCNTs can also be defined by their chiral indices (n, m), being mostly chiral ($m \neq n$) but also presenting armchair ($m = n$) and zigzag ($m = 0$) configurations. The outer walls of MWCNTs can shield

inner walls from performing chemical interactions, but both categories present similar general properties (Eatemadi et al., 2014).

The ordered arrangement of the carbon atoms of CNTs via sp_2 bonds gives unique electrical, mechanical, and optical properties to this smart material, which is the stiffest and strongest fiber known to date. High electrical conductivity, increased optical absorption, great thermal stability, and elasticity are some of the features that receive great attention from the scientific community. In special, its large surface area, great chemical stability, high electrocatalytic effect, and fast electron-transfer rate are attractive properties for the construction of electrochemical biosensors (Eatemadi et al., 2014; Tîlmaciu & Morris, 2015).

CNTs can also be modified in their outer and/or inner walls (hexahedral and endohedral functionalization, respectively). Outer modifications are simpler and more common than inner ones, being used to add functional groups to aid in nanoparticle solubilization and prevent aggregation or for the immobilization of bioreceptors, for example. Like graphene, noncovalent interactions allow CNTs to retain their original properties, while covalent interactions disrupt the nanomaterial structure and can seriously influence its features. Usually, oxidative treatments are applied to generate carboxylic groups and defects on the nanomaterial for performing covalent interactions. Other interesting properties of CNTs to be considered when developing biosensors for diagnosis include their ability to penetrate the cellular membrane, allowing the development of tools for investigating the inner portion of cells (Tîlmaciu & Morris, 2015).

Due to CNTs interesting properties that can significantly improve the analytical performance of biosensors, diverse examples of its use can be found in cancer diagnosis. Thapa et al. have applied a CNTs matrix for the highly sensitive detection of pancreatic cancer biomarker CA19-9 (Thapa et al., 2017) (Fig. 4.5A). For that, polyethyleneimine and MWCNTs were combined in a thin film on interdigitated gold electrodes. Specific anti-CA19-9 antibodies were then immobilized on the surface of CNTs using the cross-linkers EDC/NHS. In this case, the use of CNTs may increase the number of bioreceptors present on the biosensor surface and improve its electrical properties, providing greater sensitivity. The analyte was later detected using impedance spectroscopy, and the device presented a LOD of 0.35 U/mL. The biosensor demonstrated great potential for clinical use, detecting a wide range of concentrations including the standard benchmark (cancer positive samples >37 U/mL) in a much more rapid and simple manner than traditional techniques. Furthermore, the use of real blood samples from patients in different health conditions was adequately categorized, displaying low interferences even in complex biological samples.

An example of the use of CNTs to detect genetic cancer biomarkers, in its turn, was reported by Fayazfar et al. (Fayazfar et al., 2014) (Fig. 4.5B). The authors have developed

