Recent achievements and challenges on nanomaterial based electrochemical biosensors for the detection of colon and lung cancer biomarkers

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ABSTRACT

Cancer is still one of the leading diseases and causes of death in the world. More than 200 types of cancers are currently known. Early diagnosis still is an important integral part of cancer treatment. The detection of cancer biomarkers plays an essential role in clinical diagnosis and early treatment for patients. Lung and colon cancers are the most common disease. Still, they are a major cause of cancer-related deaths globally due to their difficult diagnosis in early stages resulting in late treatment. Colon cancer tumors frequently metastasize to the lung. However, identifying biomarkers such as secretory proteins is an attractive way to monitor the lung and colon cancer progression in patients at earlier stages. Nowadays, many efforts have been invested in biomarker discovery that can provide a sensitive and low-cost sensor technology using nanomaterials for non-invasive disease detection. Numerous attractive biomarker candidates such as DNA, RNA, mRNA, aptamers, metabolomics biomolecules, enzymes, and proteins can be utilized for the early diagnosis of lung and colon cancer. As the detection devices are generally highly sensitive, simple preparation, and rapid response, electrochemical biosensors are increasingly used to detect cancer markers. Many electroanalytical methods are developed for the detection of lung and colon cancer biomarkers. So, in this paper, the recent advances and improvements (2011–2021) in nanomaterials based electrochemical biosensors for the detection of the lung and colon cancer biomarkers are reviewed.

1. Introduction

Cancer is one of the most serious and life-threatening disease worldwide. Whether or not cancer cases result in patients' death, cancer cases are complex processes that reduce patients' life quality, require long and challenging treatment, and cause different adverse effects and complications with multiple drug use. While humanity is faced with such a serious disease, early diagnosis has become the most critical parameter for cancer management with a correct and effective treatment protocol. While nanomaterials-based biosensors comprise a large proportion of lung and colon cancer biomarker detection research over the last ten years, current trends show lung cancer biomarker diagnosis is gaining attention (Fig. 1). Also notable is a steady increase in the use of electrochemical nanomaterials-based biosensors. This review will focus on these recent developments in nanomaterials-based electrochemical biosensors used to detect colon and lung cancer.

Colon cancer is considered the disease of old age; however, diagnosis in younger populations is possible and associated with other hereditary diseases. It is the third most common cancer type worldwide. Early diagnosis is one of the most challenging colon cancer issues because most cases are asymptomatic at the early stages. This situation reduces the effective treatment and survival rate. Genetic factors, family history, lifestyle, and dietary habits are important parameters for colon cancer risk. Colonoscopic and non-invasive tests are used for the diagnosis [1, 2].

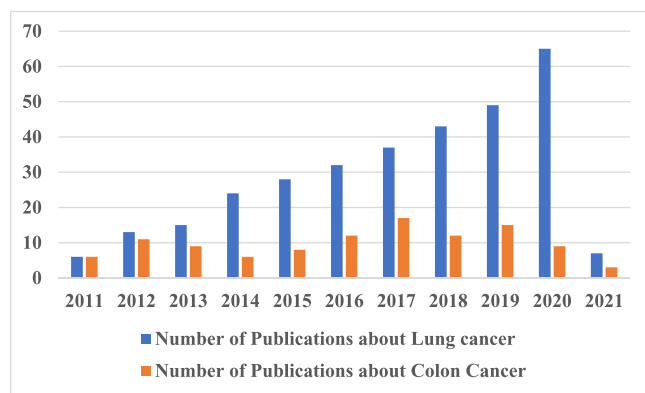Currently, almost 20% of cancer-related deaths are caused by lung

Fig. 1. Statistics of the number of publications per year related to lung and colon cancer biomarkers.

cancer. Patients' survival rate is very low, and the incident rate is increasing year by year among men and women. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (large cell carcinoma, adenocarcinoma, and squamous cell carcinoma) is a multistage and complex disease whose prognostic characteristics can be affected by genetic and epigenetic factors and tumorigenic malignancy [3,4]. Smoking history is a significant factor that causes susceptibility to lung cancer. It has a gradually worsening prognosis, and at late stages, it can cause other systemic complications. Chest X-ray, computed tomography, and magnetic resonance imagining are the most frequently used diagnostic tools for lung cancer [5,6].

Cancer biomarkers are extremely important diagnostic and prognostic tools in cancer management. All biomarkers critical for early diagnosis and treatment of lung and colon cancer will be categorized and explained comprehensively in the following sections.

Biosensors are advantageous analytical devices that provide high sensitivity, excellent selectivity, rapid analysis, low cost, and miniaturization. Therefore, they are widely used in the biomarker-based diagnostic analysis for lung and colon cancer [7,8]. The importance of nanotechnological advances in medicine and biotechnology, including medical diagnosis, has recently increased.

In particular, nanosensors that use different types of nanoparticles have received more attention in the detection of cancer biomarkers with high accuracy and sensitivity. Electrochemistry has advantages over other analytical techniques such as easy application process, short analysis time, low sample consumption, and reduced hazardous waste. Electrochemical nanobiosensors are the crossroads of the advantages of electrochemical methods, the advantages of biosensors and the use of bioreceptors, and the advantages of using nanomaterials and nanostructures [9].

Since it is an important and popular research topic, currently, there are several studies in the literature. When they are compared to this review, it can be seen that most of the reviews that were published on the similar subject of biomarker detection include a wide variety of biomarkers for different types of cancers instead of focusing on specific types [10,11]. For example; Hasan et al. [12] review the recent studies on electrochemical biosensors for the detection of cancer biomarkers. Although this kind of approach seems to be more comprehensive, it can remain vague in terms of integrity. In this review, a more focused and detailed review based on lung and colon cancers is offered.

The studies that only focus on the detection of a specific biomarker are also available in the literature [13,14]. Since a biomarker can be used in more than one disease, in this review it was preferred to evaluate the biomarkers of the most important two cancer types. Besides, it is aimed to emphasize detection with electrochemical methods and discuss them comprehensively instead of most studies that mainly explain optical sensors [15]. In a paper by Ramanathan et al. [3], multidimensional (0D–3D) nanostructures for lung cancer biomarkers were reviewed.

On the other hand, studies on electrochemical methods for biomarker detection, can concentrate only on certain techniques, such as molecularly imprinted polymers (MIPs) [16]. This review discusses nanomaterial-based electrochemical biosensors designed with the most widely used nanomaterials for the detection of colon and lung cancer biomarkers with various applications. In this review, nanomaterial-based electrochemical biosensors for the detection of colon and lung cancer biomarkers will be explained with advantages and/or disadvantages in Table 3.

2. Commonly used nanomaterials in biomarkers detection

Nanoparticles, nanowires, and nanotubes have led to the development of electrochemical biosensors, ranging from enzyme sensors to DNA-based biosensors (Fig. 2). Nanomaterials can enhance the overall sensing performance of biosensor systems [17]. Carbon nanotubes (CNT) and graphene included carbon atoms enable easy interaction with biological molecules. On the other hand, the main problem of nanomaterial-based sensors is the device-to-device variability challenging their application in the biological sample. Nanomaterials remain a challenge for their integration into lab-on-chip systems and eliminate an interfering influence of matrix in the analytical performance.

2.1. Gold nanoparticles (AuNPs)

Gold-based nanomaterials are used as valuable modification agents because of their excellent chemical stability, biocompatibility, high surface (s)/volume (v) ratio, and electrical conductivity over 106 S/m. AuNPs can be tailor-made with shape, size, and functionalization. AuNPs are preferred because of their properties such as electronic, thermal, magnetic, optical, and catalytic effects in a wide range of fields such as biomedicine, material designing, and sensors so on. Multi-functionalized Au nanomaterials can be made with organic/biological ligands, and therefore, novel Au-based nanocomposites are synthesized with the interaction between surface and analyte. A significant number of publications about Au-based nanocomposites application and synthesized for electrochemical sensors are found in the literature. The hybrid of Au-metal/metal oxide nanocomposites, Au-carbon nanotubes, Au-graphene/graphene oxide nanocomposites, Au-polymer nanocomposites, and biomolecule-Au nanocomposites are given as examples. These Au-based nanocomposites are used for a wide range, from
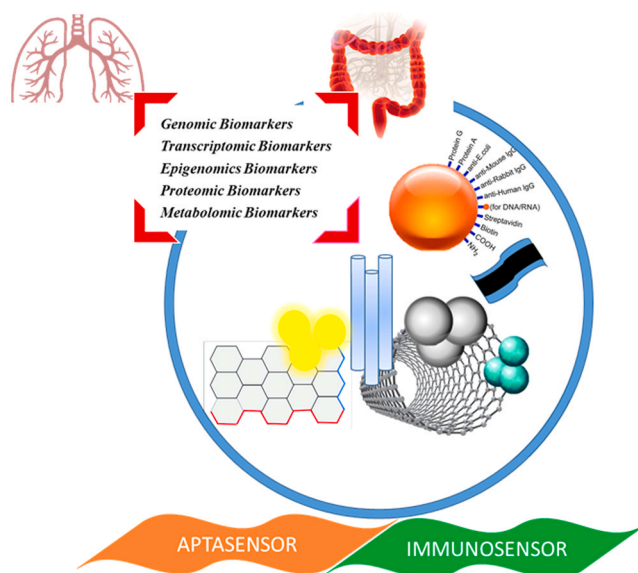

Fig. 2. Schematic representation of nanomaterial-based electrochemical biosensors.

different biomolecules to drugs.

