Biosensors And Their Applications in The Detection of Cancer Biomarkers

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Abstract. Cancer is a critical disease that seriously affects human health and has a high mortality rate. Therefore, it is very important to effectively improve the survival rate of patients. The low survival rate of cancer is because it can't be effectively diagnosed in the early stage. When most cancers are in the advanced stage after imaging examination, cancer cells have spread in the body or even metastasized, and the difficulty of treatment will be greatly increased after the metastasis occurs. Therefore, early detection of cancer is one of the key factors to improve the survival rate. However, imaging tests such as computer tomography and nuclear magnetic resonance imagining cannot accurately detect small lesions in the early stage of cancer, which also leads to low detection accuracy. In recent years, the interest in the development of biosensors has become more and more intense. Biosensors have shown excellent detection performance and real-time detection capability. Besides, biosensors can also detect biomarkers with extremely low content in detection samples, which is helpful for early cancer diagnosis. In addition, biosensors have also shown the potential to simultaneously detect multiple biomarkers.

Keywords: Cancer, Biomarkers, Biosensors.

1. Introduction

Cancer is one of the main causes of death worldwide, with nearly 10 million (or nearly one in six) deaths in 2020 caused by cancer, The transformation of a normal cell into a tumour cell is a multi-stage process, usually from a precancerous lesion to a malignant tumour, due to a combination of genetic and external factors. Many cancers have the potential to be cured if they are detected early and treated correctly and effectively.

Early detection of cancer consists of two components, namely: early diagnosis and screening. It is difficult to achieve through traditional diagnostic methods, such as ultrasound imaging and MRI, due to the limitations of detection methods and the precancerous nature of cancer itself which is not easily detected [1].

Cancer biomarkers include nucleic acids, proteins, sugars, small metabolites, cytogenetic and tumour cells in body fluids, which can not only be used to detect cancer risk and early diagnosis, but also can be used to predict treatment efficacy, toxicity and recurrence, facilitating the early detection of cancer, while cancer diagnosis based on multidisciplinary techniques may be and is gradually being used as an alternative to traditional techniques [2].

Biosensor technology has been of great interest to the scientific community because of its superior analytical and real-time measurement capabilities, its ability to reuse biometric molecules, and its ability to avoid the time lag between sample preparation and analysis. In recent years, as engineering continues to evolve, biosensor manufacturing technologies have improved and are able to detect increasingly lower minimum detection limits, allowing for the measurement of very low levels of biomarkers and small biochemical changes in physiological samples, and facilitating early cancer diagnosis [3].

2. Components for biosensors

2.1. The metal organic framework material for biosensors

MOFs (Metal Organic Frameworks) can be used as the basic body of fixed probes for electrochemical biosensors due to their stable framework structure, controllable morphology and other excellent characteristics. Many electrochemical biosensors based on metal-organic frameworks that can be used as early cancer markers have been developed to reduce the mortality of early cancer. There is a metal-organic skeleton material.

MIL (Materials of Institute Lavoisier frameworks) type MOFs materials can generally be divided into two categories: one is MIL materials formed by transition metals (such as chromium, gold, etc.) and lanthanide metals through coordination reaction with succinic acid and glutaric acid; The other is made of Fe3+, vanadium, chromium and other metals through coordination reaction with terephthalic acid (pyromellitic acid). MIL materials have the advantages of small pore size and high stability at 20 to 30 degrees Celsius. In addition, the carboxyl groups on the surface of these materials increase the polarity of molecular sieves and improve their dispersion. Because of these advantages, MILs become excellent nanofiller materials in mixed matrix membranes.

Because MIL materials have very high transition metal sites and a huge specific surface area, MIL materials are widely used in the field of catalysis [4]. Horcajada et al. [5] used Fe (III) - MIL-100 to catalyze Friedel Crafts reaction of benzyl chloride and benzene 1); the catalytic activities of Fe (Fe-MIL-100 and Cr(III)-MIL-100 were compared. The results showed that in the catalytic reaction of Fe(III)-MIL-100, the conversion depth of the substrate reached 100% in 10 min, and the selectivity was also 100%. But Cr(III)-MIL-100 had no catalytic effect. The experiment showed that when different metal central ions were used, the catalytic performance of MIL series materials would be greatly affected.

