Application of Intelligent Sensors in Biomarker Detection Using Accurate Data Measurement and Calculation

Hao Chen*
The University of Hong Kong, Hong Kong

*Corresponding author: ch990621@hku.hk

Abstract. Cancer has been one of the most serious health issues of the 21st century. Although improvements in the treatment of cancer with new pharmaceutical products and technology remain a significant challenge for cancer biologists and oncologists, early and accurate screening and analysis technology to diagnose the disease are essential for improving the survival rate and reducing mortality and morbidity. Scientists have discovered the clinical application of cancer biomarkers for cancer diagnosis and treatment. Biosensors technology appears to be the only hope for timely diagnosis and treatment of cancer, since they exhibit remarkable analytical performance. In this review, we will discuss about basic knowledge and classification of biosensors, common cancer biomarkers and some applications of biosensors in cancer biomarker detection.

Keywords: cancer, biomarkers, biosensors

1. Introduction
Cancer is a complex, highly heterogeneous and life-threatening disease, which is prevalent all over the world. Cancer is regarded as one of the leading causes of deaths throughout the globe. According to World Health Organization (WHO), in 2018 approximately 9.6 million of deaths were caused by cancer [1]. Most cancers form malignant tumors, which can spread to other parts of body, interfering the body functions. The malignant cells spread through the lymphatic or blood vessels—a process called metastasis, causing new tumors form in other parts of the body. In recent years, significant advances in cancer research have been made, including cancer diagnosis, prevention and treatments, which have successfully improved the survival rate [2]. However, cancers still pose a great threat to people. Early diagnosis is crucial for increasing the chance of treatment. The traditional methods, including chemotherapy, X-ray, computed positron emission topographies, are still inefficient at detecting early cancer. How to improve the early treatment and management remains a serious issue. Therefore, to further reduce the death rate from cancer, techniques for early stage diagnosis of cancer are urgently needed.

According to recent study, biomarkers demonstrate their potential in cancer diagnosis and prognosis, since they can provide information about the presence of cancer in the body, the cancer status and the clinical response. If cancer biomarkers can be identified and detected specifically, they would be able to assist with cancer diagnosis and cancer status monitoring. Recently, biosensors for cancer biomarker detection appeal to scientists because of their excellent analytical capability. Most of
them have high sensitivity and accuracy. In this review, some basic knowledge such as definition and composition of biosensors as well as their classification are introduced first. Then in the following sections, we provide information about common cancer biomarkers like tumor mutation burden (TMB), human epidermal growth factor receptor 2 (HER2) and microRNAs. Finally, we describe several examples of biosensors applied in cancer biomarker detection.

2. Profile of biosensors

Biosensor is an analytical device providing quantitative information of the biological recognition element, including enzymes, nucleic acids, antibodies and so on. So far, biosensors have wide applications in drug discovery and delivery, environmental pollution detection and clinical diagnostics. A biosensor is made up of three components: a typical biosensor, a bioreceptor, a transducer and an electronic system including a display. The bioreceptors are comprised of enzymes, antibodies, aptamers, nucleic acids or cells; they can interact with the analyte of interest specifically, to generate a signal that the transducer can measure. The transducer in the biosensor converts the biochemical signal to an electrical or optical signal, in order to make the signal measurable. Then in the electronic system, the transduced signal is converted into digital form, and become ready to be displayed [3]. Figure 1 presents the structure and working mechanism of a typical biosensor. Sensing systems need the multidisciplinary development in chemistry, biology and material science. It’s also worth noticing that, biosensors can help monitor blood glucose levels in diabetics, detect pathogens, and diagnose and monitor cancer.

![Fig. 1 The Scheme of a Typical Biosensor](image)

3. Classification of biosensors

The transducer converts the molecular signal into an electric or digital signal that can be quantified, displayed, and analyzed. Based on the type of bioreceptors, biosensors can be further classified [4]. According to the types of transducers, biosensors fit into three general categories: electrochemical biosensors, optical biosensors, and Piezoelectric biosensors.
3.1. Electrochemical biosensors

Electrochemical biosensor is one of the most ordinary types of biosensor in use today. It is a sensitive analytical technique that combines electrochemical and biochemical information. Using an electrochemical biosensor can transform the biochemical information of the analyte into analytically measurable signal. The concentration of the analyte can be detected directly without using additional transduction element [5]. There are three electrodes playing a role in electrochemical sensing: a reference electrode which is made up of Ag/AgCl and can maintain the stability of the potential, a working electrode which combines a biomolecular recognition system with a physiochemical transducer, and a counter electrode in which current can be passed to the working electrode [6].