Zhou et al. developed the electrochemical sensor with Au/Fe$_3$O$_4$ nanomaterials for the determination of indole, which is a metabolite of tryptophan. Au/Fe$_3$O$_4$ nanomaterials are used as a magnetic carrier for biological applications such as monitoring drug delivery, oligonucleo-tides purification, immunoassays, and protein separation. Au/Fe$_3$O$_4$ can be linked with 4-amino thiophenol via Au-S bond. Au/Fe$_3$O$_4$ nano-materials have great attention because of the broad surface area and excellent electrical conductivity in the electrochemical (bio)sensor. The magnetic beads coated with AuNPs interact with an active area of indole easily [18].

In their study, Ingrosso et al. formed a nanohybrid using AuNPs and reduced graphene oxide (RGO). Their newly developed electrochemical genosensor is highly sensitive for the determination of the biomarker miRNA-221. AuNPs and RGO based nanohybrid provides a stable, highly electroactive and sensitive sensor thanks to increased surface area [19]. Another study including AuNPs and RGO explains the developed electrochemical biosensor for miRNA-221. Kasturi et al. synthesized this dual nanocomposite using a natural solution of soapnut. This electrochemical biosensor stands out with its green perspective and potential for clinical applications [20].

Bharti et al. employed Au and Pt bimetallic nanoparticles due to their enhanced electronic and electrocatalytic properties, stability and syn-ergistic activity, in their recent work. 3-ami-nopropyltriethoxy silane (APTS) was also chosen to obtain a monolayered surface on the biosensor. The proposed electrochemical biosensor was used for the determination of miRNA in serum sample [21].

2.2. Conducting polymer nanocomposites (CPCs)

Electrically conducting polymer nanocomposites (CPCs) have a sig-nificant place in material synthesis. CPCs are made from conductive nanofillers and polymer matrices. Metal nanoparticles, carbon nano-tubes, and graphene can be given as examples of conductive nanofillers. Polypyrrole (PPy), polyaniline (PANI), polythiophene, bis(2,2′-bithien-5-yl)methane [22] and their derivatives are widely used as CPCs. They can show specific electronic properties and deliver positive and negative charge carriers through doping based on salt ions, hyaluronic acid, and peptides. CPCs linked biosensors are used in biomedical applications. The mutual interaction of nanofillers and polymer matrices leads to the enhanced surface area, increases of analytical recognition sites, and low resistance.

AuNPs can be added to the polymer matrices to accelerate the electron transfer and enhance the electrocatalytic activity of the CPCs matrix [23]. The response of AuNp/PANI hybrid nanocomposite modi-fied gold electrode gives LOD of 2.74 pg μL^{-1} and 7.43 pg μL^{-1} for HPV11 and HPV16 in the cervical specimen, respectively.

2.3. Nanosheets of graphene

The nanosheet is another two-dimensional honeycomb nanoparticle, and the size of the nanosheet is on a scale between 1 nm and 100 nm. High intrinsic current mobility and electronic conductivity, good ther-mal stability, and excellent mechanical strength are important proper-ties of nanosheets [24]. Graphene nanosheet (the size of ∼ 0.34 nm) is a typical example. The nanosheet consists of single or multiple-layer of two-dimensional array layers of carbon atoms with hexagonal lattices [25]. Graphene oxide nanosheets (GON) have recently received much attention in biosensing and biomedicine because of their unique chem-ical and physical properties such as large surface area, intense and wide optical absorption, fast electron transfer, etc [19]. GON are widely used for enzyme biosensors because of their biofunctionalization with abundant functional groups. There are two approaches for synthesizing nanosheets: One of them is the top-down approach, and another is the bottom-up approach [26].

Transition-metal oxide nanosheets are also applied as alternative

modification agents. Manganese dioxide (MnO$_2$), molybdenum disulfide (MOS$_2$), and cobaltosic oxide (Co$_3$O$_4$) nanosheets are very promising nanoplatforms for biomedical applications.

2.4. Nanomaterial modified paper-based platform

Paper-based platforms are a good alternative to classical electro-chemical sensors. Paper has gained attention among the research com-munity because it is easily accessible, disposable, affordable, and eco-friendly. Paper has a mesoporous structure and provides storage of re-agents, high surface (S)/volume (V) ratio, fast electron transfer, strong adsorption, and good stability. Paper-based device production is a simple method related to various properties such as surface roughness, mechanical strength, ink absorbance, and composition. The significance of a paper-based platform in the construction of electrochemical bio-sensors is considered for early and easy point-of-care diagnostics of cancer in biomedical applications [27]. Considering the significance of conductive nano-inks in the construction of electrochemical biosensors, they can be employed to draw patterns on the surface of the photo-graphic paper [28]. Fan et al. reported that neuron-specific enolase (NSE) as small cell lung biomarker can be determined with a micro-fluidic paper-based analytical device (μPADs) (Fig. 3). The μPADs were modified with amino-functional graphene, thionine, and gold nano-particles (NH$_2$-G/Thi/AuNPs). Android's smartphone measured the NSE in the range of 1 and 500 ng mL^{-1} with the limit of detection (LOD) of 10 pg mL^{-1} through Bluetooth in real-time [29].

3. Biomarker sensing strategies based on nanomaterial

Biomarker sensing strategies are summarized in Table 1, in terms of their advantages and applications for cancer diagnosis.

4. Biomarkers for lung and colon cancer

Lung and colon cancer biomarkers cover a wide variety of indicators that can be used in the different processes of cancer diagnosis and treatment. In Table 2, the mostly used biomarkers in the latest studies on nanomaterial based electrochemical biosensors are reviewed.

5. Label and label-free based detection method for lung and colon cancer biomarkers

For the detection of cancer biomarkers, label-based and label-free techniques are applied in biosensors. The primary strategy in label-based sensors is increasing the signal and improving the sensitivity by binding biomarkers with labels. Enzymes and fluorescent, magnetic, or electroactive compounds can be chosen as labels for label-based analysis since they quickly bind to the target molecule. Biocompatibility is one of the critical points for deciding a suitable label for the target biomarker. With labeled sensing approaches, it is possible to obtain improved sensitivity and specificity [78,79].

Label-based electrochemical detection methods may cause issues such as relatively less economic and slower analysis and a decrease of the non-specific signal. In order to overcome those challenges, label-free approaches are preferred due to the advantages such as rapid, easy-to-use, highly sensitive, selective, and low-cost analysis with decreased sample consumption for cancer biomarker detection. They are consid-ered good alternatives to conventional label-based sensors. Label-free methods also provide a saving of time without complex and time-consuming labeling processes. [78,79].

As mentioned above, label-based sensors offer an indirect approach by measuring the changes in the characteristics of labels, not directly the target biomarker. On the other hand, label-free biomarker sensing strategies directly focus on the changes in the concentration of bio-markers. Label-free biomarker sensors are suitable for the detection in complex media and for the molecules that cannot be labeled [78,79].
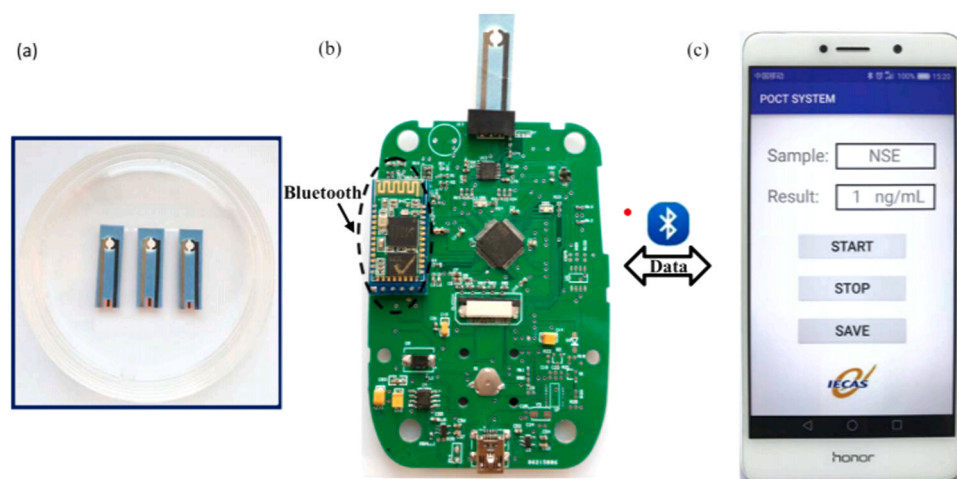
Fig. 3. Schematic representation of the prototype of the wireless POCT system. (a) Paper-based analytical devices (μPADs) (b) The electrochemical detector with μPADs. (c) The Android's smartphone for the detection results show of NSE.
Reproduced with permission from Elsevier [29].