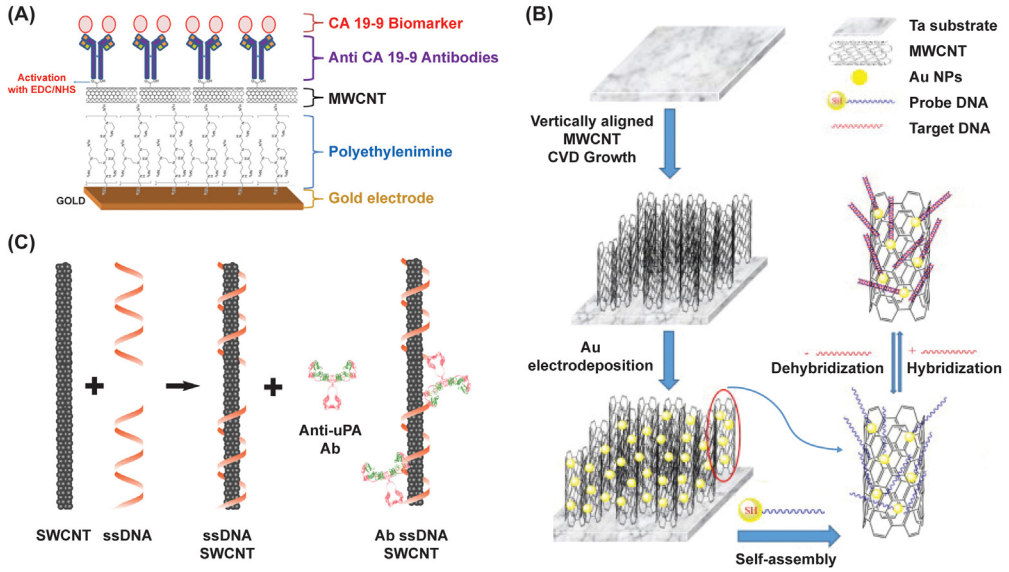

Figure 4.5 CNTs-based devices for the diagnosis of cancer. (A) CNTs attached to gold electrodes used for the efficient immobilization of anti-CA 19-9 pancreatic cancer biomarker. (B) The generation of vertically aligned MWCNTs modified with AuNPs for the immobilization of specific ssDNA and precise electrochemical detection of mutations in TP53 gene. (C) Modification of SWCNTs with ssDNA and anti uPA antibodies for the fluorescent detection of the cancer biomarker. *(Reprinted with permission from Thapa et al. Carbon nanotube matrix for highly sensitive biosensors to detect pancreatic cancer biomarker CA19-9.* ACS Applied Materials and Interfaces, *9(31), 25878–25886, © 2017 American Chemical Society, Fayazfar et al., DNA impedance biosensor for detection of cancer, TP53 gene mutation, based on AuNPs/ aligned CNTs modified electrode.* ACS Applied Materials and Interfaces, 836, *34–44, © 2014 Elsevier BV and Williams et al., A fluorescent carbon nanotube sensor detects the metastatic prostate cancer biomarker uPA.* ACS Sensor, 3*(9), 1838–1845, © 2018 American Chemical Society.)*

an electrochemical biosensor to detect mutations in the TP53 gene, a popular target in cancer research. For this, vertically aligned MWCNTs grown by chemical vapor deposition (CVD) were decorated with electrodeposited AuNPs. Then, thiol-modified specific single-stranded DNA sequences (ssDNA) were immobilized on the AuNPs, being available for hybridizing with the target sequence. The detection and quantification of TP53 were then performed by electrochemical impedance spectroscopy (EIS), with charge transfer resistance (R_{ct}) linearly increasing with higher target concentrations. The use of two combined smart materials in optimized conditions allowed the device to achieve an extremely low LOD, of 10 amol/L. Important features for the clinical use of the device were also assessed, including its long-term stability (2 weeks), reusability (4 times), and specificity (single mismatch). Therefore, the device presents great potential for clinical applications.

As previously mentioned, CNTs also present interesting properties for improving optical biosensors, including high photostability and modulable fluorescence (Sireesha et al., 2018). Williams et al. took advantage of such properties to design a fluorescent carbon nanotube sensor for the detection of urokinase plasminogen activator (uPA), a metastatic prostate cancer biomarker (Williams et al., 2018) (Fig. 4.5C). For that, the authors have involved SWCNTs with amino-modified single-stranded DNA. This functional group, in turn, was used for the covalent immobilization of anti-uPA. The presence of the biomarker caused a red-shift in the near-infrared fluorescence of the modified CNTs, with the intensity of changes being dependent on the chiral vectors of the applied nanomaterials. The authors attributed the red-shifting to an increase in the local charge density due to the presence of the biomarker. After the passivation of the CNTs surface with BSA, the developed system was successfully applied to complex samples. However, to aid in the diagnosis of cancer, further work regarding the improvement in LOD needs to be carried out. While uPA in serum may range up to 17 nM, the LOD of the developed system was 25 nM. Alternatives include the use of more sensitive, specific-chirality CNTs, site-directed antibody immobilization, and the increase of bioreceptors in the nanomaterial surface.

13. Hydrogels

Hydrogels are 3D networks composed of hydrophilic polymers that can retain large amounts of water (Ahmed, 2015). The presence of hydrophilic functional groups, such as amide, amino, carboxyl, and hydroxyl groups, in the polymer chain enables the absorption of water by ionization of these groups. The absorption of water by the hydrogel induces its swelling, turning into the gel state without compromising the structural integrity. Water availability tunes the swelling and syneresis (deswelling) processes of a hydrogel (Ferreira et al., 2018).

For biosensing applications, biocompatible hydrogels are preferable since biological components, such as the bioreceptors, can be easily incorporated into the matrix. Some of the hydrogels commonly used as biosensing platforms are polyvinyl alcohol, polyethylene glycol, polyacrylates, natural hydrogels (such as alginate, agarose, cellulose, chitosan, chitin, dextran), and electroconductive hydrogels such as polypyrrole, polyaniline, and poly(ethylenedioxy thiophene). Electroconductive hydrogels are particularly interesting for electrical and electrochemical sensing applications since the electron transport is facilitated by the charged functional groups in the polymeric structure as well as the greater diffusivity provided by the large surface area due to the presence of porous. Also, their antifouling properties contribute to the use of electrodes in these transduction methods (Tavakoli & Tang, 2017).

