Application of biosensors based on nanomaterials in cancer cell detection

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Abstract. Cancer, killing millions of people every year, is the most serious disease in the world. The survival of cancer patients is closely related to the diagnosis of cancer cells. Therefore, in the early stage of cancer, the detection of cancer biomarkers from cellular level is of great significance to improve the survival rate of cancer patients. Nanomaterials-based biosensors play the increasingly important role in the treatment of cancer, owing to their ultra-high sensitivity and high selectivity. Nowadays, it is widely used in the detection of cancer cells to provide reference for cancer treatment. Herein, the present minireview summarizes application of nanomaterials-based biosensors for the detection of cancer cells, such as colorimetric biosensors, fluorescence biosensors, surface-enhanced Raman scattering (SERS) biosensors, electrochemical biosensors and other types of biosensors. We further introduce the construction principle of these biosensing methods, and compare the advantages of these biosensors in detecting cancer cells.

1. Introduction
Cancer is the second cause of death in the world, one in six of the death cases is caused directly by cancer. Cancer is a kind of disease which is difficult to be found in time for treatment, especially in low-income countries [1]. Only 26% of low-income countries have the ability to provide complete pathology service for cancer in public sector.

The terms used for cancer are usually malignant tumors and neoplasms, which means cancer acts on one part of the human body [2] and metastasizes to other parts of human body. Compared with other diseases, cancer has a high fatality rate. And the treatment of cancer is a quiet painful process. The cost of treatment for cancer is also a whopping price for normal people. In a word, cancer is a kind of disease that makes people suffer from both spirit and physical aspect. The detection of cancer biomarkers is therefore an important method to find the initial positions of cancer cells to treat cancer in the early stages of cancer [3]. On the other hand, cancer biomarkers detection can find the concrete positions of tumors for precise drug delivery.

The regular detection methods for cancer are examination of surgically removed tissue and aspirated and exfoliated cells in the morphology aspect. The tradition methods for cancer detection in hospital are computed tomography (CT), X-ray and nuclear magnetic resonance imaging (NMRI) [4].
These methods can detect cancer by imaging the tumor in the human body. These methods have a limitation that they cannot detect the mechanical properties of cancer cells which correspond to cancer progression. The histological analysis also disregards the cancer cells with insufficient manifestations. Analytical techniques like micropipette aspiration, magnetic bead twisting or atomic force microscopy (AFM) have been widely used for single cells analysis [5]. To analyze cancer cell’s mobility, detection of cancer cell with local interest point detectors was carried out [6]. Unfortunately, these detection methods also show the disadvantage of complex operation. Development of a simple, sensitive, and selectivity detection method for cancer diagnosis has been receiving increasing attention.

Using nanomaterials as probes, various biosensors were developed in the field of cancer diagnosis and biomolecular imaging [7]. The detection strategy of cancer biomarkers based on biosensors exhibits supersensitive and high selective upon when combining nanomaterials and biological recognition molecules [8]. Among these biosensors, nanomaterials with difference optical and electrochemical properties greatly improve the sensitivity of developed biosensing method, while biological recognition molecules such as aptamers and antibodies are benefit for enhancing the selectivity of the designed biosensing detection method. Such high selectivity is very suitable for analyzing different kinds of cancer biomarkers in complex biological sample. Using the advantages of nanomaterials and biological recognition molecules, biosensors are widely used to develop virous diagnosis method for cancer monitor with cancer biomarkers as detection target [9]. In this minireview, we will focus on the application of nanomaterials-based biosensors in cancer cells detection.

2. Application of different types of biosensors cancer cells detection

2.1. Colorimetric biosensors

The key to the development of colorimetric biosensors is effectively to transform chemical or biological reactions into color signals. Using nanoparticles such as noble metal nanomaterials as colorimetric sensing probes, different kinds of colorimetric biosensors are widely developed because of their advantages of easy operation, low cost, rapid reading and no need for complex instruments. However, these colorimetric biosensors always show some defects.