Table 1
Biomarker sensing strategies on diagnostic applications.

	Advantages	Biomarkers	Applications	Ref.
Minimally Invasive Strategies	Less risky, safer, and more comfortable alternatives to traditional invasive strategies.	microRNA, circulating tumor DNAs, various proteomic biomarkers, and DNA methylation.	Liquid biopsy in blood, urine, cerebrospinal fluid, and saliva, etc. for hepatocellular carcinoma, colon cancer and prostate cancer	[30,31]
Non-Invasive Strategies	Easy and pain-free application, portability, affordability, patient compliance, highly sensitive and rapid measurements.	carcinoembryonic antigen (CEA), cytokeratin-19 (CK19) fragment 21-1 (CYFRA21-1)	Electronic noses (E-nose) for the analysis of biomarkers in exhaled breath.	[32–35]

In a study by Wang et al. [80], a label-free microfluidic paper-based electrochemical aptasensor was developed using gold nanoparticles and amine-functionalized graphene for the simultaneous detection of cancer biomarkers CEA and neuron-specific enolase (NSE). This newly developed sensor showed good linearity and low detection limits. It was also applied to the clinical serum samples evaluating its utility for early cancer diagnostics.

Li et al. [81] studied an ultrasensitive label-free electrochemical impedance detection system for the analysis of lung cancer biomarker CYFRA 21-1. Their highly selective, label-free, and anti-interference method demonstrated great sensitivity and broad linear range with a very low LOD. It can be promising for clinical applications of diagnosis and monitoring.

In another work by Abdolahad et al. [82], a label-free impedance biosensor was developed using silicon nanograss for detection of rare metastatic cells among primary cancerous colon cells. Invasive colon cancer cells were detected without biochemical labels. This method offers a better and more accurate approach to cancer staging.

6. Nanomaterial-based electrochemical biosensor applications for the ultrasensitive detection of lung and colon cancer biomarkers

Biomarker diversity is very high in lung cancer; however, a small number of biomarkers are used in nanobiosensors. The nanobiosensors consist of nanomaterials and a biorecognition elements-based sensor. In Scheme 1, several immobilization techniques are given as example for the interaction between nanomaterials and biorecognition elements. Moreover, the signified interaction is evaluated in terms of advantages and disadvantages. The biorecognition elements and nanomaterials could be combined with different methods: affinity, covalent bonding, cross-linking, entrapment, and physical adsorption. Affinity binding can be explained that two affinity parts interact with each other because of

their affinity properties. Covalent binding means that functional groups of biomolecules bind to the support materials with chemical binding. Moreover, the cross-linker chemicals such as glutaraldehyde are used for cross-linking between molecules and functional reactants. Biomaterials occupy the polymeric matrix with entrapment technique. Hydrophobic, ionic interactions and van der Waals force are given as an example for physical adsorption [83].

Miniaturized biosensors are referred to as tools for point-of-care diagnosis in hospitals. Multiplexing analysis with biosensors is very new field. The studies about multiplexing analysis are progressing rapidly. An information collected from different single biomarkers is analyzed with multiplexed biosensors. It provides the information about progression of disease and contributes to increase the sensitivity and specificity of disease detection. The combination of single or two/more markers can be analyzed in biosensors. Lab-on-chip systems have the potential for fabricating miniaturized, multiplexed and fully automated biosensors to recognize the disease.

In the regard, Chen et al. performed simultaneous detection of CEA and AFP for lung and liver cancer. The graphene modified with AuNPs and chitosan nanocomposites modified GCE are used for sensing platforms. The aim of usage of this composite is large specific surface area, excellent electron transportation, high thermal conductivity, good biocompatibility and easy modification [84]. Thus, the fabrication of simultaneous assay of multianalytes have great significance in clinical diagnosis.

Choudhary et al. developed a label-free electrochemical dual electrochemical immunosensor for determination of anti-MAGE A2 and anti-MAGE A11 lung biomarkers. In this study, single-walled carbon nanotubes-chitosan nanocomposites are dropped on graphite layer. These nanocomposites used as nanotransistors show excellent sensitivity to biomolecules. Moreover, the nanocomposites enhance the biosensing performance because of their electrical and biocompatible properties. Chitosan is good choice to disperse nanomaterials due to its excellent

Table 2

Mostly used biomarkers for lung and colon cancer.

| Biomarker	Description	Related cancer types	Ref.
Mitochondrial DNA (mtDNA) (Genomic Biomarkers)	mtDNA is involved in critical processes such as translation, transcription, and protein assembly.	Bladder, lung, colon, hepatocellular, and thyroid cancer	[36–39]
Circulating DNA (Genomic Biomarkers)	Extracellular DNA fragments that are parts of tumor cells, carriers of cancer related anomalies.	Lung, colon, and ovarian cancer	[40–42]
Plasma microRNA-21 (Transcriptomic Biomarkers)	microRNA is a type of RNA associated with critical cellular processes such as proliferation and differentiation of cells, regulation of biological development, and malignant cellular transformation. microRNA-21 has oncogenic properties and is responsible for gene expression.	Various types of solid tumors such as breast, lung, prostate, and colon	[43,44]
Non-coding RNA (Transcriptomic Biomarkers)	Non-coding RNAs have important roles in epigenetic, proliferative, and regulative processes and they can show tumor-specific expressions.	Lung and colon	[45,46]
DNA methylation (Epigenomic Biomarkers)	It is an important step for genomic regulatory processes. Nonetheless, abnormal or excessive DNA methylation can result in carcinogenesis and other serious disorders.	Colon and lung cancer	[47,48]
Lynch syndrome (hereditary non-polyposis colorectal cancer) (HNPCC) (Epigenomic Biomarkers)	Lynch syndrome (LS) is a hereditary disorder that is a result of DNA mismatch repair mutation. LS can be a primary disease in colon cancer.	Colon cancer and endometrial and ovarian cancer	[49–51]
Carcinoembryonic antigen (CEA) (Proteomic Biomarkers)	CEA is a glycoprotein antigen with higher serum levels during fetal development, and after birth, normally CEA concentration decreases to very low levels (between 2.5 and 5 ng mL^{-1}).	Lung, cervical, breast, and especially colon cancer	[52,53]
Sialyl Lewis X antigen (SLX) (Proteomic Biomarkers)	SLX take part in processes related to inflammation.	Colon, stomach, lung, and bladder cancer	[54–56]
Squamous cell carcinoma (SCC) antigen (Proteomic Biomarkers)	It is possible to obtain useful information about the stage and progression of the tumor and the course of the treatment by measuring the serum	Lung, neck, head, anal canal, and esophagus squamous cell carcinomas	[57,58]

Table 2 (*continued*)

Biomarker	Description	Related cancer types	Ref.
	level of SCC-Ag in patients.		
Cytoskeleton-associated protein 4 (CKAP4) (Proteomic Biomarkers)	CKAP4 is a protein associated with the endoplasmic reticulum. It can also be found on the surface of vascular smooth muscle cells.	Pancreatic, kidney, and lung cancer	[59,60]
Cytokeratin fragment (CYFRA) 21-1 (Proteomic Biomarkers)	It shows various expression patterns in different cells and tissues and is related to the cell apoptosis process.	NSCLC	[61–63]
N-succinyl-2,6-diaminopimelate, deoxycholic acid glycine conjugate, glutamine, taurine, isoglutamine, choline, lactate, phenylalanine, and tyrosine (Metabolomic Biomarkers)	Metabolomic approaches for cancer biomarker detection are perspective and dynamic techniques that provide valuable data about diagnosis, prognosis, response to treatment, and cancer metabolism.	Colon and lung cancer	[64–67]
Cancer antigen 125 (CA-125)	CA-125 is a mucinous glycoprotein related with tumor proliferation.	Ovarian cancer and NSCLC.	[68,69]
Tissue polypeptide antigen (TPA)	It is another cytokeratin fragment related with cancer and it can be found in epithelial tissues.	Lung, ovarian and pancreatic cancer	[70–73]
Vascular endothelial growth factor (VEGF)	VEGF is a cytokine with high angiogenic activity. It is associated with tumor angiogenesis.	Colon cancer and	[74,75]
Neuron specific enolase (NSE)	NSE belongs to an enzyme group named glycolytic enolases. It can be found in nerve, neuroendocrine and muscle cells.	SCLC	[70,76, 77]

capability of film formation, nontoxicity, biocompatibility, mechanical strength, and good water permeability [85].

