Biosensors For Cancer Diagnosis Based on Quantum Dots

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Abstract. Cancer, one of the most dangerous illnesses threatening people's lives, caused nearly 2.4 million death last year in China. Effective and timely detection is the key to relieving and even successfully treating this disease. In order to realize the aim of early and sensitive diagnosis, multiple nanomaterials have been used, including the rising materials in recent years, quantum dots. Compared with other materials, quantum dots (QDs) have various advantages, like cheap fabrication expense, great fluorescent behavior, high sensitivity to targeted cells or related markers, versatile recognizing strategies, no toxicity and low detection limits. These properties enable this material to become the popular choice for biosensors. In this review, two main streams were chosen in the quantum dots-based biosensors: graphene quantum dots (GQDs) based biosensors and carbon quantum dots (CQDs) based biosensors. The character of each kind of quantum dot was reported and the recent development of these two nanomaterials was introduced, including their synthesis and application of optical and chemical analysis. Therefore, this report can offer great value to the research in the field of quantum dots-based biosensors, which will be used for cancer diagnosis in the future.

Keywords: Graphene Quantum Dots, Carbon Quantum Dots, Biosensor, Cancer.

1. Introduction

Cancer disease has become the top cause of killing people, especially in developing countries. According to estimates from GLOBOCAN, the number of deaths will be about 3,210,000 in China. What's worse, the increasing population age, limitations of many objective factors and multiple risks bring the task of early cancer detection and treatment a great burden [1]. Nowadays, the application of cancer biosensors is mammography and ultrasound scanning. However, these two technologies have respectively drawbacks and limitations. Mammography has a limited detecting capability which may lead to false results. Moreover, due to exposure to radiation, extra harm can be produced. Although ultrasound scanning is a safe method, it cannot take the place of mammography totally [2]. Therefore, a technology that can realize not only timely and sensitive cancer detection but also be accessible to most countries in the world. Quantum dots are microscope semiconductors which can bind excitons in three spatial directions. When this semiconductor is hit by the photon of visible light, this material's electrons can be stimulated to a higher level. When these electrons return to their origin, they can emit photons with special frequency [3].

In recent years, quantum dots have been considered an ideal choice for cancer biosensors. Various materials of QDs have contributed to the development of cancer diagnosis. Firstly, as a viable substitute, semiconductor quantum dots (semi-QDs) have been used., however, as they are toxic and difficultly soluble, some side effects could be caused. Moreover, their fluorescent character is not stable enough to perform molecule tracking well, and the too-big size of the body can also result in a cluster with nonspecific molecules when being used. Due to these drawbacks, better materials of quantum dots should be found for bio-imaging [4]. In this review, carbon-based quantum dots which are below 10nm was introduced. These nanomaterials mainly consist of two particles: carbon quantum dots and graphene quantum dots. Compared with traditional QDs, they have a series of merits such as superior stability and strength in lighting, a lower limit of detection, simpler methods to synthesis, no toxicity and better bio-compatibility. Numerous biomedical applications have been created based on these features. For example, graphene quantum dots were used to detect and track biomarkers of cancer cells through the specialized chemical link between probe and target, and their glowing characters can sensitively amplify the signal. Additionally, carbon quantum dots have been used in vivo and vitro imaging due to their ability to excite continuous and strong wavelengths [5]. The goal of this review is to examine the most recent advancements in various GQD- and CQD-based biosensors for cancer diagnosis. It will also focus on the ideal performance of GQDs and CQDs for biosensors, take into account the techniques used in their synthesis and functionalization, and discuss potential applications, such as cancer diagnosis and management.

2. Main cancer diagnosis biosensors based on quantum dots

2.1. Graphene quantum dots-based biosensors

Because of their advantages in physical and chemical properties, Graphene quantum dots have been considered suitable materials for cancer diagnosis. These sensors have different strategies for detecting cancers.

