Study of the Electrochemical Impedance Immunosensor

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Abstract. Colloidal gold has been extensively studied for its unique properties in various fields, including diagnostics and therapeutics. The colloidal gold electrochemical impedance immunosensor is a promising tool in antigen detection. It has high sensitivity, specificity, and rapid results. However, it also faces certain challenges, such as the stability of colloidal gold particles and optimization of assay conditions. To address these challenges, this paper suggests several recommendations. First, advancements in colloidal gold synthesis and surface modification techniques can enhance the stability and reproducibility of the particles. Second, optimizing assay conditions, including sample pre-treatment and antigen binding conditions, can improve the overall performance of the electrochemical impedance immunosensor. Furthermore, research is needed to develop the full potential of this technique in different fields and expand its practical applications. In conclusion, the colloidal gold electrochemical impedance immunosensor shows great promise in antigen detection. By comparing it with other methods and proposing solutions to its limitations, this review paper aims to contribute to the advancement of this technique and its applications in various industries. Continued research and development in this field will undoubtedly lead to further improvements and broaden the scope of this powerful tool.

Keywords: Colloidal gold; electrochemical impedance immunosensor; antigen detection.

1. Introduction

In biomedical research, antigen detection is an essential method for diagnosing diseases and monitoring disease progression. Traditional antigen concentration detection methods, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and immunofluorescence, are often complex and time-consuming [1]. Therefore, finding a convenient, rapid, sensitive, and cost-effective antigen detection method has become a hot topic in current research. The inclusion of these methods in electrochemical impedance immunosensor research serves several purposes. Firstly, it allows for comparative studies to evaluate the performance of impedance-based techniques against established methods. Secondly, it provides insights into the advantages and limitations of each method, aiding in the selection of the most appropriate approach for specific applications. Lastly, it facilitates the development of hybrid or integrated platforms that combine the strengths of different detection techniques, leading to enhanced sensitivity, specificity, and versatility. By considering and incorporating these established antigen detection methods in electrochemical impedance immunosensor research, researchers can advance the field by harnessing the strengths of multiple techniques while addressing their respective limitations. This comprehensive approach will contribute to the development of robust and efficient immunosensing platforms for various applications in diagnostics, biomedical research, and beyond.

Colloidal gold antigen detection technology is an emerging antigen detection method. Colloidal gold has unique optical properties, and when a large number of colloidal gold particles aggregate, the absorbed light wavelength is approximately 510nm to 550nm blue light, resulting in a reddish or pale red color in solutions with high colloidal gold particle concentrations. Compared to traditional antigen detection methods, colloidal gold colorimetric assays have advantages such as high sensitivity, strong specificity, simplicity of operation, and intuitive display of results. However, most techniques that use colloidal gold colorimetric assays for antigen detection cannot provide quantitative results and can only provide information on the presence or absence of the target substance [2]. In order to apply colloidal gold detection methods in biosensors, many researchers are looking for a method that can quantitatively detect antigens and convert the results into electrical signals. The electrochemical

impedance immunosensor of colloidal gold has emerged as a solution. Due to the unique electrochemical properties of colloidal gold particles, when they become part of a circuit, they can cause changes in the resistance value, which is the basis of the electrochemical impedance immunosensor.

In recent years, the electrochemical impedance immunosensor of colloidal gold has been flourishing and has become a research hotspot. Therefore, the study of the application of the electrochemical impedance immunosensor of colloidal gold in the pharmaceutical field is of great importance. In the following sections, this paper will provide an analysis and introduction of the principles, applications, and advantages and disadvantages of this method.

2. Mechanisms

2.1. Preparation and Coloration Principle of Colloidal Gold

2.1.1. Preparation

The essence of colloidal gold is that chloroauric acid can be polymerized into colloidal gold of a certain size under the condition of reducing agents, and form a negatively charged hydrophobic colloid solution, which is the origin of the name colloidal gold [3].

2.1.2. Coloration Principle

The coloration principle of colloidal gold comes from the different dispersion of light by colloidal gold of different diameters, which causes significant changes in the depth of color of the colloidal gold solution. As the diameter of gold particles increases, the wavelength of the absorbed light also increases. The surface of colloidal gold carries a negative charge, and colloidal gold with different particle sizes can be prepared by reduction. The solution that contains colloidal gold with a particle size of 5-20nm absorbs a wavelength of 520nm and the solution is ruby red, while the solution with a particle size of 60nm mainly absorbs orange-yellow light with a wavelength of 600nm, and the solution is blue-purple [4]. These colloidal gold particles have a strong adsorption function for proteins, so colloidal gold is a very good marker for immune responses [5].