It is known that there is a correlation with aberrant expression of microRNAs and occurrence of lung cancer. microRNAs determination takes an attention for lung cancer early diagnostics. Li et al. reports that 3D DNA origami of novel design is immobilized at the Au NPs modified gold disk electrode. The developed electrochemical genosensor has a great potential in highly sensitive clinical cancer diagnosis application [86].

Non-small cell lung cancer (NSCLC) with EGFR mutation accounts for 80% of all lung cancer cases. EGFR value in patients in lung cancer should be followed by different analytical methods. The microfluidic paper-based electrochemical DNA biosensor (μ-PEDB) is one of the alternative determination techniques for EGFR biomarkers. μ-PEDB was fabricated for sensitive detection of EGFR mutations in patients with saliva. Firstly, DNA sequences are immobilized with different interaction strategies. Then, EGFR mutation is detected through the analysis of DNA hybridization reaction and then, amperometric signal is obtained. Tian et al. fabricated μ-PEDB for determination of EGFR mutations gene for point-of-care testing. The concentration range of target DNA is 0.5–500.0 nM, with LOD of 0.167 nM [87].
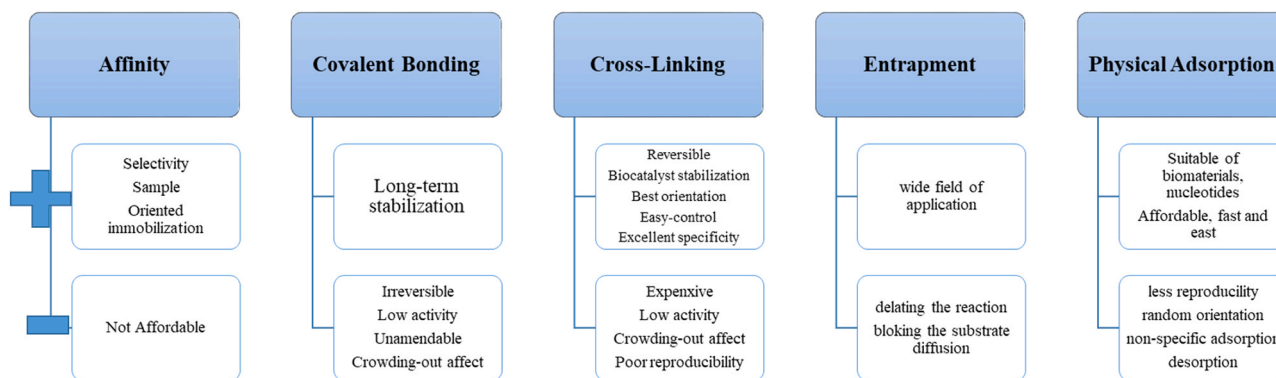
Scheme 1. The several immobilization techniques between nanomaterials and biorecognition elements. Reproduced with permission from Elsevier [83].

7. Biological matrices used for detection

Biomarkers in body tissues and fluids are analyzed with biosensor techniques. The biosensor techniques could analyze these biological samples accurately. Especially, assay of biomarkers by electrochemical biosensors have been most preferred in literature for the cancer diagnosis because of their excellent advantages such as their versatility, accurate quantification, fast response, and amenability for multiplexing and miniaturization [88]. The proteins in the serum play a very important role in the immune system and circulation. Most of proteins in the serum are composed of albumin and globulins, while the remaining proteins are present in coagulation.

Cell lines are used in drug casing and therapy development. Since the molecular characterizations of these lines reflect genetic and epigenetic changes, an idea can be obtained about chromosomal changes and gene methylation [89–92]. Cancer cell lines are widely used in critical gene discovery research, where they can show the level of carcinogenesis (initial or advanced) in the cell. Hence, human cancer cell lines are preferred in clinical studies.

Since urine testing is non-invasive, it is widely used in routine applications. A urine test can be used in the detection of diseases and the analysis of large structure biomolecules. Sample collection methods should be applied carefully to prevent contamination from the environment [93,94]. Urine is an attractive biological matrix because of easily repeatable, collection of samples, and unlimited volumes. Among the different biological fluids (such as breath, blood, and bronchoalveolar fluid), urine has several advantages: Sample collection is affordable, a pre-treatment process is not required, prolonged frozen storage, and there is usually no side effects or complications [95]. Proteins and peptides excreted in the urine are less complex and more stable than plasma proteins, thus conferring an advantage for biomarker monitoring [96].

Another non-invasive and reproducible biological fluid is saliva. Saliva contains proteins, DNA, and RNA that can be evaluated to identify abnormalities and diseases in the body. It is easier to monitor the treatment response with the patient saliva samples retaken [97]. Breath samples may also contain biomarkers of cancer disease. The exhaled breath contains volatile organic compounds, and these compounds can be used to detect some cancer-related markers [98]. Bronchoscopy is used in the detailed examination of all segmental bronchi and as a primary diagnostic method for lung cancer. It is applied under local anesthesia. When any lesion is noticed, the diagnostic yield can be increased by applying the endobronchial biopsy [99]. The invasive liquid biopsies have been replaced with appropriate specific receptors because of the developments in technology [100].

Sputum has been investigated in numerous studies because it contains biomarkers used in the diagnosis of lung cancer. It can contribute to the overall decline in lung function and mortality. The most studied biomarkers in pulmonary exacerbations are neutrophil elastase and interleukin (IL)-8 [101–103]. As a result, the detection of early biomarkers is very important for the effective treatment of cancer in the mentioned biological samples.

Before collecting saliva from patients, consents from the patients or voluntary participants have to be obtained. Saliva is considered the best and easy research tool for scientific investigations on humans from an ethical perspective. Saliva testing gives information about the hormonal, immunological, metabolic, and nutritional state of a person. Saliva testing, which is fast screening non-invasive and easy to collect properties, helps the early diagnosis and surveillance of disease. However, the levels of some biomarkers are not fully clarified and understood in saliva and serum when pathologic event occurs. Therefore, compared to other body fluids, the therapeutic intervention based on the detection and quantification in the saliva is more difficult. Complicated samples contain many proteins, especially high-abundance proteins and undesirable levels of proteolytic enzymes and mucin [104]. The mixtures are so complex that you need to clean them up before you use the LC-MS. Furthermore, the concentration of biomarkers can be at undetectable level in serum.

Early clinical detection of biomarkers in blood or cerebrospinal fluid may allow earlier diagnosis compared to MIR or CT results. Thus, it will enable earlier initiation of intervention to increase the survival rate and speed up the recovery time. In summary, the detection of biomarkers will be an important basis for initiating drug use and evaluating the severity of the disease [88,105].

Bronchoscopy is a recommended procedure inserted through the nose or mouth to collect several pieces of lung tissue for all patients suspected of having lung cancer. Bronchoscopy contributes the surgical map through the evaluation of the extent of the tumor surface. Nowadays, innovative probe-based bronchoscopic techniques are developed such as autofluorescence bronchoscopy (AFB), narrow band imaging (NBI) and high magnification bronchovideoscopy (HMB). These innovative techniques can detect pre-invasive malignancies in the short examination time [106].