Fan, Zetan, et al. reported a new imaging tool, pH-responsive fluorescent graphene quantum dots (pRF-GQDs), to strengthen the diagnosis and treatment of cancers. According to the Warburg effect, tumor cells energize themselves through aerobic glycolysis, different from common oxidative phosphorylation of mitochondria. In this way, tumor cells can a large amount of lactic acid, and the pH values change reasonably. The pH values greatly differ between healthy tissues and tumours, respectively, in the range of 7.0 to 7,4 and 6.4 to 6.8. By electrolyzing graphene rods in sodium p-toluene sulfonate acetonitrile solution, pRF-GQDs were created. This probe exhibits a clear transition between green and blue at pH 6.8, matching the acidic extracellular state of cancer cells, and it is not harmful. Furthermore, pRF-GQDs have the property of upconversion photoluminescence, which can realize the aim of early cancer detection in different organs. Therefore, these unique fluorescence characters enable pRF-GQDs to work as a tool to distinguish malignant cells from normal ones [6]. Li, Nan, et al. published a fluorescent probe based on GQDs coupled with (2,4-dinitrophenol) tyrosine to detect the presence and amount of H2S, a key signal molecule involved in the growth and development of the cancer cell line. H2S and its Cystathionine βSynthase and cystathionine γ Lyase (CSE) are expressed in bladder cancer cell lines and higher than normal bladder epithelial cells. H2S promotes the proliferation of bladder cancer cells and reduces the apoptosis-promoting effect of cancer drugs. The theory of this sensor can break the dinitrophenol group to restore the photoluminescence of the nanomaterial when combined with H2S. This technique has been approved for use in the MCF-7 breast cancer marker experiment. As GQDs-based biosensors have the advantages of high fluorescence quantum production, biocompatibility, light stability and easy absorption by cells, dynamic supervision of living tumour cell lines can be achieved with higher accuracy [7].

In addition to using chemical reactions between cells to detect cancer, GQDs, also known as immunosensors, are capable of measuring signals related to antibody-antigen interactions. Traditional immunosensor-like enzyme-linked immunosorbent assay has several steps, which are time-consuming and may possibly cause error-positive results. GQDs are able to make stable chemical interactions with a variety of biologically linked compounds thanks to their enormous surface area, superior electrical and thermal conductivity, and other properties. GQDs show themselves as qualified candidates for this field [8]. Ogaidi, Israa, et al. created an immunosensor that utilizes the cancer antigen 125 markers to identify ovarian cancer (CA-125). CA-125 is a glycoprotein tumour-associated antigen which exists in epithelial cells of ovarian tumours. When patients with epithelial ovarian cancer and endometrial cancer, the serum CA-125 level can be significantly increased. As opposed to the conventional nanometal surface energy transfer method, which necessitates the use of an external excitation source to energize the energy transfer process. In order to reduce the signal-to-noise ratio, the author employed the chemiluminescence resonance energy transfer (CRET) to GQDs. The capture antibody (cAb), which can link the CA-125 antigen, was conjugated to the graphene dots that were glued on an amino-modified glass chip. The whole signal transition was based on CRET mechanism, involving the catalyzing of horseradish peroxidase. Firstly, the 3-aminopropyl-trimethoxysilane layer was salinized by amino-modified glass chips. As the amine group was positively charged, the GQDs could be well linked on the layer. Secondly, the CA-125-specialized capture antibody was covalently linked with GQDs. Bovine serum albumin (BSA) was used to reduce unspecific reactions. When the CA-125 antigen was absent, reactive oxygen species would be produced by the horseradish peroxidase enzyme, thus stimulating electrons. Chemiluminescence could be emitted when the electrons returned to their original state; then, the blue light would be recorded by a reader. Inversely, the fluorescence would be eliminated when the CA-125 antigen was present.

As GQDs could easily facilitate biological conjugation and linked with cAb on the transparent surface, these nanomaterials had the potential to contribute to the application analyzing huge amounts of data automatically. Additionally, fewer washing steps were required during the manipulation process [9]. The carcinoembryonic antigen (CEA) is commonly over-expressed by mucosal cells of tumours. An acidic glycoprotein called CEA resembles the human embryonic antigen in its properties. It is created in the cytoplasm, secreted via the cell membrane, and then reaches the bodily fluids around it. Therefore, this antigen is often used as a signal to detect cancers [10]. Figure 1 by Yang, Yuying, et al. depicts a nanocomposite made of gold nanoparticles and PtPd nanoparticles that included GQDs combined with the nitrogen. In this study, the nanocomposite was mounted on a Gold Circuit Electronics (GCE) and chemically joined to anti-CEA via the amine groups of the antibody. The BSA solution was added to reduce other unspecific bindings. H2O2 can be reduced to H2O due to the interaction between CEA and N-GQDs. The systems could be used to detect and magnify the signal from the analysis of CEA levels in serum samples [11].

