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Application of electrochemical biosensors in colorectal cancer research

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Abstract: Malignant tumors are one of the diseases that seriously threaten human health, among which colorectal cancer is one of the common malignant tumors with high morbidity and high mortality. Early diagnosis and treatment is the main way to reduce the mortality of colorectal cancer, so the accurate detection of colorectal cancer-related substances has important clinical significance in recent years. With the rapid development of electrochemical biosensors and nanomaterials, electrochemical sensors based on nanomaterials can realize the detection of tumor-related indicators, which has the advantages of high sensitivity and strong specificity.

Keyword: Electrochemical biosensors, Colorectal cancer, Applications

1 Introduction

Colorectal cancer (CRC) is the third largest cancer in the world. According to statistics, in 2020, about 1.9 million new cases and 900,000 deaths occur in the world (Sung et al., 2021). About 3.3 million new cases are expected by 2040 (Xi et al., 2021; Zheng et al., 2022). In recent years, the incidence and mortality of colorectal cancer in China have shown an increasing trend year by year, and it is one of the common malignant tumors in the digestive system. According to the results of the 2020 China Cancer Statistics Report, among malignant tumors, the incidence and mortality of colon cancer rank second and fifth respectively (Zheng et al., 2022). Most CRC patients are diagnosed at an advanced stage, which means the cancer cells have metastasized and formed secondary tumors, resulting in a high mortality rate. The 5-year survival rate of patients with advanced colorectal cancer is only 5-10%, while the 5-year survival rate of patients with early colorectal cancer can reach 90% (Benson et al., 2021). Therefore, early detection of colorectal cancer will reduce its associated mortality, providing greater intervention potential and probability of treatment.

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Patients with early-stage colorectal cancer have no obvious symptoms (such as intestinal bleeding, abdominal pain) and usually require 7-10 or even decades of development to be diagnosed (Simon, 2016). There are many traditional methods to detect colorectal cancer, including occult blood test (OB Test), colonoscopy and so on. OB test is not highly specific, and its sensitivity needs to be improved, while colonoscopy is an effective tool for CRC screening, which can clearly detect intestinal lesions under endoscopy, directly remove tumor lesions, and stop bleeding (Hazewinkel & Dekker, 2011; Kuipers et al., 2013). However, people are often reluctant to undergo colonoscopy because of its invasive nature and the cumbersome process required for bowel preconditioning. In addition, there is a risk of bleeding, perforation and even death during colonoscopies. CT colonoscopy is an auxiliary imaging method for the diagnosis of polyps with a radiation dose of 8.8 mSv, which is less invasive (Hoshino et al., 2019). This test provides an opportunity for early detection of colorectal cancer. In addition, there are some tumor markers detections, such as common colorectal cancer biomarkers including carcinoembryonic antigen, carbohydrate antigen 199, 50, 724, mucin, interleukin, p53, EGFR, APC gene and microRNA and so on. Detection methods include enzyme-linked immunosorbent assay (Arya & Estrela, 2018; Robinson et al., 2014), immunohistochemistry (Howat et al., 2014; Rizk et al., 2019), radioimmunoassay (Fourkala et al., 2016). chemiluminescence assay (Yang et al., 2018; Liu et al., 2017), and polymerase chain reaction (PCR) (Mehta et al., 2014).

Although these methods can obtain accurate results, there are obvious disadvantages, such as the detection process is long, the operation procedure is complex, and the sample size is still large. In addition, adequate amounts of biomarkers may not be detected in the early stages of colorectal cancer. Therefore, a simple, rapid, accurate and low-cost biomarker detection method was developed in this paper to promote the early diagnosis and treatment of colorectal cancer (Quinchia et al., 2020). This detection method is the detection of electrochemical biosensors. This method has the advantages of portability, small size, high sensitivity and good specificity, and can be used to achieve specific detection of colorectal cancer with very small samples (Grieshaber et al., 2008). When designing electrochemical biosensors, more rapid, simple, specific and sensitive methods can also be provided for the modification of different nanomaterials (Wang, 2005; Filik & Avan, 2019). With the wide application of electrochemical methods in the design of biosensors in clinical practice, the detection efficiency of CRC biomarkers has also been improved and has been readily accepted by medical researchers (Zhang et al., 2021).