8. Applications of biomarkers in medicine

8.1. Diagnosis

Cancer biomarkers can help diagnose a specific disease, especially when necessary, to determine the primary or metastatic origin of tumors. For example, Vrba et al. used the DNA methylation biomarker for the detection of lung cancer in liquid biopsies from non-small cell lung cancer (NSCLC) patients [107]. Since the change of miRNA level can be detected in human plasma, many studies have aimed to identify a reliable diagnostic tool by examining the differential expression of circulating miRNAs in NSCLC patients, patients with benign tumors, and

healthy controls [108]. Liao et al. have applied miRNA biomarkers to diagnose NSCLC in sputum and plasma biofluid [109]. For diagnosis of NSCLC, the integrated panel of biomarkers, which were included one plasma miRNA (miR-21-5p) and two sputum miRNAs (miRs-31-5p and 210-3p), are more sensitive (85.5%) and more specific (91.7%) in comparison to the individual panels. The performance of the bio-recognition in the experimental group was evaluated, and it was concluded that the function of the integrated biomarker panel was in-dependent of age, sex, and race of patients as well as histology and NSCLC stage. To screen for NSCLC and monitor disease progression, long non-coding (lnc) RNAs have been proposed as biomarkers. There is an urgent need for new, rapid, and cost-effective lncRNA biosensors that can be used in the clinic. For example, a novel electrochemical biosensor based on a gold nanocage and a screen-printed carbon electrode deco-rated with amidated multiwalled carbon nanotubes (Au NCs/MWCNT-NH$_2$) has been developed by Chen et al. [110]. The SPCE Au NCs/MWCNT-NH$_2$ lncRNA biosensor possesses a wide linear range (10^{-7}–10^{-14} M) and a LOD of 42.8 fM due to its large surface area, su-perior conductivity, and excellent biocompatibility. Compared to traditional RT-PCR, the suggested technique displays good selectivity, acceptable stability, is easier to run, faster, and utilizes less expensive raw materials than the previous method. There is a strong correlation between the presence of the biomarker CYFRA21-1 and the diagnosis and prognosis of NSCLC. Through the use of RAFT (reversible addition-fragmentation chain transfer), an ultrasensitive electro-chemical immunosensor for detecting CYFRA21-1 was developed. An antigen-antibody interaction allowed CYFRA21-1 to be preferentially bound to GO fixed on a glassy carbon electrode (GCE) surface through an amide bond (see Fig. 4). This was followed by the addition of a chain transfer agent-conjugated secondary CYFRA21-1 antibody. The result was an immunocomplex that can be linked to multiple monomers by RAFT polymerization, which is similar to a sandwich. It was then decided to attach to the immunocomplex a high-density electroactive polymer chain that would greatly intensify the electrochemical signal provided by CYFRA21-1. By employing the approach, the current response increased linearly with rising logarithmic concentrations ($R^2 = 0.998$), and the LOD is 0.14 µg/mL (S/N = 3) [111].

The specificity and sensitivity of a single tumor antigen are insuffi-cient to fulfill reliable diagnostic criteria, and single antigen measure-ment is prone to false-negative and false-positive results. As a result,

simultaneous monitoring of numerous tumor antigens in blood samples linked to specific tumors has emerged as an intriguing and promising analytical technique. Yang et al. developed an electrochemical biosensor based on various signal amplification techniques to detect two lung cancer markers, carcinoembryonic antigen (CEA) and cytokeratin 19 fragments 21-1 (CYFRA21-1). The presence of a large number of gold nanoparticles on the surface of three-dimensional graphene (3D-G), poly-thionine (pThi), and poly-m-Cresol purple (pMCP) not only pro-vides a large number of binding sites for antigen and antibody, but also enhances the electrochemical signal of the biosensor and greatly im-proves its sensitivity. For CYFRA21-1 and CEA, the detection linear range is 0.5–200 ng/mL, with low detection limits (LOD) of 0.18 ng/mL and 0.31 ng/mL, respectively. Therefore, this type of immunosensor has a lot of promise for detecting many targets at the same time in early clinical diagnosis [112]. Some selected biosensors for the detection of biomarkers and their analytical parameters are summarized in Table 3. As exemplified in Table 3, electrochemical methods-based biosensors provide the opportunity to make highly sensitive measurements that can be down to the fM level compared to other methods. Therefore, elec-trochemical sensors are often preferred for the detection of lung and colon cancer biomarkers as highly sensitive, selective and easy to apply sensors. When electrochemical techniques are evaluated among them-selves, it is seen that LSV, SWV and DPV techniques provide lower LOD values.

8.2. Prognosis and treatment predictions

A biomarker may be used to directly assign patients to different treatment regimens in a clinical trial. Some biomarkers are specific for the determination of certain cancer types. In this case, biomarkers can be useful in determining how aggressive a cancer is, as well as the likeli-hood that it will respond to a particular treatment. In part, this is because tumors that exhibit specific biomarkers may respond to treat-ments associated with biomarker expression or presence. Circulating tumor cells (CTCs) can be applied as a biomarker for predicting disease progression. CTC collection from blood is non-invasive. Thus, CTCs can be used to assess "real-time" tumor dynamics. High CTC levels are often associated with increased metastasis, invasive disease, and shorter disease-free survival (DFS). Some morphological changes in CTCs can be related to chemotherapy resistance. Besides, genomic analysis of CTCs in
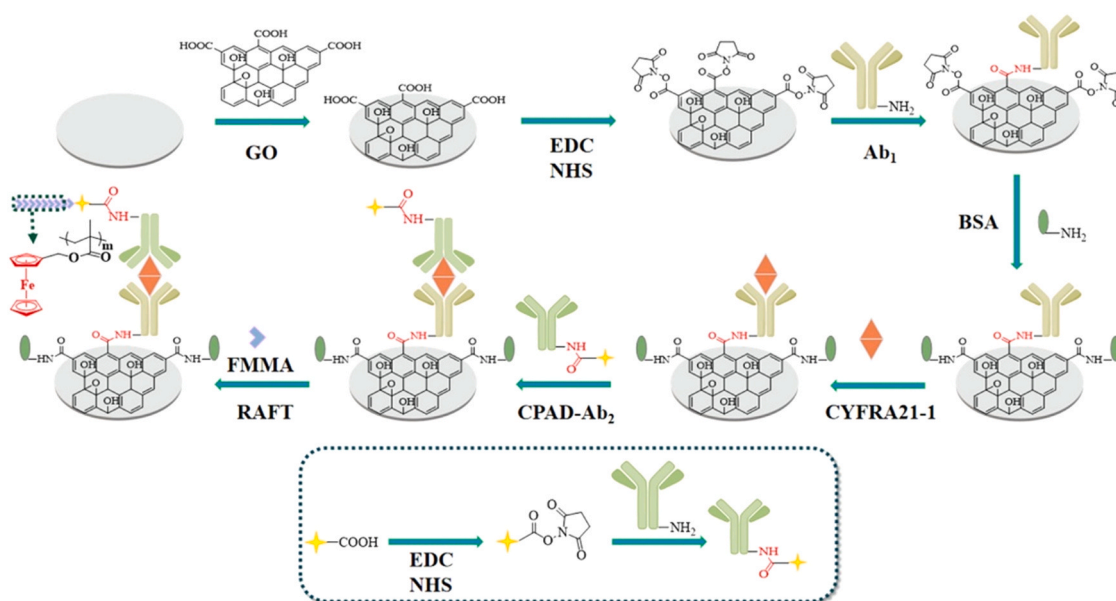

Fig. 4. Scheme of the electrochemical immunosensor for the CYFRA21-1 detection via RAFT polymerization signal amplification. Reproduced with permission from Elsevier [111].

Table 3

Analytical performance of some colon and lung cancer biomarkers based biosensors.