Different electrochemical biosensors have been developed to allow a sensitive detection of biomarkers. There are three types of electrochemical biosensors classified based on their distinct reactions: amperometric, potentiometric and conductometric transducers [7]. Amperometric transducers measure current produced when a potential is placed between two electrodes. The amperometric-based biosensors measure the oxidation of the product on the working electrode. They can be divided into two types according to the systems they are based on: direct or indirect. The direct system is based on a biological redox reaction which closely links biology and electrochemistry, while the indirect system involves conventional detectors measuring the biological material [8]. And the potentiometric biosensor measures the potential difference, comparing the working electrode with the reference electrode. The ions bind to the electrode surface selectively, creating an accumulation of charge at zero current, which will be monitored by the device [8]. Lastly, the conductometric biosensor can measure the analyte conductivity of the electrical current. They are often used in combination of enzymes and can be applied to study enzymatic reactions [7].

3.2. Optical biosensors

Besides from electrochemical biosensors, optical biosensors are the another most common type of biosensors. Optical biosensors are light-based sensors that measure changes in specific wavelengths of light. Their function is using optical transducers to convert the biological activities into electronic signals under the light source; and the signal strength is in proportion to the analyte concentration. To achieve the optical detection, the optical field will interact with the biorecognition element [9]. According to TD Martins et al [10], optical biosensors can be classified into two groups according to their recognition methods: probing biosensors and reacting biosensors. Probing biosensors base on the interactions between analyte and recognition elements, while reacting biosensors base on chemical processes that can generate optical changes. More commonly, optical biosensors are classified into two modes: label-free mode, which generates signals by the interaction of the analyte and the transducer, or label-based mode, which requires a label and use a colorimetric, fluorescent or luminescent method to generate the optical signal [9].

As one of the most investigated biosensors, the surface plasmon resonance (SPR)-based biosensors are widely used. Surface plasmon (SP) is a phenomenon that free electrons oscillate in the conduction band of metals [11]. The electrons can interact with the photons from an incident wave, generating a SP wave. The SPR technology utilizes the SP wave to detect the change in the refractive index due to the molecular interactions on the metal surface [12]. SPR biosensing techniques are widely applied in biomedical research, because of their advantages in monitoring the affinity binding of biomolecules. They are also often used for primary screening of molecules that are medicinally available [13].

3.3. Piezoelectric Biosensors

Piezoelectric biosensors are a class of mass-based biosensors. They can convert changes in pressure, acceleration, temperature or force into electrical charge and then measure them. Decades ago, Jacques Curie and Pierre Curie discover the piezoelectric effect; they recognized that under mechanical pressure, anisotropic crystals can generate electric dipole, namely piezoelectricity [14]. Scientists consider piezoelectricity ideal for constructing biosensors. Alternating the voltage given on the surface by two electrodes can excite the biosensor by generating mechanical oscillations of crystal. When the
crystal is put into the oscillation circuit, the frequency of oscillations is measured. By applying the piezoelectricity to biosensors, the affinity interactions can be recorded easily and no specific reagents are required [15].

4. Cancers and biosensors
Cancer, also called malignant tumor, is a larger group of diseases, and any part of the body has the possibility to be affected. Cancer is caused by abnormalities in the mechanisms that control cell division and proliferation. The abnormally grown cells, called cancer cells, can spread all over the body, invade normal tissues and damage the circulatory system and lymphatic system in the body. According to the WHO, cancer is the second leading cause of death [1]. Lung, colorectal, stomach, liver and breast cancers are the most common cancers with a high death rate. To reduce cancer mortality, early diagnosis and screening are very important.

Biomarker is defined as any measurable indicator in a bodily fluid or tissue which can indicate a normal or abnormal process. A biomarker can be used to see how well the body responds to a treatment for a disease or condition Therefore, a cancer biomarker can indicate the presence of cancer in the body. Table 1 shows some common cancer types along with their corresponding biomarkers. In most cases, a cancer biomarker is defined as a molecule, especially protein, that is secreted by the tumor and can be measured in the body fluid.

Biosensors can be applied in cancer research, including cancer diagnostic and prognostic. They can provide information about the disease status by monitoring the change in cancer biomarkers level. In the following sections, we will introduce several common cancer biomarkers and some applications of biosensors for cancer research.

4.1. Candidate biomarkers for cancer
Cancer cells are associated with genetic or epigenetic, protein alteration. The cancer biomarker refers to the considerable change of a protein, DNA and metabolite. Such changes are useful for treatment and testing. According to their usage, cancer biomarkers can be divided into three classes: predictive biomarkers, prognostic biomarkers and diagnostic biomarkers. Predictive biomarkers can respond to specific treatment and can help to select therapeutic methods. Prognostic biomarkers can provide information about the risk of clinical outcomes. They can provide precise and direct information. Diagnostic biomarkers can determine whether the patient has a particular disease [16].