Smart or intelligent hydrogels are capable of responding *reversibly* to external stimuli besides water availability. The reversibility of the smart hydrogel is an attractive characteristic for biosensing applications since it enables the reuse of the sensor. The external stimuli to which smart hydrogel response can be classified as physical, chemical, and biological. Physical stimuli consist of temperature, light, electric fields; chemical stimuli consist of pH, ionic strength, solvents, electrochemical field; and biological stimuli relate to molecular recognition and/or biological interaction by receptors (Samal et al., 2014). For sensing cancer biomarkers, smart hydrogels that respond toward biological stimuli are particularly interesting to achieve specific and sensitive biosensors.

The binding of the molecule of interest leads to molecular reorientation or displacement events in the polymer matrix producing useful and detectable signals (Culver et al., 2017). Since hydrogel interacts with biological components at a molecular level (Tavakoli & Tang, 2017), bioreceptors can be easily immobilized into the matrix for molecular recognition of cancer biomarkers.

DNA strands can be incorporated into the hydrogel matrix, known as DNA hydrogels, which combine the skeleton function of a hydrogel with the biological function of the DNA (Liu et al., 2018). Liu et al. utilized a DNA hydrogel to detect microRNA miR-21, a lung cancer genetic biomarker (Fig. 4.6A). Two DNA strands grafted onto linear polyacrylamide and a third DNA sequence (probe) specific to miR-21 were used as a cross-linker for the DNA hydrogels. Upon hybridization, the polymer solution transformed into the gel state, which was later immobilized on the ITO electrode. The recognition probes were previously tagged with ferrocene, an electrochemical label monitored by DPV. In the presence of the miR-21, the hybridization of the probe with the target DNA released the polyacrylamide chains and the hydrogel structure collapsed. Upon sequential washing of the electrode surface to remove any physically adsorbed molecule, there was a decrease in the signal from ferrocene, which correlated with the concentration of miR-21. The DNA hydrogel biosensor achieved a LOD of 5 nM and proved to be selective when tested against highly homologous sequences to miR-21. The miR-21 biosensor also showed good stability when stored in a vacuum box at 4 °C, up to 85.67% after 14 days of storage, indicating it can be promising for early lung cancer diagnosis (Liu et al., 2018). A similar approach integrated with surface-enhanced Raman spectroscopy (SERS) was used for multiplex analysis of 9 microRNA (miR-21, miR-221, miR-224, miR-205, miR-155, miR-141, miR-25, miR-18, and miR-183), allowing the diagnosis for several types of cancer (Si et al., 2020).

DNA hydrogels can incorporate aptamers instead of DNA probes for the detection of protein biomarkers. However, aptamer's functionality resembles more like an antibody's; the nucleotide composition functions as a cross-linker to structure the hydrogel scaffold, just like the DNA probes. The replacement of DNA probes with aptamers widens the