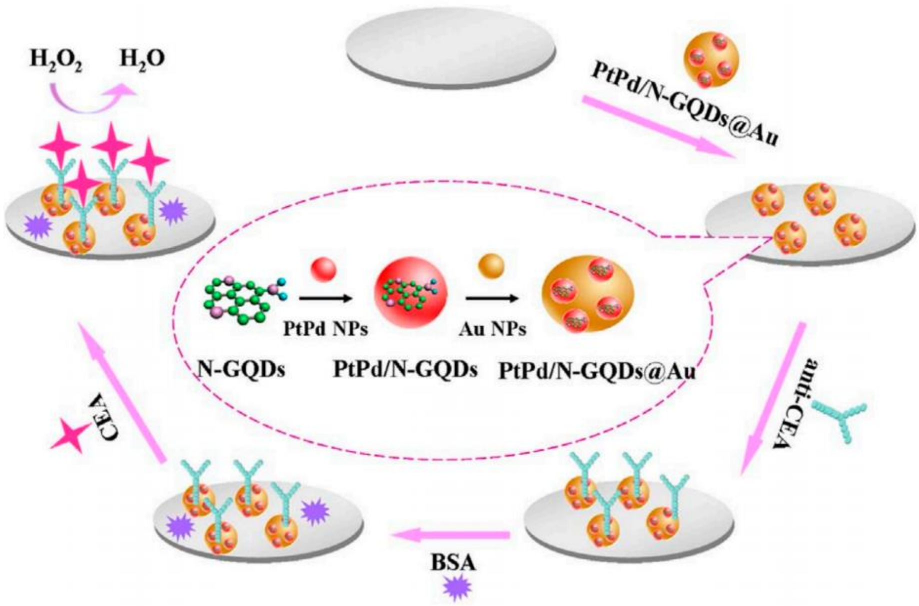

Figure 1. PtPd/N-GQDs@Au label-free electrochemical immunosensor [11].

2.2. Carbon quantum dots-based biosensors

The other member of the family of carbon-based quantum dots is called carbon quantum dots, or CQDs. Smaller than 10nm nanoparticles are known as CQDs. Xu et al. first discover CQDs. In 2004, after preparative electrophoresis separates and purifies single-walled carbon nanotubes. Ya-Ping Sun at el. reported CQDs' characterization of strong photoluminescent in fluid and solid conditions in 2006 after surface passivation [12]. CQDs own a variety of properties enabling these materials to become popular in the field of biosensor. For example, the fluorescence emission is controlled by the particles' size. CQDs are also free of toxicity and avoid side effects for patients. The procedure of CQDs is relatively simple and inexpensive, which can reduce the overuse of rare elements [13]. Multiple methods of synthesis of carbon-based quantum dots like candle burning, laser ablation with low cost and some green methods without pollution have been reported. This review is going to discuss different applications of biosensors based on the properties of CQDs to detect cancer cells and markers. These materials are used in the process of electrochemiluminescence, fluorescence, electrochemical biosensors and cell imaging as particles without toxicity.