For example, in clinical diagnosis, the relative low sensitivity and selectivity limits the application of colorimetric biosensors in cancer cell detection. To solve this defect, Xiao et al. used Au nanoparticle-decorated Bi$_2$Se$_3$ (Au/Bi$_2$Se$_3$) nanosheets as sensing probe to develop a novel colorimetric biosensor for detection of cancer biomarker carcinoembryonic antibody (CEA) [10], as shown in Figure 1. The enhanced sensitivity of such colorimetric biosensor in this study is attributed to the low redox potential and typical topological insulating properties of Bi$_2$Se$_3$ nanosheets, which allows electrons to gather on the surface to form synergistic catalytic effects. Through this effect, the detection limit of the developed colorimetric biosensor was 160 pg/mL for CEA.

![Figure 1. Schematic illustration of the colorimetric biosensing platform for the detection of cancer biomarker [10].](image-url)
As for pancreatic cancer (PC) antigens detection, Yang et al. used a magnetic iron oxide (IO)-coated gold nanorods (MGNRs) nanocomposite to design an enzyme-free colorimetric immunosensor for ultrasensitive detection of carbohydrate antigen 19-9 (CA19-9) and mucin 1 (MUC1) [11]. Owing to the high electron transfer capability of the used MGNRs, catalytic rate of colour reaction was accelerated greatly, resulting in the use of a small volume of real sample in experiments. And the detection limits for CA19-9 and MUC1 were $3.5 \times 10^{-5}$ U/mL and $5.2 \times 10^{-6}$ U/mL, respectively.

2.2. Fluorescence biosensors

Application of fluorescence biosensors for detection of cancer cell shows super sensitivity and high selectivity. Among these fluorescence biosensors, fluorescence resonance energy transfer (FRET)-based design ideas have attracted more and more attention. The key to develop FRET-based fluorescence biosensors is to regulate the distance between molecules (or nanoparticles). Taking the fluorescent molecule (i.e., organic dyes) as an example, they are often toxic and easily photobleached, which limits their application in cancer cell detection. Thus, nanoparticles with fluorescence effect are synthesized and used to construct fluorescent sensors.

Afzalinia and Mirzaee used FRET strategies and “sandwich-type” hybridization of aptamer to design a ultrasensitive fluorescence biosensor for the detection of MicroRNA-155 (miRNA-155) [12]. The functionalized La (III)-metal-organic framework (MOF) and silver nanoparticles (Ag NPs) were used to construct the energy donor-acceptor pairs to utilize successfully FRET strategies. By optimizing experimental conditions, the detection limit was 0.04 ppb (ng. mL$^{-1}$) or 5.5 fM of the miRNA-155 cancer biomarker. The reported fluorescence biosensing method can be used as a candidate for cancer diagnosis in clinical treatment.

Using FRET principles, Zhao et al. developed a ratiometric fluorescence biosensor for the detection of cancer cells [13]. Compared to conventional fluorescent biosensing method, in this work, they designed a quencher- and fluorophore-labelled dsDNA and another fluorophore-labelled dsDNA. The used ratiometric fluorescence oligonucleotide probe can effectively eliminate the influence of other experimental parameters, and exclude false positive results. Thus, the telomerase activity was qualitative and quantitative analysis in cell extracts with a detection limit of 102 HeLa cells.

2.3. SERS-based biosensors

Surface-enhanced Raman scattering (SERS) is powerful analysis tool in clinical diagnosis, biomedical science, and environmental field. In recent years, SERS-based biosensing techniques have been successfully designed for the detection of cancer biomarkers, pathogen microorganisms, and viruses [14].