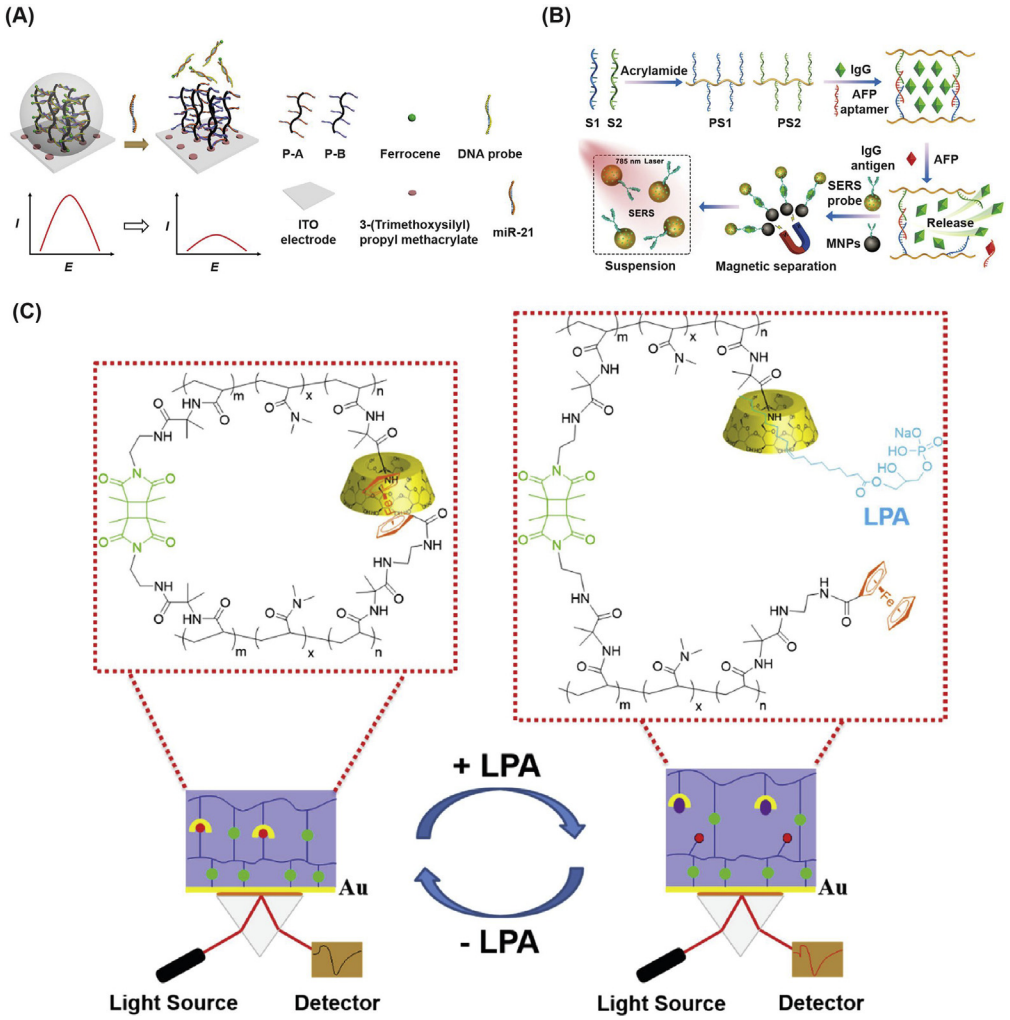

Figure 4.6 Hydrogel-based devices for the diagnosis of cancer. (A) DNA Hydrogel with DNA probe for sensing of miR-21, lung cancer biomarker. (B) DNA hydrogel with aptamers for optical detection of AFP, a biomarker for hepatocellular carcinoma. (C) Dually cross-linked hydrogel functionalized with β-cyclodextrin to detect ovarian cancer biomarker LPA. *(Reprinted with permission from Liu et al., Manufacturing of an electrochemical biosensing platform based on hybrid DNA hydrogel: Taking lung cancer-specific miR-21 as an example.* Biosensors and Bioelectronics, 103*, 1—5, © 2017 Elsevier BV, Wang et al., (2020). Preparation of aptamer responsive DNA functionalized hydrogels for the sensitive detection of α-fetoprotein using SERS Method.* Bioconjugate Chemistry, 31*(3), 813—820, © 2020 American Chemical Society and Li et al., (2020). Dually crosslinked supramolecular hydrogel for cancer biomarker sensing.* ACS Applied Materials and Interfaces, 12*(33), 36873—36881, © 2020 American Chemical Society.)*

range of biomarkers that DNA hydrogels biosensors can detect. Wang et al. developed a sensitive detection for α-fetoprotein (AFP), a biomarker for hepatocellular carcinoma, using DNA hydrogel coupled with surface-enhanced Raman spectroscopy (SERS) (Fig. 4.6B). AFP aptamers were used as linker strands and immunoglobulin G (IgG) proteins were embedded into the DNA hydrogel. Upon binding of AFP by the aptamers, the hydrogels were disentangled and released the IgG in an accurately controlled way. The IgGs, in turn, were captured by SERS-probes and magnetic capture probes, which were previously functionalized with IgG antibodies, forming a sandwich-like structure with the IgG in the middle. The magnetic capture probes were utilized to concentrate the amount of SERS-probe attached to the IgG and enhance the detection sensitivity. The SERS-probe consisted of AuNPs labeled with 4,4′-dipyridyl molecules, a Raman signal reporter. The DNA hydrogel biosensor achieved a LOD of 50 pg/mL and a good recovery rate when detecting AFP in spiked serum samples, showing the applicability for cancer diagnosis (Wang et al., 2020).