Type of biomarkers	Detection technique	Nanomaterials	LDR	LOD	Matrices	Application	Advantage and/or disadvantages of method	Ref.
Lung Cancer								
CYFRA21-1	Electrochemical (SWV, CV, EIS)	Graphene oxide	0.5 fg mL^{-1}–10 pg mL^{-1}	0.14 fg mL^{-1}	Clinical serum samples	Clinical diagnosis and analysis	Advantages: high sensitivity, environmental benignity, and low cost	[111]
NSE	Electrochemical (EIS, LSV)	Porous 3D graphene-starch architecture	0.02 pg mL^{-1}–35 ng mL^{-1}	8 fg mL^{-1}	Clinical serum samples	Clinical diagnosis and analysis	Advantages: acceptable accuracy, good stability, and high sensitivity	[113]
SCCA	Electrochemical (SWV)	Magnetic mesoporous Fe$_3$O$_4$	1 pg mL^{-1}–4 ng mL^{-1}	0.33 pg mL^{-1}	Clinical serum samples	SCCA detection with CRSHIP using magnetic mesoporous Fe$_3$O$_4$ as a nano container and aminated polystyrene microspheres as a molecular gate	Advantages: simple, fast and sensitive approach	[114]
SCCA	Electrochemical (Amperometry, CV, EIS)	Au/Ag/Au core/double shell nanoparticles	0.5 pg mL^{-1}–40 ng mL^{-1}	0.18 pg mL^{-1}	Human serum	Determination of SCCA by electrochemical immunosensors using Au/Ag/Au core/double-shell nanoparticles as enzyme-mimetic labels	Advantages: good reproducibility, high selectivity and stability	[115]
SCCA	Electrochemiluminescence	magnetic graphene oxide and AuNP/graphitic-phase carbon nitride	1 pg mL^{-1}–10 ng mL^{-1}	0.4 pg mL^{-1}	Human serum	SCCA diagnosis with immunosensing strategy based on electrochemiluminescent AuNPs/g-C3N4 nanocomposites	Advantages: high sensitivity, a low detection limit, good stability, and acceptable precision and accuracy	[116]
SCCA	Photoelectrochemical	MoSe$_2$ nanosheets and hollow gold nanospheres	1.0 pg.mL^{-1}–50 ng mL^{-1}	0.21 pg mL^{-1}	Clinical serum samples	SCCA diagnosis with photoelectrochemical immunosensor based on MoSe2 nanosheets and hollow gold nanospheres	Advantages: good reproducibility, selectivity and stability	[117]
CA 125	Electrochemical (SWV, EIS)	Prussian Blue-Platinum nanoparticles/polyaniline	10 mU mL^{-1}–5000 U mL^{-1}	4.4 mU mL^{-1}	Human serum	CA 125 diagnosis by label-free voltammetric immunosensors based on Prussian Blue-platinum nanoparticles (PB-PtNPs) incorporated into a polyaniline	Advantages: satisfactory accuracy compared to a commercial chemiluminescent microparticle immunoassay (CMIA)	[118]
CA 125	Electrochemical (EIS)	gold array microelectrodes	0 U mL^{-1}–100 U mL^{-1}	7 U mL^{-1}	Human serum	CA 125 detection with micro-flow Immunosensor Based on Thin-film Interdigitated Gold Array Microelectrodes	Advantages: good analytical performance	[119]
Tissue Polypeptide Antigen (TPA)	Electrochemical (Amperometry, CV, EIS)	graphene sheet	5 pg mL^{-1}–15 ng mL^{-1}	1.2 pg mL^{-1}	Serum samples	TPA detection with a sandwich-type immunosensor using Pd–Pt nanocrystals as labels	Advantages: wide linear range, a low detection limit, good reproducibility, good selectivity and acceptable stability	[120]
TPA	Electrochemical (Amperometry, CV, EIS)	gold nanoparticles magnetic graphene nanocomposites /single ferriferous oxide nanoparticles (Fe$_3$O$_4$ NPs)	10 fg mL^{-1}–100 ng mL^{-1}	7.5 fg mL^{-1}	Human serum	TPA detection with an ultrasensitive sandwich-type electrochemical immunosensor based on dual signal amplification strategy using multifunctional graphene nanocomposites as labels	Advantages: good reproducibility, high selectivity and stability, indicating potential application promising in clinical monitoring of tumor markers	[121]
NSE; CYFRA21-1; CEA; SCC; CA 125	Electrochemical (SWV)	polymerized dye-gold composites and polyaniline-gold composite	NSE: 1–150 ng mL^{-1} CYFRA21-1: 1–150 ng mL^{-1} CEA: 1–150 ng mL^{-1} SCC: 1–150 U mL^{-1} CA 125: 0.1–100 ng mL^{-1}	NSE: 0.9 ng mL^{-1} CYFRA21-1: 0.4 ng mL^{-1} CEA: 0.2 ng mL^{-1} SCC: 0.9 U mL^{-1}	Human serum	Simultaneous detection of five biomarkers of lung cancer by electrochemical immunoassay based on polymerized dye-gold composites	Advantages: simultaneous detection of other multiple proteins	[122]

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Table 3 (*continued*)

Type of biomarkers	Detection technique	Nanomaterials	LDR	LOD		Matrices	Application	Advantage and/or disadvantages of method	Ref.
GM2 activator protein (GM2AP)	DPV	polyethyleneimine-coated gold nanoparticle and phosphomolybdic acid	0.005–25 ng mL^{-1} 25–400 ng mL^{-1}	0.51 pg mL^{-1}		Both human urine and serum samples	A label-free immunosensor of GM2AP using a phosphomolybdic acid/ polyethyleneimine coated gold nanoparticle composite	Advantages: simple fabrication, low cost, rapid analysis, satisfactory stability, high selectivity and sensitivity, and good reproducibility	[123]
1-propanol Isopropyl alcohol	CV	Co and Ni doping in tin oxide	1-propanol: 0–15 ppb isopropyl alcohol: 0–10 ppb	1-propanol: Isopropyl alcohol:	SnO$_2$: 0.6 ppb Co-SnO$_2$: 0.14 ppb Ni-SnO$_2$: 0.28 ppb SnO$_2$: 1.53 ppb Co-SnO$_2$: 0.27 ppb Ni-SnO$_2$: 0.24 ppb	Breath sample	E-Nose Based Electrochemical Sensing of 1-propanol and isopropyl alcohol in breath samples based on SnO$_2$ nanomaterials	Advantages: highest sensitivity of 2.99 µA/ppb for isopropyl alcohol and 3.11 for 1-propanol as well as Co-SnO$_2$ shows selectivity for IPA Disadvantage: Ni-SnO$_2$ is only selective to 1-propanol against all other volatile compounds analyzed	[124]
Cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1)	CV	silicon nitride -molybdenum disulfide (MoS2) composite on multi-walled carbon nanotubes	0.01–1.0 pg mL^{-1}	2.00 fg mL^{-1}		Plasma sample	CYFRA 21-1 immunosensor based on Si$_3$N$_4$/MoS$_2$ incorporated MWCNTs and core-shell type magnetic nanoparticles	Advantages: low detection limit	[125]
CD59	CV	graphene oxide nanoparticles	1 fg mL^{-1} to 10 ng mL^{-1}	1 fg mL^{-1}		Urine samples of lung cancer patients	An electrochemical CD59 targeted noninvasive immunosensor based on graphene oxide nanoparticles	Advantages: good storage stability and specificity	[126]
Ethyl Acetate	CV	Ni and Cu doping on the electrochemical characteristics of the SnO$_2$ nanomaterial	1–20 ppb	SnO$_2$: 0.376 ppb Cu-SnO$_2$: 0.377 ppb Ni-SnO$_2$: 0.398 ppb		No real sample	Ethyl Acetate Chemical Sensor as Lung Cancer Biomarker Detection	Advantages: great potential for early lung cancer detection	[127]
CYFRA21-1	DPV	3D graphene functionalized with Ag nanoparticles	1.0×10^{-14} to 1.0×10^{-7} M	1.0×10^{-14} M		Non-small cell lung cancer	Three-dimensional electrochemical DNA biosensor based on 3D graphene-Ag nanoparticles for sensitive detection of CYFRA21-1	Advantages: highly sensitive, economical, simple, and timesaving	[128]
CYFRA 21-1 DNA	EIS	thiolated peptide nucleic acid/Zr^{4+}/poly(ε-caprolactone)	1.0×10^{-16}–1.0×10^{-9} M	1.073×10^{-17} M		Serum samples	early clinical diagnosis of lung cancer	Advantages: real time monitor, label-free analysis, simple operation, high sensitivity and selectivity	[81]
Colon cancer									
CEA; VEGF	Electrochemical (EIS, DPV)	Au Np, Pb Np CuNp and γFe2O3 Np	CEA: 25–600 ng mL^{-1}; VEGF: 0.2–12.5 ng mL^{-1}	CEA: 4.31 ng mL^{-1} VEGF: 14 pg mL^{-1}		Human serum	Fabrication of a bimetallic nanomaterial-based electrochemical immunosensor for simultaneous detection of CEA and VEGF	Advantages: accurate, sensitive, practical and robust electrochemical platforms	[129]
Gene sequence-associated with CRC	DPV	CeO$_2$/Chitosan composite matrix	1.59×10^{-11} to 1.16×10^{-7} M	1.0×10^{-11} mol L^{-1}		NA	Colorectal cancer DNA sequence-selective electrochemical biosensor based on nano-porous CeO$_2$/chitosan composite film	Advantages: wide linear range and low detection limit, high sensitivity and satisfactory reproducibility	[130]
KRAS mutation (KRAS G12D)	DPV	dendritic DNA nanostructure	0.01 fM to 1 pM	2.4 aM		plasma sample	Potential applications in clinical cancer screening and prognosis	Advantages: satisfactory specificity and acceptable accuracy	[131]

Table 3 (*continued*)