In antigen detection, we generally use colloidal gold lateral flow immunochromatography. First, the antibody that binds to the antigen is labeled with colloidal gold and placed on the binding pad, and another antibody for detecting the antigen is fixed on the test line. In addition, prepare a secondary antibody that can specifically bind to the labeled antibody with colloidal gold and fix it on the control line. In this way, if an antigen is detected, both the test line and the control line will turn red due to the enrichment of colloidal gold. On the contrary, if no antigen is detected, only the control line will turn red due to the enrichment of colloidal gold [6].

2.2. Page Numbers Operational Requirements of Colloidal Gold

The operation of colloidal gold requires certain conditions. The first is a relatively fixed pH value. Higher or lower pH values may cause colloidal gold to fail. Adding buffer solutions allows pH values to be controlled within a certain range to provide a good environment for colloidal gold. In addition, salt components in the solution can also cause aggregation and precipitation of colloidal gold, causing it to lose its ability to bind to proteins. Therefore, before using colloidal gold to treat proteins, it is necessary to reduce the ion concentration in the protein solution to be tested [7].

In addition, after labeling is completed, there are often unbound proteins in the solution and incompletely labeled colloidal gold. Therefore, labeled colloidal gold must undergo purification before it can be used. Generally, the technique is using high-speed or ultra-speed centrifugation methods to centrifuge colloidal gold at low temperatures 2-3 times repeatedly, remove the upper clear liquid, dissolve the precipitate in other solutions, and save it for later use [6,8].

2.3. Principle and Function of the Electrochemical impedance immunosensor of Colloidal Gold

The electrochemical impedance immunosensor of colloidal gold is based on the specific binding of colloidal gold-labeled antibodies to the target substance. When the antigen is present, it binds to the colloidal gold-labeled antibodies, forming a complex. This binding is specific, meaning that only the antigen corresponding to the antibody can bind to the colloidal colloidal gold. This specific binding leads to a change in the degree of colloidal colloidal gold aggregation, resulting in a change in resistance. By measuring the change in resistance, the presence or concentration of the antigen can be indirectly determined. This interaction mechanism forms the basis of the electrochemical impedance immunosensor of colloidal gold, making it a commonly used detection and analysis method [9]. Alternatively, colloidal gold-labeled proteins can be used and bound to antibodies on the electrode surface, ultimately leading to a change in resistance. By using this method, the degree of colloidal gold aggregation can be converted into an electrical signal, transforming the qualitative tracing method, which relies on visual observation to determine results, into the quantitative electrochemical impedance immunosensor that can accurately measure concentration. [10,11].

3. Applications

3.1. Importance of Antigen Concentration Detection and Limitations of Existing Methods

3.1.1. Importance of Antigen Concentration Detection

Antigen detection plays a crucial role in disease prevention and control. It helps us to detect the presence of pathogens early and take appropriate measures to contain the spread and outbreak of diseases. Antigen detection can also be used for monitoring vaccine efficacy, evaluating treatment effectiveness, and predicting disease progression.

3.1.2. Colloidal gold tracing method

Colloidal gold technology is a commonly used method for antigen detection. It utilizes the reflective properties of metal on the surface of nanoparticles to detect the target antigen. This technology has many advantages, including high sensitivity, high specificity, rapid response, and simplicity. By combining colloidal gold with specific antigens, visible color changes or optical signals can be generated, enabling quantitative or qualitative detection of antigens [12]. Colloidal gold technology is widely used in clinical diagnosis, such as detecting pathogens of infectious diseases, tumor markers, and autoantibodies in immune-related diseases. It is also used in food safety testing, environmental monitoring, and rapid identification of bioterrorism events [13,14].

3.1.3. ELISA

ELISA (Enzyme-linked immunosorbent assay) is a commonly used method for antigen concentration detection. It is based on the reaction between antigens and specific antibodies and utilizes enzyme-labeled secondary antibodies or substrates to generate measurable signals. ELISA has the advantages of high sensitivity and high specificity, allowing for quantitative detection of antigen concentration. However, ELISA also has some limitations, including long operating time, dependence on specific laboratory equipment and conditions such as an ELISA reader and washer, and limited dynamic range for accurately measuring very low or very high antigen concentrations [15].