In this paper, various electrochemical biosensors for CRC biomarker detection are introduced, and the advantages and uniqueness of the detection are introduced in order to diagnose timely and monitor the prognosis of CRC. Based on the classification of different CRC biomarkers, the different electrical analysis methods of biomarkers are introduced, and the advantages and amplification strategies of biosensors, as well as their advantages and limitations in electrochemical analysis are discussed. In general, the advent of electrochemical biosensors provides more powerful support for the detection of colorectal cancer.

Electrochemical biosensor is an analytical device that combines biometric elements with electrochemical transducers and converts biological signals into electrochemical signals for detection through the specific interaction between biometric elements and objects to be measured. Recently, the detection and diagnosis of biomarkers for colorectal cancer have received extensive attention (Cruz-Pacheco et al., 2024; Lișcu et al., 2024). Electrochemical biosensors have the characteristics of high sensitivity, strong specificity and rapid response. Because of the high sensitivity and high specificity of biological response, they provide a powerful means for detecting colorectal cancer markers. The schematic diagram of an electrochemical biosensor is shown in **Figure 1**. The electrode usually has a biometric element on its surface. The electrode specially differentiates biomolecules from target substances and converts their reaction signals into different electrical signals to achieve qualitative or quantitative detection of target substances (Hammond et al., 2016; Ronkainen et al., 2010). Samples containing target biomarkers (such as CEA, miRNA, etc.) are added to electrochemical sensors, which can be widely used in rapid and simple clinical detection and analysis methods. Biosensor applications in cancer diagnosis usually consist of three parts: biomarkers, bioreceptors, signal amplification methods and materials, all of which are crucial to the performance of the overall biosensor.

Biometric element is the core part of electrochemical biosensor, which is used to specifically identify the object to be measured. Common biometric components include antibodies, antigens, DNA, enzymes, peptides, etc. (Sadik et al., 2010). These biometric elements bind to the object to be tested through immune responses, base complementary pairing, or other specific actions to achieve the detection of the target substance.

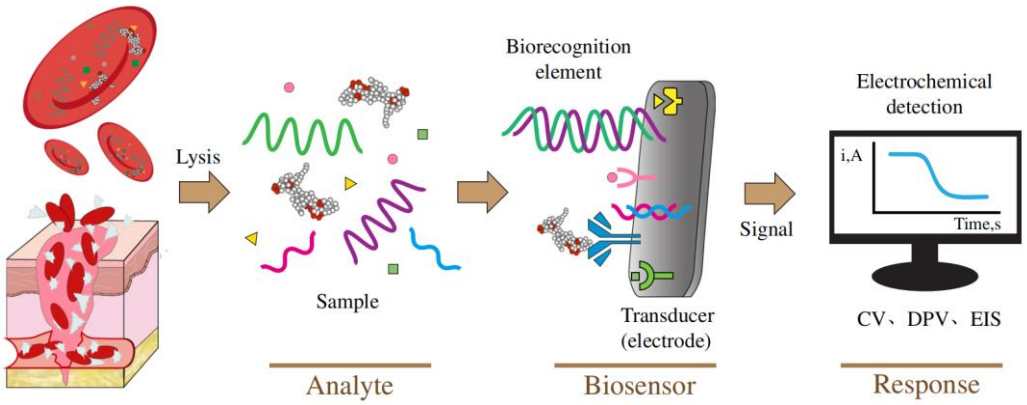

Figure 1

The occurrence and development of colon cancer involve the changes of various biomarkers, including tumor markers, related oncogenes, tumor cells, etc. Electrochemical biosensors show great potential in colon cancer detection due to their high sensitivity and specificity (Quinchia et al., 2020). Electrochemical DNA sensors have high sensitivity and selectivity in nucleic acid detection, providing a new detection method for the diagnosis of colorectal cancer and other cancers (Yang et al., 2019). The fixation of single-strand DNA probes is a key step in electrochemical gene sensors. It is necessary to ensure its stability on the electrode

surface and avoid non-specific adsorption. Due to the complexity of DNA sample preparation and reagent processing, microfluidic technology can be combined with nucleic acid extraction, amplification and detection in the future, making electrochemical DNA sensors more convenient, simple and automated (Rashid & Yusof, 2017).