MIL series materials are one of the typical representatives of MOFs materials. Because of their large specific surface area, high crystallinity and controllable ordered pore structure, they have become a hot spot in the research field of MOFs materials. Now people are trying to use MIL series materials in biosensors to test the adsorption and separation of some gases, as well as some heterogeneous catalytic reactions, and it has achieved certain results.

2.2. Transducer

Transducers are devices that can convert biological responses (recognition signal events) generated by the interaction of biometric molecules with biomarkers into measurable signals by means of biomolecular recognition elements, whose functions are mainly achieved by electrochemical, optical, piezoelectric and thermometric methods [6].

Electrochemical transducers are the most widely used, which can convert the electron transfer rate, surface conductivity and redox potential generated by the reaction into measurable electrochemical signals（current, potential, conductance, impedance, etc.）as the electrochemical signal generated by the interaction of biometric molecules and biomarkers is linearly with the concentration of the biomarker [7].

Optical transducers have the potential to replace conventional transducers for their huge advantages in terms of detection, measurement, performance, and sensitivity. Optical biosensors are classified as colourimetric, fluorescent, photometric, fibre optic, and SPR biosensors, which have the ability to test and analyze chemical and biological categories using light absorption, fluorescence, luminescence, total internal reflection, and other optical phenomena [8].

Mass-sensitive transducers that can directly detect mass changes caused by binding between biomarkers and receptors on the sensor and have the advantage of not requiring the use of markers are mainly classified into three types: piezoelectric, surface acoustic wave and micro-cantilever and of which the piezoelectric transducer, which converts mass changes on its surface into frequency changes based on the piezoelectric effect of quartz crystals, is the most widely used [9].

3. Categories of cancer biosensors

Cancer has always been one of the most important health problems besetting the world. Such detection methods are an important part of research on cancer diagnosis and treatment. Now the electrochemical biosensor, which is being actively developed, realizes a remarkable sensitive cancer biomarkers detection, strong repeatability and high accuracy through portable and low-cost equipment, which can replace traditional technology. Electrochemical biosensors based on different substances offer different advantages.

3.1. Electrochemical Biosensor

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3.1.1 Electrochemical biosensor based on nucleic acid

Due to gene regulation, normal cells can't appear, and unlimited division. If the body is under the action of carcinogens and abnormal gene regulation, there may be an abnormal proliferation of new creatures-tumor, which originated from epithelial tissue malignant tumors become "cancer". Multiple abnormal accumulations occur during the transformation of normal cells into cancer cells and significant changes in the content of some genetic materials can be used as nucleic acid-based markers for diagnosis [3], which can be diagnosed at an early stage, even in the absence of obvious physical signs.

In recent years, the electrochemical sensor based on nucleic acid has developed rapidly. In 2019, a novel homogeneous electrochemical biosensor was introduced by Jiafu Chang et al. [10]. This electrochemical sensor utilizes the homogeneous electrodeposition principle of MOFs, and MOFs based on double-stranded DNA termination can simultaneously and sensitively detect multiple single-stranded small molecule RNA in a single detection. The biosensor has high sensitivity and excellent selectivity as well as excellent performance. The use of a biosensor based on MOFs material for the analysis of human serum samples in this report has the advantage of high accuracy and reliability. In addition to the above method, the detection of other tumour markers can be realized only by changing the DNA structure of the sealed end, and the device provides a homogeneous electrochemical platform for the detection of a plurality of biomarkers so that the device has great advantages in early diagnosis of various indicators with cancer precursors in the body, and cancers can be detected and treated in advance.

In the 2020 article, Pan Fu et al. reported an electrochemical sensor that can detect miRNAs in a variety of cancer cells was introduced [11]. This method proved the feasibility of sensitive detection of miRNA21 and miRNA155 in cancer cells. At the same time, this method can also significantly increase the intensity of electrical signals. Has high selectivity to miRNAs with similar sequences, improves the detection limit to 249fM and 11.63fM for the miRNAs 21 and 155, enhances the sensitivity and accuracy of equipment, reduces the manufacturing difficulty, and enables the biomarker to be capable of simultaneously detecting a plurality of miRNAs in clinical practice to have great potential.