Type of biomarkers	Detection technique	Nanomaterials	LDR	LOD	Matrices	Application	Advantage and/or disadvantages of method	Ref.
BRAF mutation (BRAF V600E)	DPV	Fe_3O_4/Au NPs	50–0.8% of V600E alleles	Not reported	Cell line HT29	Sensitive electrochemical analysis of BRAF V600E mutation based on an amplification-refractory mutation system	Advantages: high sensitivity, simplicity, low cost, and easy validation of assay procedures	[132]
5-hmC MGMT	Amperometry	streptavidin-magnetic microbeads	77–7500 pM (ProtA-polyHRP80)	23 pM	Cell lines SW480 SW620	Amperometric bioplatforms to detect regional DNA methylation	Advantages: versatility, quick execution, ease of implementation, and low cost	[133]
			44–5000 pM (Histostar)	13 pM	Colorectal tissues			
miRNA-21	Amperometry	AuNPs	5–5000 pM	3.96 pM	Cell line HT29	Rapid and easy method for early cancer marker detection in clinical diagnostics	Advantages: direct, sensitive and selective determination of miRNA-21 in total RNA extracted from human hepatocarcinoma HepG2 cells and human colon cancer HT-29 cells	[134]

CYFRA21-1: Cytokeratin fragment antigen 21.1
NSE: Neuron specific enolase
SCCA: Squamous cell carcinoma antigen
CA 125: Carbohydrate antigen 125
CEA: Carcinoembryonic antigen
VEGF: Vascular Endothelial Growth Factor
LRG1: Leucine-rich α-2-glycoprotein-1
KRAS: Kirsten rat sarcoma viral oncogene homolog
BRAF: v-Raf murine sarcoma viral oncogene homolog B
CRC: Colorectal cancer
CV: Cyclic voltammetry
DPV: Differential pulse voltammetry
SWV: Square wave voltammetry
EIS: Electrochemical impedance spectroscopy
NA: Not apply.

small cell lung cancer can be used for the prediction of response in chemotherapy. To better understand disease diagnosis and prognosis, it is becoming increasingly important to understand the physiological role of ribonucleic acid (RNA) biomarkers, which include different coding and noncoding transcriptome such as microRNA (miRNA), messenger RNA (mRNA), and long noncoding RNA (lncRNA) [135]. miRNAs are more stable than mRNA [136], which explains their positive predictive activity in cancer. A vast number of miRNAs have been identified as useful markers for cancer prognosis in recent years [137,138]. Overexpressed miR-21 has been associated with a bad prognosis and treatment outcome in patients with colon cancer [137], whereas downregulated let-7 miRNA has been linked to a poor prognosis and therapeutic outcome in patients with lung cancer. An electrochemical detection method for lung cancer-related microRNAs has been developed by Liu using a 3D DNA origami structure. A ferrocene-tagged stem-loop DNA structure coupled with a thiolated tetrahedron DNA nanostructure at the bottom make up the 3D origami structure. On one hand, it hybridized with the lung cancer associated microRNA, while on the other, it self-assembled on a gold disc electrode surface that was modified with gold nanoparticles (Au NPs) and blocked with mercaptoethanol (MCH). At its most sensitive and linear, the newly designed genosensor detected microRNA concentrations from 100 pM to 1 μM at optimum conditions [139]. In another work, Povedano created the first bioplatform capable of electro-chemically determining the occurrence of five hydroxymethylcytosins (5-hmC) at located locations and the sensitivity to the single base. The proposed bio-platform uses a particular anticuerpo (anth-5-hmC) to recognize the epimark in target DNA, which was captured in hybridization on streptavidin-magnetic microbead (Strep-MBs), loaded with numerous horseradic peroxidase (HRP) molecules. Amperometry (-020 V versus Ag pseudoreference electrode) is used to detect the analyte in the presence of H_2O_2/hydroquinone at disposable screen-printed carbon electrodes (SPCEs). The application of the commercial biomass agents ProtA-polyHRP80 and Histostar, very hardly studied in electrochemical biosensors till now, gives remarkable sensitivities in the promoter region of MGMT tumor suppressor genes in respect of the synthetic target DNA sequence of a unique 5-hmC. The amplification factors of ProtA-polyHRP80 or Histostar were reached at 43.6 and 55.2 compared to typical secondary antibody markings. The magnification was essential for the identification of methylation events at single-nucleotide resolution, which was achieved without any target DNA amplification at 23.0 and 13.2 p.m. respectively. This platform will contribute substantially to the early detection of (hydroxy)-related illnesses, to the biology of nucleic acid methylated bases and to the propensity for cancer and progression of tumors [133].

8.3. Pharmacodynamics and pharmacokinetics

Cancers vary in response to treatment, and in a variety of cancers, few patients benefit from specific systematic methods. Therefore, if we can select patients correctly in terms of response to treatment, we can probably avoid unnecessary therapies and use the most beneficial therapies for patients, which is why prognostic markers are so important in oncology. These biomarkers are factors that indicate cancer's response or sensitivity to treatment during treatment. Predictive biomarkers are important in helping patient selection, while pharmacodynamics biomarkers (PDs) can provide information about a drug's therapeutic effects on its target. There is an increasing need for a combination of predictive and PD biomarkers in drug development. PD studies may provide information on the biologically optimal dose or planning of a targeted agent [140]. PD studies may also provide insights into conceptualization (e.g., does hitting the target of the drug lead to the desired biological effect?) and give evidence of the mechanism (e.g., does the agent reach its intended target?) [140]. Mononucleotide polymorphisms (SNPs), inherited germline DNA sequences can evaluate individual differences in drug distribution in the metabolism in some diseases. SNPs can be used as PD biomarkers for predicting specific

susceptibility in toxicity or drug activity [141].

8.4. Monitoring treatment response

The use of biomarkers is a trusted way to periodically monitor patients for controlling the possible relapses or other events that are difficult to detect at this time. Biomarkers may be determined to monitor patients with lung or colon cancer to detect an event earlier than possible with standard clinical approaches. Although tissue-based biomarkers may be controlled, such a strategy is invasive, and the use of secreted or circulating biomarkers is preferred and widely used in this strategy [140,142]. Carcinoembryonic antigen (CEA) is the most common blood biomarker for monitoring CRC after treatment. CEA levels can offer predictive data and can be helpful in the implementation of treatments. Studies by Nan et al. [143] indicated that doctors introduce a regime which more carefully monitors patients with blood CEA values of ~ 2.885 μgL^{-1} for pre-operative treatment. Chi et al. [144] developed a new strong colorectal carcinoembryonic antigen (CEA) enzyme-free voltammetric aptasensor without the presence of any antibodies. This aptasensor has been constructed using a thiolated CEA aptasensor immobiliser on a glassy carbon electrode coated with gold nanoparticles. The silica nanoparticles thionine doped have been produced by means of reverse micelle and functionally administered to p-aminophenylboronic acid by a special CEA glycoprotein conjugation epoxy-amino reaction. The analyte initially reacted to an aptamer CEA complex in the presence of objective CEA with immobilized aptamer on the electrode. Specifically identified with CEA glycoprotein, phenylboronic acid is immobilized on a silica nanoparticle based on the sugar boronic acid interaction to make the electrode a sandwich-style complex. The electron mediators were employed for the production of well-defined voltammetric signals (compared to Ag/AgCl) with the doped thionine molecules into silica nanoparticles. The peak current ranged from 1.0 pg mL^{-1} to 10 ng mL^{-1} and the limit for detection of 0.49 pg mL^{-1} at 3δ, under optimal circumstances. In batch-to-batch mode, repetitiveness and intermediate precision of 8.7% were achieved at the CEA standards.

8.5. Point of care testing

Point of care testing offers an easier and practical approach for the analysis of biomarkers without needing laboratory analysis. Thanks to point of care analysis, the management of many diseases such as cancer will be easier in terms of diagnosis, treatment and monitoring [145]. Point of care devices can employ various strategies for the determination of different analytes including biomarkers as seen in Fig. 5 [145].