3.1.4. RIA

Radioimmunoassay (RIA) is a method that uses radioisotope-labeled antibodies to detect antigen concentration. RIA has high sensitivity and high specificity, enabling the detection of extremely low levels of antigen concentration. However, RIA also has some limitations, including the use of radioactive materials which pose risks and difficulties in handling and disposal, long operating time

and complex procedures, and dependence on specific laboratory equipment and conditions such as a gamma counter [16].

3.1.5. Immunofluorescence assay

Immunofluorescence assay is a method that uses fluorescent-labeled antibodies to detect antigen concentration. It has high sensitivity and high specificity, allowing for quantitative or qualitative detection of antigens. However, immunofluorescence assay also has some limitations, including the need for a microscope or fluorescence analyzer to observe and analyze fluorescent signals, susceptibility to background interference such as autofluorescence or non-specific binding events that may lead to false positive results, and requirements for sample processing such as fixation and staining which increase the complexity and time of operation [17].

3.2. Application of the Electrochemical impedance immunosensor of Colloidal Gold

To illustrate the practical application of the Electrochemical impedance immunosensor of colloidal gold electrochemical impedance immunosensor, let's consider its use in the diagnosis of Forest-Spring encephalitis. This technique involves utilizing a thick-film graphite electrode that is printed by the screen as the transducer. On this electrode, a layer of the Forest-Spring encephalitis antigen is immobilized, serving as the bio-recognition substance. Forest-Spring encephalitis is a viral disease that affects the central nervous system [18].

By utilizing the colloidal gold electrochemical impedance immunosensor, healthcare professionals can detect and diagnose this condition accurately and efficiently. The process begins by collecting a patient's sample, such as blood or cerebrospinal fluid. The sample is then subjected to appropriate pre-treatment to remove any interfering substances. Next, the sample is introduced to the electrode with the immobilized Forest-Spring encephalitis antigen. If the patient has been infected with the Forest-Spring encephalitis virus, the specific antibodies present in their sample will bind to the antigen on the electrode surface. This binding event causes a change in the impedance of the colloidal gold particles, leading to a measurable signal. The transducer then converts this signal into a quantifiable result, which can be analyzed and interpreted by healthcare professionals. The impedance observed directly correlates with the presence and concentration of Forest-Spring encephalitis antibodies in the patient's sample. This allows for the accurate diagnosis of Forest-Spring encephalitis [18].

The use of the colloidal gold electrochemical impedance immunosensor in the diagnosis of Forest-Spring encephalitis showcases its potential in clinical settings. By providing a rapid, sensitive, and specific detection method, this technique aids in the early detection and timely treatment of this viral disease [18,19].

4. Advantages and Challenges

4.1. Advantages

The colloidal gold electrochemical impedance immunosensor is a commonly used detection method. This technique has many advantages, including high sensitivity, high specificity, rapid response, and simplicity. By combining colloidal gold with specific antigens, visible color changes or optical signals can be generated, enabling quantitative or qualitative detection of antigens [20]. In clinical diagnosis and biosensing, the colloidal gold electrochemical impedance immunosensor can be applied to detect pathogens of infectious diseases, tumor markers, autoantibodies in immune-related diseases, and other areas [6,10].

4.2. Challenges

However, the colloidal gold electrochemical impedance immunosensor also faces some challenges. For example, the stability of colloidal gold particles and storage conditions may affect the accuracy of detection results. In addition, there are technical difficulties in sample processing and operation,

such as sample pre-treatment and optimization of conditions for colloidal gold and antigen binding. Furthermore, due to the immaturity of the related technology, there are relatively few practical application cases for this technique [6,10].

5. Conclusion

Colloidal gold-based impedance biosensors can be widely used to detect various biological molecules due to their high sensitivity, specificity, and low cost. The method is based on the principle that the combining of target molecules and the surface of colloidal gold causes changes in the impedance of the solution, which will be detected by measuring the impedance of the solution. The method has been used to detect proteins, DNA, viruses, and bacteria. The advantages of this method include its simplicity, low cost, and high sensitivity. However, it also has some limitations such as its inability to detect small molecules and its dependence on the size and shape of the colloidal golds.

In terms of future research directions, it is important to focus on developing new methods for the synthesis of colloidal gold by controlling size and shape. This will enable the optimization of the sensitivity of the method. In addition, it is important to develop new methods for the functionalization of colloidal gold with specific ligands that can selectively bind to target molecules. This will enable the detection of a broader range of biological molecules. Finally, it is important to explore new applications of colloidal gold-based impedance biosensors in areas such as environmental monitoring, food safety, and clinical diagnostics.

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