2 Detection of tumor markers

Tumor markers refer to a class of substances that reflect the existence and growth of tumors during the occurrence and proliferation of tumors, which are secreted by tumor cells themselves or produced by the body's reaction to tumor cells. Tumor markers exist in blood, body fluids or tissues, and are of great practical value in tumor diagnosis, prognosis judgment, efficacy evaluation and physical examination screening of high-risk groups (Duffy, 2007). In recent years, with the development of electrochemical biosensors detection, more types of electrochemical biosensors have been used for the detection of tumor markers. **Figure 2** is a schematic diagram of specific detection of tumors based on surface plasmon resonance (SPR) biological markers.

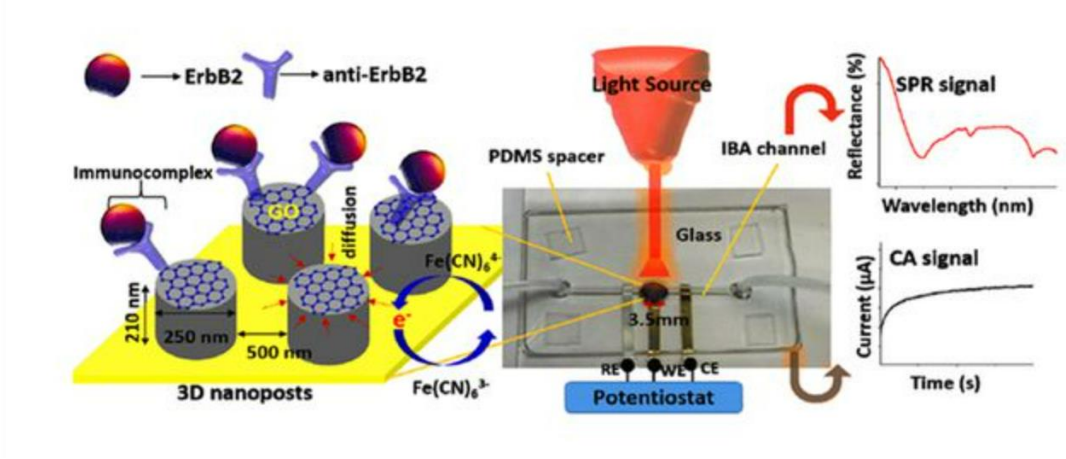

Figure 2

This unique property enhances the interaction of two-dimensional nanomaterials with chemical substances, thereby improving the sensitivity and selectivity of the sensor (Khan et al., 2021). The higher charge mobility of two-dimensional nanomaterials increases the heterogeneous electron transfer, thus improving the response time of the biosensor. Moreover, 2D has good biocompatibility. Biometric element is the core part of electrochemical biosensor, which is used to specifically identify the object to be measured. Common biometric components include antibodies, antigens, DNA, enzymes, peptides, etc. These biometric elements bind to the object to be tested through immune responses, base complementary pairing, or other specific actions to achieve the detection of the target substance (Guo et al., 2024).

3 Detection of related oncogenes

The occurrence and development of colorectal cancer are closely related to the abnormal expression of many oncogenes. Detection of the expression level of related oncogenes can provide an important basis for the early diagnosis and treatment of colon cancer. miRNA is a single stranded RNA molecule with a length of about 22 nucleotides encoded by endogenous genes. Mirnas can regulate the expression of other genes involved in cell growth and differentiation. Abnormal expressions of miRNA can lead to cancer and cardiovascular diseases (Maqbool & Hussain, 2014). Studies have shown that in the development of colorectal cancer, the up-regulation, down-regulation or deletion of miRNA is closely related to the impact of colorectal cancer. Therefore, many of these factors can be used as biomarkers for the diagnosis and prognosis of colorectal cancer (Shirafkan et al., 2018; Ng et al., 2009). For example, miR-21, a carcinogenic miRNA, regulates the development of CRC by acting on target genes and has become one of the most widely studied mirnas in the diagnosis, prognosis and treatment of CRC (Li et al., 2013; Jang et al., 2021). At present, the quantitative detection and analysis of related mirnas by various methods play an important role in the early diagnosis of CRC. This study focused on the relevant tumor markers related to colorectal cancer.