Before a definitive diagnosis, biomarkers can be used for screening or risk assessment. In some cases, despite pathological examination, medical examination, routine blood, and serum tests, and x-ray examination, they are still unable to identify the primary tumor tissue. Fortunately, several tumor markers have been identified in such cases, and examining their levels in the blood and serum may help identify the primary tumor tissue. During diagnosis, biomarkers are used to monitor the progression, recurrence of the disease and select appropriate treatment methods. Therefore, the progression stage and activity of the disease can be determined. Naturally occurring short RNAs (about 22 nucleotides in length) play essential roles in a wide range of biological processes, including carcinogenesis, which they have been linked to. They are a significant target for future medical diagnostic technologies. Bettazzi et al. [146] developed an electrochemical technique for miRNA detection based on paramagnetic beads and enzyme amplification. They investigated miR-222 in human glioblastoma and non-small cell lung cancer cell lines. As a model sequence, miR 222 was selected because of its role in brain, lung, and liver malignancies. On streptavidin-coated paramagnetic beads, biotinylated DNA capture probes are immobilized. Biotinylated RNA was used to hybridize with the capture probe on the beads. A streptavidin–alkaline phosphatase reaction followed, and

the beads were exposed to the appropriate enzyme substrate. It was electrochemically monitored to see what came out of the enzymatic process. Researchers utilized a small microfluidic device to test the experiment. This device enables multiplexed analysis of eight distinct samples with a LOD of 7 pmolL^{-1} and an RSD of 15%. This method provides an innovative way of measuring miRNA expression by simultaneous processing of eight samples, with appropriate attention to the principle of multiplexed point of care testing (POCT) as seen in Fig. 5.

9. Conclusion

Early diagnosis is really difficult in most cases; hence delayed treatment, recurrence risk, and late detection of recurrence are the major issues causing cancer to be the leading disease that has the highest mortality rate. Therefore, early diagnosis, proper treatment, and careful monitoring of the treatment process can be life-saving for patients.

The pathogenesis of cancer and the causes of mutations in genes are difficult to understand topics and are still being studied. Therefore,

identifying specific biomarkers and distinguishing healthy and pathological processes with these biomarkers are important and challenging issues. Studies continue on standardization of both biomarkers and detection methods, finding sensitive, easily applicable and inexpensive methods, and developing easy-to-apply diagnostic and prognostic tests for patients [147,148]. In this case, OMICS technologies are new approaches for the better understanding of biomarkers and oncology relations. They can offer more specific and targeted treatment for patients [147].

The use of cancer biomarkers is one of the newest methods in the early diagnosis of the disease, the diagnosis of the current stage, the evaluation of the effects of treatment, monitoring the patient during treatment, predicting the return of cancer, and so on. Since biosensors are made of biological materials, they do not have any side effects and destructive effects on living tissue. On the other hand, their special design leads to good performance and very accurate results. Due to the placement of the biorecognition and transducer in a sensor, this method has a high speed and can continuously control the desired elements. In
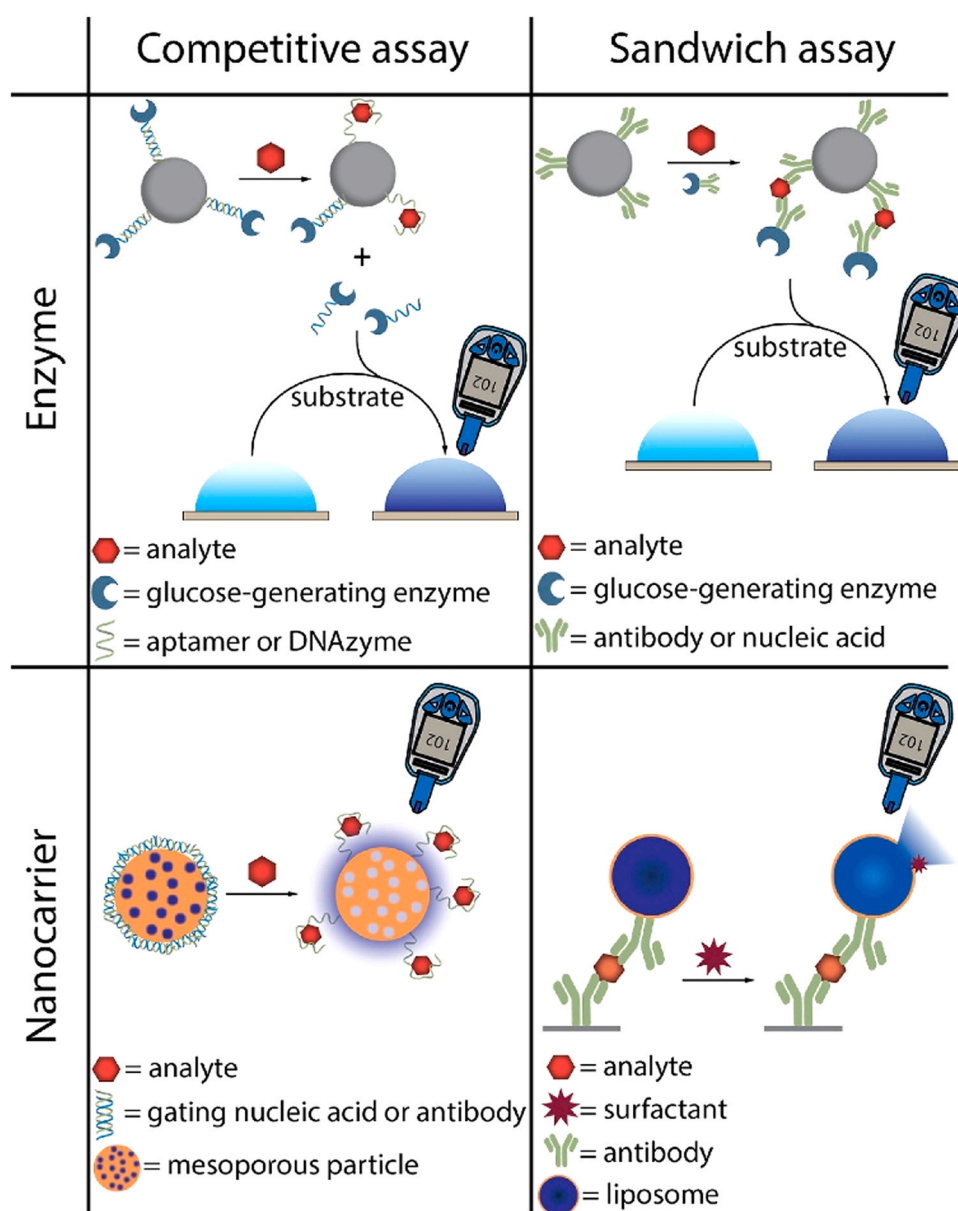

Fig. 5. Classification of the strategies used in point of care devices.
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this case, the use of nanoparticles in the construction and design of a biosensor or immunosensor increases the efficiency of the sensor in detecting cancerous biomarkers. Considering the studies carried out in the last 10 years on this topic, studies on the determination of lung cancer biomarkers with electrochemical biosensors are predominant, especially due to the increase in the incidence of lung cancer.

10. Future perspectives

Innovative primary screening is very important for a decrease in cancer mortality. The progression of cancer can be prevented or decelerated through preventive measures focused on identifying biomarkers in a timely manner with the biosensors applications. Biomarker detection-based research focusing on the development of new biosensor platforms has always been a significant and popular field of study over the past years. Discovery of novel potential biomarkers for various types of cancers in addition to currently identified ones has increased the importance and vitality of the sensitive, selective, and accurate detection of cancer biomarkers with biosensors and created new perspectives for cancer-based researches. Technological developments that show their effect in every field will also be effective in the development of biosensors for cancer biomarkers. In this context; it can be predicted that point of care devices, which will provide ease of application for healthcare professionals, and diagnostic and monitoring kits that patients can use without applying the healthcare organizations, will become common applications of the future. Moreover, Point-of-care (POC) testing integrated with electrochemical methods could be applied for successful clinical application in a hospital, doctor's office, clinic, or home. Miniaturized and portable electrochemical systems could effectively help emergency doctors because of getting fast and reliable patient results. Innovative novel nanotechnologies could contribute to the enhancement of sensitivity and detection limit of electrochemical biosensors. Moreover, multi-assay biosensors would be used for the simultaneous detection of biomarkers. Smartphone health applications are attracted attention from scientists. So, they would be focused on the integration with mobile phone applications and biosensors systems. Moreover, remote control of these kinds of smartphone-based biosensors would change the concept of the entire biosensor market. Miniaturized saliva-based diagnostic technologies will enable the use of trace amounts of biofluids to provide quick and reliable results for clinical decisions. Finally, the developed biosensors would be applied directly to the human body thanks to visible measurement techniques.

CRediT authorship contribution statement

Sariye İrem Kaya: Investigation, Conceptualization, Writing – original draft. **Goksu Ozcelikay:** Investigation, Conceptualization, Writing – original draft. **Fariba Mollarasouli:** Investigation, Conceptualization, Writing – original draft. **Nurgul K. Bakirhan:** Investigation, Conceptualization, Writing – original draft. **Sibel A. Ozkan:** Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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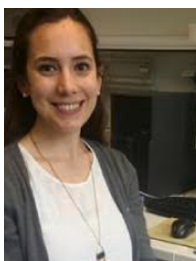
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