4.1.1. Tumor mutation burden (TMB).
Tumor mutation burden (TMB) is identified as a novel predictive biomarker in lung cancer, although its potential use in clinics is still unclear, which requires further research. Previous studies have found out that TMB has developed the application as a predictive biomarker in tumor immunotherapy for non-small cell carcinoma lung (NSCLC) patients [17]. Chalmers et al reported that the clinical availability of TMB can be used as a biomarker; in the same study, they found that a large proportion of patients with different diseases have a high TMB and they can benefit from immunotherapy [18]. Specifically, a large number of patients with different cancers have received treatments with immune checkpoint inhibitors (ICPIs) for which TMB is established as a predictive biomarker. The basic principle of ICPIs treatment of cancer is that ICPIs can revitalize suppressed immune cells affecting the immune response against tumor cells, and thus motivate an antitumor immune response [19]. The programmed cell death protein 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are two of the most common ICPIs. TMB is discovered to be predictive of ICPIs treatment outcomes independently, supported by several studies. After treated NSCLC with PD-L1 plus CTLA-4 blockade and examined the results using whole-exome sequencing, Hellmann et al [20]. found out that positive outcomes were predicted by TMB, including improved objective response, durable benefit and progression-free survival. Rizvi et al [21] also found out the predictability of TMB by treating NSCLC patient with PD-L1 and monitoring the TMB level in patients with durable clinical benefit (DCB) and no durable benefit (NDB). The result
shows that patients with DCB had greater TMB than with NDB. Therefore, they concluded that TMB could predict the probability of clinical benefit from ICPIs.

4.1.2. HER2. The human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase. Activated HER2 can mediate cell signaling pathways, which control the cell proliferation, motility and survival. The prognostic value of HER2 in breast cancer was first reported by Slamon et al. in 1987 [22]. They found that. HER2 amplification could predict the overall survival and time of recurrence in breast cancer patients. This finding was supported by subsequent studies. HER2 overexpression and HER2 gene amplification can cause uncontrolled and unregulated cell growth, which is related to poor outcome of HER2-positive breast cancer patients [23].

4.1.3. MicroRNAs. MicroRNAs (miRNAs) are 19–25 nucleotides RNA molecules with important roles in gene silencing. miRNAs are a class of non-coding RNA which can regulate gene expression. miRNAs often have different expression patterns, sometimes even aberrant, when they are under different pathological and physiological conditions, such as cancer. MicroRNAs have been reported to be significant in cell growth as well as cancer prognosis. These molecules are developed to be potential biomarkers in cancer diagnosis. At the early stage of cancers, more circulating miRNAs are dysregulated. By means of polymerase chain reaction (PCR), microarray or deep sequencing, the differential expression of miRNAs can be monitored, and thus can be applied in cancer clinics [24]. In addition, when circulating miRNAs are acting as diagnostic biomarkers, they can help distinguish different subtypes of cancer. Taking NSCLC as an example, it can be sub-classified into two types: adenocarcinoma (ADC) and squamous cell carcinoma (SCC). The expression of miR-9-5p falls significantly in ADC patients compared with SCC patients. Also, it was discovered that some ADC- and SCC-specific miRNAs express differently regarding to RNA sequencing [24].

| Biomarker type | Cancer | Biomarker name |
|---------------|--------|----------------|
| Predictive    | Breast cancer | BRCA 1/2, HER2, TOP2A |
|               | Colorectal | RAS, BRAF |
|               | Squamous cell carcinoma | HER, KRAS |
|               | NSCLC | ROS1, BRAF V600E |
|               | Melanoma | BRAF V600E/K |
| Prognostic    | Breast cancer | HER2, TOP2A, 70-gene expression profile, 58 gene RNA expression profile |
|               | Prostate cancer | tPSA |
|               | Acute Myeloid Leukemia | EGR1 |
| Diagnostic    | Bladder cancer | Aneuploidy for chromosomes 3, 7, 17, Loss of 9p21 |
|               | Breast cancer | MG, CK19 |
|               | Colorectal cancer | Multi-target DNA, Hemoglobin assay |
|               | B-cell chronic lymphocytic leukemia | Alpha satellite region, 12p11.1-q11 |
|               | Ovarian cancer | BRCA1 and 2 gene mutation |
|               | Prostate cancer | PCA3 |
4.2. Biosensors for cancer detection.
As is mentioned in the previous section, cancer treatment requires early and accurate diagnosis. Therefore, efficient and accurate methods for cancer detection and treatment are in demand. In recent years, applications of biosensors in cancer research have been developing given that they exhibit high-quality analytical performance and real-time analysis in early stage of the disease. Specifically, biosensors show strong capability for cancer biomarker detection [25]. In this section, several types of biosensors that are used for cancer biomarker detection are introduced.