4 Detection of colorectal cancer-related tumor cells

Direct detection of tumor cells is one of the important methods for the diagnosis of colon cancer (Zhong et al., 2019). Electrochemical biosensors show great potential in the detection of tumor cells due to their high sensitivity and specificity. Chemotherapy is the core treatment of any cancer and involves the use of various types of chemicals. In most cases, such as when colon cancer has spread to the lymph nodes, chemotherapy is performed after surgery but can also be used before surgery to reduce the size of the tumor and make it easier to remove. The primary purpose of chemotherapy is to destroy cancer cells by targeting the inhibition of DNA replication, interfering with chromosome separation in the cell cycle, or directly providing a cytotoxic environment for the tumor (Hanahan & Weinberg, 2011). Conventional treatments for colon cancer are site-specific and cause adverse side effects, such as gastrointestinal toxicity, hematological disorders, hepatotoxicity, anemia, and hand-foot syndrome. So far, no satisfactory results have been achieved. However, associated side effects lead to the development of drug resistance. Since all the rapidly dividing cells are involved, they cannot distinguish between normal and cancer cells, and only a small amount of the drug can reach the cancer cells, again reducing the desired effect of the drug and causing serious side effects in other tissues (Bukowski et al., 2020). Currently, potential systemic therapies for patients with advanced colorectal cancer include cytotoxic chemotherapy as well as targeting vascularendothelial growth factor, VEGF and epidermal growth factorreceptor (EGFR) biologics (Van Cutsem et al., 2009). Cytotoxic chemotherapeutic drugs active in the treatment of colon cancer include 5-Fluorouracil (5-FU), oxaliplatin, irinotecan and fluorouracil/Tipiracil, etc. [28]. The approved monoclonal antibodies against EGFR are cetuximab and panizumab (Bourhis et al., 2021). Anti-vegf drugs were classified according to target specificity and route of administration, including

bevacizumab, abexipril, ramumab and regorafenib (Li et al., 2021; Hurwit et al., 2004).

4.1 Alpha-fetoprotein (AFP)

Alpha-fetoprotein, a plasma protein, is one of the most widely used clinical tumor markers (Duffy, 2012). As an important tumor marker of liver cancer, AFP content is low in the serum of normal people, but significantly increased in patients with liver cancer. In addition, AFP can also be used for early diagnosis and evaluation of early treatment effect of other cancers, such as yolk sac cancer and gastric liver metastasis (Lu et al., 2024). Due to its unique physical and chemical properties, such as high mechanical strength, large specific surface area, good electrical and thermal conductivity, high thermal stability, high transmittance, and good biocompatibility (Bridgewater et al., 2020), nanomaterial graphene has shown a good development trend and application potential in the detection of tumor-related indicators by electrochemical methods in recent years. By combining graphene oxide with the traditional antigen-antibody reaction, using graphene to fix the primary antibody and mesopore silica nanoparticles to fix the secondary antibody, AFP detection through the classical immune sandwich complex can significantly improve the sensitivity and specificity of the detection, and can be applied to the detection of colorectal cancer (Abolhasan et al., 2021).

4.2 Carcinoembryonic Antigen (CEA)

Carcinoembryonic antigen is a broad-spectrum tumor marker, which mainly indicates digestive system tumors and lung cancer, and also plays a good indicator role in metastatic liver cancer (Duffy, 2001). Nitrogen-doped graphene shows good results in CEA detection because of its better catalytic effect and electrical conductivity. By modifying streptomycin on the basis of nitrogen-doped graphene, an electrochemical immunosensor for CEA detection can be prepared, which can achieve high sensitivity detection of CEA.

4.3 Glycoantigen 15-3 (CA15-3)

Breast cancer is one of the malignant tumors threatening the health of women of all ages, and the mortality rate of breast cancer has been increasing in recent years. As the main specific biomarker of breast cancer, CA15-3 can provide help for the early diagnosis and treatment of breast cancer. Three-dimensional graphene aerogel (3D Gas) has better electrical conductivity and a larger specific surface area compared to ordinary graphene materials. β-cyclodextrin (β-CD) was polymerized on the 3D Gas surface by electrochemical polymerization, and the anti-CA15-3 antibody was fixed on the sensing interface through the host-guest interaction between β-CD and antibody, so as to construct a labeling free immune sensor, which can achieve high sensitivity detection of CA15-3.