3.1.2 Electrochemical sensor based on protein

At present, the most commonly used detection method in clinical practice is enzyme linked immunosorbent assay. This method takes a lot of time and is costly, so there is a high demand for developing low-cost, fast and efficient alternatives. Since detecting only one biomarker does not have enough specificity, the accuracy for specific cancer detection is greatly reduced, so that a biosensor for detecting a plurality of biomarkers at the same time is very important, and detecting a plurality of

substances at the same time can reduce cost and save time, and the research and development purpose of the protein-based biosensor which has been developed now is to simultaneously detect a plurality of cancer protein biomarkers.

As an ideal nanomaterial, carbon nanotubes have been widely used for the fabrication of electrochemical biosensors due to their independent structure, low cost, high stability and good biocompatibility. A variety of protein-based carbon nanotube electrochemical sensors based on carbon nanotubes were introduced in the 2017 article Jiafu Chang et al. [10]. In order to further improve the performance of electrochemical immunosensors in clinical diagnosis, all of the sensors are designed based on novel promising nanomaterials. The method has the advantages that (1) the carbon nano tube or the carbon nano tube hybrid material is used as a support platform, and high-efficiency electron transfer from the surface of the modified electrode to the center of an electronic medium is realized; (2) using carbon nanotubes or functional carbon nanotubes as markers to enhance detection signals

Overall, whether based on nucleic acid or protein electrochemical sensors in recent years have made great progress in the study, after adding new materials to improve the accuracy, reduce the cost, after years of research, electrochemical sensors for early cancer detection have made great contributions to meet the greatest demand for early detection of cancer-the detection of multiple markers at the same time.

3.2. Optical Biosensors

3.2.1 SPR/LSPR based biosensor for cancer detection

Surface plasmon resonance (SPR) based biosensor uses an optical phenomenon absorption of light energy to detect the optical signal(refractive index) change of solution on metal surface due to complex formation or dissociation by immobilizing molecules (ligands) with specific recognition properties on the surface of the metal film detection of biomarkers in testing solution [12]. Due to their ability to detect molecular interactions lively and without the need to label molecules, SPR systems are developing rapidly and are used in a wider and wider range of biological applications [13].

In 1990, BiacoreAB in Sweden developed the world's first commercially available SPR biosensor, BiacoreTM, and the US, Japan, Germany, the Netherlands, and China have distributed their own SPR products since then. Robert B et al. (1996) demonstrated bispecific antibody and its dual specificity for carcinoembryonic antigen and tumour necrosis factor alpha by two-step analysis with BiacoreTM and targeted cytokines in tumours using bispecific antibodies against the carcinoembryonic antigen and tumour necrosis factor α [14]. Sato et al. developed a biosensor to detect the specific point mutations of K-ras gene by SPR mechanism, and Their research results prove that SPR sensor can detect DNA with super sensitivity when fitted with special methods [15]. Piliarik et al. developed an SPR biosensor to detect human chorionic gonadotropin and activated leukocyte adhesion molecules in plasma, which allows protein markers to be screened out at low levels in plasma [16].

In addition, when conducting nanoparticles are small enough to be below the incident wavelength, conducting a mixture of incident light with electrons and electromagnetic waves produces localised surface plasmon resonance (LSPR), whose absorption and scattering spectrum significantly influenced by the composition, category and size of the nanoparticles [17]. Based on this phenomenon, Qianying Zhao et al. developed a new method for the detection of squamous cell carcinoma antigens that allows the LSPR biosensor to be reused, and its rapid, simple and reusable features make it promising for clinical diagnosis and serological diagnosis [18]. Nguyen et al. developed an LSPR-based biosensor and proposed an ultrasensitive detection strategy for tumour-specific mutations and circulating tumour DNA methylation of the PIK3CA gene based on LSPR and gold nanoparticle demonstrating that circulating tumour DNA carrying tumour-specific mutations and methylation is a promising biomarker for non-invasive cancer assessment [19].

3.2.2 SERS-based biosensing of cancer cells and biomarkers

Raman spectroscopy is molecular vibrational spectroscopy that senses the characteristic vibrational patterns of molecules, by which the detailed information on the structure and conformation of macromolecules is provided [20]. When the intensity of the Raman signal increases dramatically due to the sorption of an enhancer onto a rough electrode surface, which is called Surface-enhanced Raman scattering (SERS), and biosensing tools based on this effect have shown high sensitivity and applicability of cancer biomarkers detecting. Sujuan Ye et al. developed a combined biobarcode probe and hybridization chain reaction amplification method for amplification of asymmetric signals, which improves the detection limits of microRNA and ATP by surface-enhanced Raman scattering. As a result, the detection limit of microRNA and ATP by SERS exceeded 11 orders of magnitude, enabling the simultaneous detection of multiple biomarkers at different concentrations [21]. Guofeng Wang et al. developed an immunoassay to investigate the simple diagnosis of mucin MUC4 and for the first-time detected mucin MUC4 in serum samples from cancer patients. The results showed that sera from PC patients were able to produce a measurable SERS response to MUC4 and also demonstrated that the assay was superior to conventional analysis in terms of detection limit, readout time and sample size required [22].