Dually cross-linked hydrogels are advanced hydrogel systems with improved mechanical properties for various biological applications (Gong et al., 2003). In this system, the cross-linking of the polymer involves covalent, as common hydrogels, and noncovalent bonding, such as hydrogen bonding, metal coordination, and host–guest interaction. Dually cross-linked hydrogels respond toward target molecules without losing their original structure or mechanical properties, unlike the DNA hydrogels biosensor, which collapsed upon binding of the target (Li, Ji, et al., 2019). This technology has been used for sensing small molecules, including lysophosphatidic acid (LPA), a metabolic level biomarker for ovarian cancer (Li, Ji, et al., 2020) (Fig. 4.6C). In this sensor, β-cyclodextrin (β-CD) was used for recognition of the LPA. β-CD is a macrocyclic oligosaccharide with a 3D structure resembling a hollow cone (Healy et al., 2021). The ability of cyclodextrins to host a variety of guest molecules inside their 3D structure makes them an attractive alternative for common bioreceptors, known as biomimetics receptors. The β-CD was tailored in one of the two copolymers that composed the dually cross-linked hydrogel and hosted a ferrocene molecule, tailored to the other copolymer, forming the hydrogel network. The hydrogel was then deposited into a gold surface to be analyzed by SPR combined with optical waveguide spectroscopy (SPR-OWS). In the presence of LPA, the host β-CD released the ferrocene to bind to LPA since the pair β-CD/LPA showed a higher affinity constant. This event resulted in the swelling of the hydrogel, which was quantitatively measured by SPR-OWS as an increase in the hydrogel layer thickness and a decrease in the refractive index. LPA was detected in synthetic plasma solution at a concentration as low as 2 μM. This approach opens a new strategy of using hydrogels as smart materials for biosensing other cancer biomarkers.

A summarization of the devices presented in this chapter, their characteristics, and their applications can be found in Table 4.1.

Table 4.1 Summary of the biosensors for the detection of cancer biomarkers using smart materials discussed in this chapter.

Smart material	Strategy	Bioreceptor	Cancer type	Biomarker	References
AuNPs	Electrochemical	Antibody	Colorectal cancer	Carcinoembryonic antigen	https://doi.org/10.1021/acsami.6b15750
AuNPs	Electrochemical	ssDNA	Breast cancer	ERBB2c and CD24c	https://doi.org/10.1016/j.bioelechem.2017.07.002
AuNPs	Optical	Aptamer	Prostate cancer	Prostate cancer −derived exosomes	https://doi.org/10.1007/s00604-020-04573-4
Graphene	Electrochemical	Antibody	Prostate cancer	PSA	https://doi.org/10.1016/j.ab.2011.12.047
Graphene	Electrochemical	ddDNA	Breast cancer	BRCA1	https://doi.org/10.1016/j.snb.2018.03.120
Graphene	Optical	Folic acid	Cancer	FAP	https://doi.org/10.1016/j.bios.2016.01.076
CNTs	Electrochemical	Antibody	Pancreatic cancer	CA19-9	https://doi.org/10.1021/acsami.7b07384
CNTs	Electrochemical	ssDNA	Cancer	TP53	https://doi.org/10.1016/j.aca.2014.05.029
CNTs	Optical	Antibody	Metastatic prostate cancer	uPA	https://doi.org/10.1021/acssensors.8b00631

DNA hydrogel	Electrochemical	DNA	Lung cancer	miR-21	https://doi.org/10.1016/j.bios.2017.12.021
DNA hydrogel	Optical	DNA	Breast cancer, pancreatic, cancer, lung cancer, ovarian cancer, liver cancer, leukemia thyroid cancer, glioblastoma, colon cancer	miR-21, miR-221, miR-224, miR-205, miR-155, miR-141, miR-25, miR-18, and miR-183	https://doi.org/10.1021/acs.analchem.9b04606
DNA hydrogel	Optical	Aptamer	Hepatocellular carcinoma	AFP	https://doi.org/10.1021/acs.bioconjchem.9b00874
Dually cross-linked hydrogels	Optical	Biomimetic receptor	Ovarian cancer	LPA	https://doi.org/10.1021/acsami.0c08722

14. Conclusions

The use of biomarkers for aiding in cancer diagnosis is a growing trend that may bring relevant features for improving patients' health, including early detection through population screening, accessible disease monitoring, increased precision, and the possibility to use minimally invasive biofluids. The development of biosensors to detect such biomarkers may bring further improvements, including rapidness, lower costs, multiplexed detection, and the possibility to perform measurements even in low-resource settings. Smart materials, in turn, are being commonly applied to biosensors to improve their performance, allowing the sensitive detection of specific biomarkers in low concentrations even in complex biological samples. Therefore, the use of such a combination is extremely promising for aiding in cancer diagnosis and improving patients' health conditions. Advancements regarding the discovery of new biomarkers, the use of innovative bioreceptors (as DNA origami, artificial antibodies), and the combination of smart materials hold great potential for building even more stable and efficient devices shortly. For these technologies to achieve the market, drawbacks including biomolecules stability and complex building are being addressed.

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