4.4 Prostate Specific Antigen (PSA)

Prostate cancer is one of the major cancers that cause high mortality in men (Siegel et al., 2018). Serum PSA concentration can provide evidence for early prostate cancer screening and postoperative prostate cancer prognosis (Lowrance et al., 2023). Using graphene and horseradish peroxidase/gold nanofunctionalized secondary antibodies (HRP-Ab2/AuNPs) to construct a novel carbon screen printed electrode for PSA detection, which can achieve high sensitivity detection of PSA.

4.5 P53 gene

The p53 gene is an important tumor suppressor gene, and its mutation is closely related to the occurrence and development of various tumors. By electrochemical detection of wild-type p53 protein in the lysoid of colon cancer cells, a small and wide oxidation peak could be observed, in which the concentration of wild-type p53 protein was about 50 times lower than that of normal umbilical epithelial cells, indicating serious mutations in p53 gene in colon cancer cells. Because of its high sensitivity and specificity, electrochemical methods show great potential in the detection of p53 genes.

4.6 Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases are a class of enzymes that can degrade extracellular matrix and are closely related to the occurrence, development and invasion of tumors. Among them, membrane-type matrix metalloproteinase-14 (MMP-14) plays an important role in the process of tumor metastasis and invasion in the human body and is considered to be an important marker of whether some malignant tumors have metastasized (Overall & Kleifeld, 2006). By constructing electrochemical biosensors based on polypeptide inhibitors, high sensitivity detection of MMP-14 can be realized, which provides an important basis for the early diagnosis and treatment of colon cancer.

5 Folate receptor-mediated tumor cell detection

Folate receptor is a glycosylated phosphatidylinositol linked membrane glycoprotein, widely distributed in normal and tumor tissues, but its quantity and activity in tumor tissues are much higher than that in normal cells. Based on the differences in the expression of folate receptors and their high affinity for folate, folate molecules are ideal for the specific capture and detection of folate receptor-positive tumor cells. By constructing an electrochemical biosensor based on folic acid receptor, high sensitivity detection of colon cancer cells can be realized.

The invention relates to an electrochemical biosensor for rapid detection of tumor cells, which comprises a flexible screen-printed electrode and a gold nanoparticle deposited on the surface of a flexible screen-printed electrode. The surface of the gold nanoparticle is modified with merphyto-folate molecules. The sensor has a strong specific binding ability to the folate

receptor on the surface of tumor cells, which realizes the rapid detection of tumor cells. Electrochemical biosensors can enable early diagnosis of colorectal cancer by detecting tumor markers (such as CEA, CA19-9, etc.) in blood or other biological samples. These sensors are often highly sensitive and selective, enabling the detection of markers at low concentrations.

Electrochemical biosensors can be used to monitor biomarker levels in patients in real time, helping doctors assess the effectiveness of treatment and the risk of cancer recurrence. For example, by monitoring specific metabolites or proteins, treatment regiments can be adjusted in time. Targeted drug delivery in the treatment of colon cancer, electrochemical biosensors can be combined with targeted drug delivery systems to achieve precision treatment. The sensor is able to detect changes in the tumor microenvironment in real time to regulate drug release.

6 Conclusion

Electrochemical biosensors have shown extensive application prospects in the study of colorectal cancer, not only improving the possibility of early diagnosis, but also providing new ideas for personalized treatment. Electrochemical biosensors can also be used to study the biological mechanisms of colorectal cancer. For example, by monitoring changes in extracellular matrix components, metabolites, or cell signaling molecules, researchers can reveal the mechanisms by which tumors develop and metastasize. Modern electrochemical biosensors enable multiple detection, that is, simultaneous detection of multiple biomarkers on the same platform. This approach can improve the accuracy and efficiency of diagnosis and help doctors assess patients' conditions more comprehensively.With the development of nanotechnology and materials science, the emergence of portable electrochemical biosensors has made the detection of colon cancer more convenient and widespread. The device can be used in primary care facilities or at home, improving access to early screening. Looking to the future, as technology advances, electrochemical biosensors show greater potential for early screening, prognostic assessment and personalized treatment of colorectal cancer. Researchers are exploring novel materials and signal transduction mechanisms to improve the sensor's sensitivity and reliability. As technology continues to advance, it is expected to play a more important role in future clinical applications.

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