3.3. Mass sensitive transducer

3.3.1 Piezoelectric sensors

Piezoelectric sensors include quartz crystal sensing coated with gold electrodes. Piezoelectric quartz crystals are extracted from single crystals that vibrate at frequencies that depend on their own physical properties and are influenced by the electrodes, producing a detectable signal, a principle that is used as a microbalance (QCM) to detect changes in the quality of the sensor surface [23]. Laetitia Nowacki et al. developed a sensing method to detect cell's morphological changes between the cell and matrix during programmed cell death lively using QCM technology, demonstrating that for low doses. They found that recording dissipation shifts could detect early cellular responses and allow quantifying the efficiency of drug effects in less than 4 hours without the need to set additional markers [24]. Xueming Li et al. (2015) developed a method to study the binding thermodynamics and kinetics of non-immobilized cancer cell surface carbohydrate-protein interactions based on QCM biosensor, monitoring the binding and dissociation between cell surface carbohydrates and a range of lectins lively without the need for labelling to assess cell surface glycosylation [25].

3.3.2 Micro cantilever sensor

The microcantilever-mounted sensor is a more advanced biosensor relative to other types, and the cantilever sensor is a simple structure that can detect very small changes in mass. It is ideal for high-accuracy and high-sensitivity sensors [26]. small number of molecules attached to the surface of the cantilever beam will make the bending deflection and vibration frequency of the cantilever beam change. Since its invention, it has been widely studied and applied in the biomedical detection field with the advantages of small volume, low cost, high sensitivity and so on. The research indicates that the micro-cantilever sensor can be added on the basis of carbon nanotubes to realize the rapid detection of cancer markers [27]. Firstly, a detection probe containing a tumour marker aptamer is carried out on a carbon nanotube cantilever beam, And then put that detection probe into a sample to be detected. The tumour mark in that sample forms a complex with the nucleic acid adaptor on the detection probe through a specific reaction and is attached to the cantilever, And the mass is positively correlated with the concentration of the tumour marker in the sample to be detected, and the mass change of the compound on the micro-cantilever beam causes the bending displacement or the resonance frequency of the cantilever beam to be changed so that the detection of the tumour marker can be performed.

In addition, when the size of the conducting nanoparticles is smaller than the incident wavelength, the mixed incident light of the conduction band electrons and electromagnetic waves produces an

optical phenomenon known as LSPR, an absorption and scattering spectrum significantly influenced by the composition, shape and size of the nanoparticles.

4. Conclusions

This paper introduces the materials that can be used to make cancer biosensors, the converters that can be used to carry on biosensors, and the research progress of various cancer biosensors in recent years. These techniques diagnose the presence of cancer cells in the patient's body by invasive means in the case of biopsies, by fixing the cells or examining the tissue by morphological methods. However, as early-stage cancer cells are difficult to detect by tissue excision, these tests are not able to detect early-stage cancer and it is important to develop non-invasive means of cancer detection. Compared with the electrochemical biosensor and the optical sensor with relatively mature technology, the development of the mass-based sensor is still in the initial stage, and the mas-based sensor has wide application prospects due to the advantages of small volume, low cost, high sensitivity and the like, and should be taken as one of important research directions of scholars.

Cancer, as one of the most common malignant tumours originating from epithelial cells, has seen an increasing death rate in the past few years. If cancer can be found in time at the early stage, the survival rate can be greatly improved. Therefore, it is particularly important to develop a cancer biosensor that is fast, effective and easy to popularize. With the development of biotechnology and medical technology, tumour biomarkers emerge at a historic moment. Current research progress indicates that biosensors still remain in the complex process of detection object acquisition. The detection method is not suitable for the non-professional stage, but it still has made great progress in recent years. Future research can be further toward the direction of wearable detection equipment and tactile sensor at a lower cost to produce convenient and fast cancer biosensors.

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