Although SERS-based biosensing techniques are widely used in different fields, development of the real SERS-based sensors for practical application is rarely reported. Benefiting from ultra-sensitivity of SERS sensing method, Kim et al. designed a portable SERS spectrometer to detect breast cancer from human tears [15]. The Au-deposited SERS substrate was shown in Figure 2. Combined with the identification algorithm based on multivariate statistics, they could control the standard deviation of reliability and reproducibility to within 5%.

Wang et al. used porous CuFeSe$_2$/Au heterostructured nanospheres to construct a SERS-based sensor for lung cancer and the corresponding cancer biomarkers detection [16]. In this study, p-aminothiophenol (4-ATP), as the Raman-active sensing molecule, was modified onto the surface of
CuFeSe₂/Au heterostructured nanospheres. And thus, the gaseous aldehyde molecules can easily adsorb onto nanoparticles surface by C=N bond, and the detection limit was 1.0 ppb. Additionally, folic acid (FA)-modified CuFeSe₂/Au heterostructured nanospheres could be used to recognize and detect A549 cells.

2.4. SPR-based biosensors

As one of label-free detection methods, surface plasmon resonance (SPR)-based biosensing strategies are used to design a series of SPR biosensors for the detection of cancer cells [17]. As shown in Figure 3, Tothill et al. designed a SPR biosensing platform for cancer biomarkers detection in human serum samples [18]. In this study, total prostate-specific antigen (tPSA) was used as prostate cancer biomarker. The detection limit for tPSA detection in 75% human serum was 2.3 ng mL⁻¹ if antibody-functionalized 20 nm gold nanoparticles was used while that was 0.29 ng mL⁻¹ with 40 nm gold nanoparticles. The obtained detection results from SPR biosensor were compared with quartz crystal microbalance (QCM), demonstrating the developed SPR biosensor chip can be used for cancer biomarker screening.

Breast cancer biomarkers, such as breast cancer gene-1 early onset (BRCA1) and breast cancer gene-2 early onset (BRCA2), can also be detected by using SPR based biosensors. Hossain et al. used a numerical simulation of the graphene-coated fiber SPR biosensor for detection of breast cancer biomarkers, BRCA1 and BRCA2 [20]. To achieve successfully breast cancer biomarkers detection, the attenuated total reflection (ATR) method was used in SPR biosensor, and the changes in SPR angle and surface resonance frequency (SRF) was applied to probe deoxyribonucleic acid (DNA) hybridization. Using such SPR biosensor, breast cancerous cell and non-cancerous cell were distinguished, and offer a new detection method for breast cancers detection.
2.5. Electrochemical biosensors

Development of more sensitive and selectivity biosensing method for cancer cells detection is still necessary in clinical diagnosis. Electrochemical devices exhibit efficient signal transduction, such as from voltage to current. When electrode surface was modified by nanomaterials or other conductive molecules, the sensitivity of the proposed electrochemical detection method is greatly enhanced. And thus, electrochemical biosensors are widely used for the detection of various targets [21]. In this section, we focus on the application of electrochemical genosensors and electrochemical immunosensors in cancer cells detection.

For cancer detection, MicroRNAs (miRNAs) can be used as an ideal cancer biomarker [22]. In general, miRNAs are a kind of RNA which is naturally generated during cell metabolism, and the expression levels of circulating miRNAs are able to correlate with cancer [23]. Therefore, the number of miRNAs can be used for monitoring human cancers.

Labib et al. developed a three-mode electrochemical biosensor for the detection of ultralow miRNAs concentration [24]. They used three detection modalities including hybridization (H-SENS), p19 protein binding (P-SENS), and protein displacement (D-SENS) to analyse five miRNAs. Their results showed that such electrochemical biosensor can respond to a wide range of concentrations from 10 aM to 1 μM and the detection limit was up to \( \sim 90 \) molecules miRNAs per 30 μL sample. Increasingly, fluorescent labelling or PCR amplification is not required to acquire such a low detection concentration. As for cancer cells detection, electrochemical nucleic acid biosensors, or so-called electrochemical genosensors, has been widely designed for cancer diagnosis using miRNAs as excellent cancer biomarkers. But we need to point out that detection of miRNAs in real human samples are still challenging. Besides miRNAs, DNA can also be used as a candidate to develop nanotechnology-enhanced electrochemical biosensors to detect cancer cells [25].