Fluorescence resonance energy transfer (FRET), a new fluorescence-based technique, has recently been created in the biosensor industry. FRET is a non-radiative energy transition that involves the electric dipole interaction of molecules to transfer the excited energy of the donor to the exciting energy of the receptor. This mechanism is not radiative because photons are not involved. Although organic materials such as fluorescein isothiocyanate, rhodamine and blue pigment are low in cost and have rich types, they cannot offer stable and strong fluorescence and also easily cause interference due to their high spectrum widths. However, the CQDs applied in fluorescent nano sensors can offer a consecutive and stable spectrum. Therefore, this technology has been used in vitro analysis and vivo monitoring on the level of cellular supervision and light capture [14]. A physical technique to identify molecular contact is the FRET process. It takes place when a donor fluorophore transfers its energy to an acceptor chromophore in a non-radioactive manner while still in an excited condition through prolonged interactions between dipoles [15]. Mohammadi et al. reported a new method to detect microRNA based on FRET. A DNA probe that functions as a fluorophore and MnO2 nanosheets that serve as quenching sites are both incorporated into CQDs. The fluorescent output by CQDs is quenched by MnO2 nanosheets and restored when combing with the target microRNA, which shows a high specialty. The use of MCF-7 cells monitoring this technology has been used in the detection of breast cancer cells [16].

Hamd-Ghadareh et al. synthesized two CQDs (CD-1, CD-2) and functionalized antibodies to simultaneously detect two tumour markers (CA125, CA15-3) through the FRET strategy. In this process, MoS2 was used as quenching points. Additionally, MoS2 was used to absorb Abs as a FRET acceptor. This strategy could observe strong PL emission to realize the imaging of the cancer cells MCF-7 and OVCAR-3 [17]. Electrochemiluminescence (ECL) has been a useful application in research because this technology can combine electrochemistry and luminescence merits. Although common metal-based QDs have been applied for ECL, they own several disadvantages, such as weak ECL behavior and high toxicity, which may limit the dimension of use [18-19]. Liu et al. designed a paper-based ECL biosensor for detecting cancer cells. This facility was convenient and disposable. The functionalized Au@Pd nanoparticles served as paper electrodes. The electrodes were covered with an aptamer that was toxic to MCF-7 cells. As the label of ECL, PtNi alloy was loaded with C-dots to create PtNi@C-dot composite. Using ECL to label concanavalin A (Con A) as Con A can specially recognize mannose on the tumor cell surface. In this way, the number of MCF-7 cells can be linked with ECL [20]. Liver cancer, as well as a number of other malignancies, are all intimately associated with alpha-fetoprotein. Due to its high concentration in a number of malignancies, it can be utilized as a positive detection indicator for certain tumours. Zhou et al. Diethylenetriamine pentaacetic acid (DTPA) was carbonized to create nitrogen-doped carbon quantum dots (N-CQDs), which were used to detect alpha-fetoprotein... Due to ECL resonance energy transfer, these dots displayed excellent luminescence, and their ECL could be successfully suppressed by a secondary antibody tagged NH2-G [21]. Qiu et al. used solid-state zinc-coadsorbed carbon quantum dots (ZnCQDs) to make ECL probe for breast cancer detection and CD44 expression evaluation because this protein is closely related to tumor cell transduction. The probe was constructed with ZnCQDs and loaded with magnet beads for signal amplification. This probe can show a 120 times development and offer more stable and effective labelling [22].

3. Discussion

3.1. Advantages and disadvantages of graphene quantum dots-based biosensors

GQDs, a class of particles with fluorescence, belong to the carbon nanomaterial family. Due to their special physical and chemical characteristics, they have been used in biomedical sensors. Because they retain the characteristics of both graphene and carbon dots, GQDs can be made in a variety of ways. The top-down method and bottom-up method are the two primary categories into which the preparation techniques for GQDs may be separated. Large graphene sheets or nanosheets can be cut into smaller GQDs by physical or chemical means (hydrothermal method, electrochemical method and chemical stripping method). In addition, taking small organic molecules as precursors, the larger GQDs can also be gradually synthesized through a chemical reaction. In the second method, a few GQDs with precise molecule structures can be made by using small organic molecules as precursors to make key intermediates. Currently, the majority of quantum dots are created by heating a tiny organic molecule over its melting point, enlarging them to form the GQDs with certain sizes and figures. However, this synthesis cannot produce highly accurate GQDs. The structure of graphene is a combination of pentagon and hexagon, so it is stable enough to enable specific bonds with other molecules, such as antibodies, proteins and nuclear acids on the surface. This mechanism allows GQDs-based biosensors to sensitively detect various cancer cells by recognizing different biomarkers. Moreover, GQDs are also applied in optical detection because of their fluorescent features. With an absorption rate of 2.3% over a broad wavelength range and excellent optical characteristics, graphene seems to be practically transparent. The absorptivity rises by 2.3% for every extra layer of graphene within the range of several layers in thickness. GQDs' size, shape, and a number of layers can be altered to alter the luminous intensity. Therefore, a system of artificial optical changing can be realized by controlling the particles' figures. Similar to graphene, GQDs also have outstanding electrochemical properties, so they can be used as an electrochemical probe in biosensors. Based on the merits of luminance and electrochemistry, GQDs are expected to be a good assistant in the field of the biosensor. However, present technologies can not satisfy with highly sensitive detection of any cancer cells by GQDs biosensors, and this detection method is almost accessible in labs. Therefore, a more sensitive and portable application with better accuracy is needed.