4.2.1. Electrochemical biosensors in cancer detection. There are several electrochemical biosensors that are developed for miRNA detection. In 2013, Wang et al [26]. reported an electrically magnetic-controllable electrochemical biosensor for the detection of miRNAs. The biosensor was based on a homemade electrically magnetic-controllable gold electrode, and was highly sensitive for low concentration miRNAs. It was also able to distinguish similar miRNAs. In the work of Lin et al. [27], they demonstrated an enzyme-based E-DNA sensor for miRNAs detection. Basically, the sensing process involved conformational change, electrocatalytic reaction and surface control. They used a DNA tetrahedral probe and enzymatic amplification to establish the biosensor. The tetrahedral structure could control the orientation and density of the probe. High sensitivity to detect low concentration of miRNAs and great ability to differentiate similar miRNAs were well achieved.

4.2.2. Optical biosensors in cancer detection. Optical biosensors are commonly applied for cancer biomarker detection. In 2016, Ding et al [28]. reported a FRET-based (fluorescence based) optical biosensing technique for the detection of telomerase activity in cancer cells. The sensor involved a fluorescence probe DNA, a quencher DNA and a telomerase substrate (TS) primer. The quencher DNA would be replaced from the probe DNA when interacting with the telomerase, enhancing the fluorescence, and thus the telomerase activity could be monitored. This method was highly sensitive and could successfully measure the telomerase activity in crude cancer cell extracts. Another type of optical biosensor is the opto-fluidic ring resonator (OFRR). In 2010, Gohring et al. [29] reported a label-free biosensing method to detect the HER2 in human serum, by means of the OFRR. Microfluidics and optical ring resonator were combined to achieve the rapid label-free detection. By using this biosensing technique, HER2 at medically relevant concentrations in serum could be detected, and therefore the disease progression could be monitored.

4.2.3. Piezoelectric biosensors in cancer detection. Another common type of biosensors is piezoelectric biosensors, and they also have some applications in cancer biomarker detection. Capobianco et al. [30] developed a piezoelectric microcantilever biosensor, which achieved real-time and label-free detection of HER2. They used 3-mercaptopropyltrimethoxysilane (MPS)-coated (PbMg1/3Nb2/3O3)0.63–(PbTiO3)0.37 (PMN-PT)/tin and lead zirconate titanate/glass, and on the MPS surface the single-chain variable fragment was immobilized. Therefore, HER2 could be detected sensitively in a background of 1 mg/mL bovine serum albumin. In 2013, Su et al. [31] developed a new piezoelectric biosensor, of which the transducer was lead zirconate titanate (PZT) ceramic resonator. The sensor included a sensing unit and a control unit, both of which were ceramic resonators and were connected parallelly. This biosensor could successfully detect some cancer biomarkers such as prostate specific antigen (PSA) and α-fetoprotein (AFP), with high sensitivity and effectiveness.
Table 2. Biosensors for Cancer Biomarker Detection [25, 32, 33]

| Biosensor type | Cancer biomarker | Transducer       |
|----------------|------------------|------------------|
| Electrochemical | mir-126          | Voltametric      |
|                | NSE              | Voltametric      |
|                | VEGF165          | Voltametric      |
|                | EGFR             | Amperometric     |
|                | MiR-21           | Voltametric      |
|                | PSA              | Amperometric     |
| Optical        | VEGF165          | Fluorescent      |
|                | CEA              | SPR              |
|                | p53              | SPR              |
|                | HER2             | Optical fluorescence |
|                | CA-125           | Chemiluminescence |
|                | PSA              | SPR/Optical fluorescence |
| Mass-based     | AFP              | Piezoelectric    |
|                | PSA              | Piezoelectric    |
|                | HER2             | Piezoelectric    |
|                | Ferritin         | Quartz crystal microbalance (QCM) |
|                | TIMP1, HER2      | Surface acoustic wave (SAW) |
|                | ALCAM            | Suspended microchannel resonator (SMR) |

5. Conclusions
With the development of biosensing techniques, more and more cancer biomarkers can be detected and monitored and can be utilized for cancer diagnosis and treatment. Hopefully, the application of biosensor in cancer research can lead to effective treatment, decreased mortality and improved survival rate. However, there are still some challenges faced by scientists. The sensitivity, selectivity and cost-effectiveness of biosensors still need to be further improved in order to achieve commercialization. Also, the long-term stability of the biosensor needs to be taken into consideration for clinical use [25]. In addition, since some of the biomarkers often express non-specifically, wrong diagnosis and treatment are likely to occur. Therefore, we should obtain a deeper understanding of the biomarkers in cancers [34]. Nevertheless, in general, it is doubtless that biosensing technique is a promising tool in cancer research which enables early-stage diagnosis and treatment of cancer.

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