As shown in Figure 4o, the use of electrochemical immunosensor to detection of cancer cell is additional choice because such biosensing method shows high sensitivity [4]. The combination of electrochemical detection method and immunosensors to design novel biosensor for cancer cells detection are fast and sensitive, allowing us to detect ultralow concentrations of cancer biomarkers. Especially, upon introducing different nanomaterials, we can detect simultaneously multiple cancer biomarkers. Limoges et al. first revealed that colloidal gold nanoparticles could be used as direct labels in electrochemical immunoassay [26]. Application of different inorganic nanocrystal tracers, Collins et al. used electrochemical immunoassay to detect multiple proteins [27]. Taking advantages of electrochemical immunoassays, electrochemical immunosensors based on nanomaterials is widely developed for cancer cells detection [28]. For example, Rusling et al. used gold nanoparticles film electrode combined with multienzyme-particle amplification strategy to develop an ultrasensitive electrochemical immunosensor for detection of cancer biomarker proteins [29]. In this work, the sensitivity of such biosensing method was greatly enhanced by using horseradish peroxidase (HRP)-containing magnetic bioconjugates particles. Thus, the sensitivity of developed electrochemical immunosensors and the corresponding detection limit were 31.5 μA mL ng\(^{-1}\) and 0.5 pg mL\(^{-1}\), respectively.
2.6. Other biosensors

Except for colorimetric biosensors, fluorescence biosensors, SERS biosensors, and electrochemical biosensors, there are some other biosensors in the field of cancer detection. For example, electromagnetic metamaterials-based biosensor is another choice for cancer detection. Farmani et al. reported a simple, supersensitive, and tunable compact footprint biosensor for the detection of cancer [30]. In their work, the used theoretical model was constructed by using split ring resonators (SSRs) array on a dielectric substrate, and three-dimension finite element method (3-D FEM) was used to simulate the as-prepared structure. They optimized dielectric material and different kinds of cells to obtain the optimal operating conditions to achieve the best performance of the developed biosensor. In detail, there dielectric materials, silicon dioxide (SiO₂), titanium dioxide (TiO₂), and polymethyl methacrylate (PMMA), were utilized to evaluate the performance of biosensor. Under the help of high resolution of the biosensor, the values of computed sensitivity for SiO₂, TiO₂, and the PMMA are 658, 653, and 633 nm/RIU, respectively. The simulated results showed that a supersensitive biosensor could be obtained if the refractive index of the sublayer is near to that of the samples. Additionally, the proposed biosensor could be used to detect nanoscale samples because of the nanometer-sized SSRs.

Danaie and Kiani designed a label-free photonic crystal refractive index biosensor for detecting cancer cells [31]. Using a 2-D square lattice of GaAs rods, they developed two kinds of photonic crystal optical refractive index biosensors for detection of basal cell cancer. In addition, the finite difference time domain method and plane wave expansion methods were used to simulate and analyse the designed structure. The photonic crystal ring-shaped resonator coupled waveguide filter was applied to monitor the change in refractive index. Under the optical conditions, the sensitivity of the developed biosensors was up to 720 and 638 nm/RIU.

3. Conclusion

In conclusion, we summarize the application of different types of nanomaterials-based biosensors for detecting cancer cells. In detail, the designed biosensing sensors, including colorimetric sensors, fluorescence sensors, SERS sensors, SPR biosensors, electrochemical biosensors, and other biosensors, are present in this minireview. In this paper, the construction principle of each biosensor for detection of different cancer cells is systematically discussed. Compared to conventional detection method, the use of biosensors based on nanomaterials for diagnosis of cancer shows better clinical application value.
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