3.2. Advantages and disadvantages of carbon quantum dots-based biosensors

CQDs, another type of fluorescent particle, have been thought of as ideal materials for biomedical applications. Many advantages of these nanomaterials have been developed and certificated in imaging and sensing fields. As many nanomaterials do not have enough fluorescent strength to offer stable bioimaging, the emergence of CQDs could help to solve these problems. These particles have good optical absorption in the region of 230nm-320nm, which is suitable for bioimaging. Besides, CQDs can be doped with other atoms and oxidized on their surfaces. This feature enables the stimulation of luminescence and can also change the excitation wavelength and quantum yields. Due to their electrochemical character, CQDs can realize the separation of assembled charge in the interface, so an electron pathway could be offered to the electrode surface. This feature enables CQDs to be good electrodes because electrodes can absorb more analyte through CQDs' hydrophilic margin and hydrophobic planes. When the electrode surfaces return to their original state, the ECL signals can be released as markers of cancer detection. Moreover, CQDs are low in cytotoxicity, so they can be used in high concentrations in vivo and in vitro imaging. Lastly, these materials can provide stable and continuous fluorescence emission when they are applied as probes. Although these characteristics and advantages help CQDs to have a prosperous future, the research in this field is still not mature enough. For example, there have not been experiments on this biosensor in human bodies. Therefore, firstly the research should be done to test this biosensor on a solid platform, and then the application should be tried to settle down in human bodies to realize the point-of-care testing. Furthermore, as this biosensor still requires complex facilities to be manipulated in the lab, a more portable and convenient tool is needed to be invented for timely and fast cancer detection in common hospitals.

4. Conclusions

Although present medical techniques have been developed to cure cancers, the early detection of cancers is still the most effective way to treat cancers. Because cancer cells can quickly invade and spread, which worsens the condition in a short time and even cause diseases in other organs, making the treatment more complex and difficult. However, the general methods of cancer detection may have the drawbacks of long-time reactions, usually costing several hours. Additionally, common biosensors like MRI cannot have the limitation of detection, which is low enough to detect every tumour cell accurately and even make wrong positive results. Worse, patients may be exposed to the risk of radiation that threatens their health. Quantum dots, a type of inorganic nonmaterial, are becoming more and more popular in the field of cancer biosensors. This material is a semiconductor nanoparticle with a diameter of 1~100nm and can receive excitation light to generate fluorescence. Their unique electrical and optical characteristics have great value in medical research. A variety of biosensors have been created based on graphene quantum dots and carbon quantum dots. Compared to traditional ones, they have higher sensitivity and can monitor and identify the relevant biomarkers in the early stages of cancer cells. Moreover, the capability to stimulate stable and strong fluorescence and judge the spectrum's width by changing their sizes. Consequently, biosensors based on quantum dots have a promising future in the realm of medical diagnosis. The review discussed the fabrication and function of GQDs-based biosensors, which can detect the change of different biomarkers sensitively, such as pH values, antigens, enzymes and proteins. Besides, the CQDs-based biosensors' utilization in FRET and ECL processes was also discussed. These two dots' properties and the synthesis, working mechanism and future development of nano biosensors were introduced, which can offer a reference in the cancer detection field.

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