Study of nano-electrochemical immune biosensors for the detection of pathogenic bacteria

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Abstract. A biosensor is a type of technological device that brings together a number of diverse fields of study, including biology, chemistry, physics, medicine, and electrical. It combines the sensitivity of a biological sensing element with the signal converter of an analytical device, an analytical device that provides a signal proportional to the density of the substance that is being measured. This makes it possible to more accurately determine the concentration of a substance. It is important to point out that nanotechnology is currently the most concerned technology throughout the world since it is both more sensitive and faster than the traditional technologies that have come before it. In the course of this study, both the benefits and drawbacks of biosensors were investigated by bringing together conventional approaches to the detection of pathogenic bacteria and nano-electrochemical biosensors. This was done with the intention of presenting an informative summary of the application of biosensors to future development prospects.

Keywords: Biosensor, pathogenic bacteria, Nano electric chemical biosensors, immunological techniques, rapid detection.

1. Introduction

Bacteria that cause disease in humans are collectively known as pathogenic bacteria. Some pathogenic bacteria are so virulent that a very small amount of invasion can cause the organism to become ill, such as the plague bacillus, where infection can occur with the invasion of several bacteria. Infectious diseases are caused by pathogenic infections, and the clinical signs of the condition are intricate and pose challenges in terms of diagnosis. In recent years, the incidence of infectious diseases has been increasing. In recent times, there has been a notable rise in the occurrence of infectious diseases, primarily attributed to the improper utilization of antibiotics. Consequently, the management and treatment of these infections have grown progressively challenging, emerging as a significant concern for public health on a global scale.

Currently, there are a number of common microbial detection methods, such as plate counting, polymerase chain reaction, etc. But there are strengths and weaknesses in each of the current testing methods. For example, there are some disadvantages of requiring pre-treatment and disconnecting the detection (easy to cause the loss of target molecules, resulting in false-negative), requiring the participation of specialized personnel, and the long detection cycle. Therefore, this paper summarized the methods used to detect typical pathogenic bacteria to provide some recent research advances for researchers in this field.

2. Mainstream methods for detecting pathogenic bacteria

2.1. Immunological Techniques

2.1.1. Enzyme-linked immunosorbent assay, ELISA

Enzyme-linked immunosorbent assay (ELISA) is a technique that use enzymes to visualise antigen-antibody reactions, and it was developed and improved by the radioimmunoassay (RIA) [1]. The two different types of ELISA are: (1) competitive assays, which combine an antigen-enzyme or an antibody-enzyme; and (2) noncompetitive assays, which measure the antigen between two antibodies and are also known as sandwich ELISAs [2].

Despite its numerous advantages, including high specificity, stability, cheap testing cost, ease of operation, a high degree of uniformity, and the capacity to conduct simultaneous testing of large volumes of samples. ELISA is difficult to analyze multiple components at the same time, has high selectivity of reagents, analyzes structurally similar compounds with cross-reactivity, and false-negative results can occur.

2.1.2. Immunomagnetic separation, IMS

Immunomagnetic separation (IMS) is the technique that elevates a target organism to the lowest concentration of the target, then it makes the target organisms can be rapidly detected without pre-enrichment. According to the notable efficacy demonstrated, IMS may be effectively integrated with other molecular techniques such as PCR, real-time PCR, and multiple PCR, among others, in order to enhance the detection capability for a wider range of microorganisms [3]. The IMS-PCR is mostly used in the detection of food. Vinayaka's research shows that IMS-PCR can detect the Salmonella Typhimurim exclude the bacteria culture, DNA isolation, which speed up the process of PCR in 3 hours [4].

IMS can highly reduce the disturbance of the food subsequent detection. It is a fast and simple method of separation, and meanwhile it would not affect the biological feature and the function of the isolated cell and other biological materials. However, there are many differences kinds of the microorganisms in one sample, so far there will be rich in more other bacteria, the growth of target bacteria will be inhibited by the other bacteria, then fail to detect the target bacteria [5].

2.1.3. Immunofluorescence technique, IFT

The immunofluorescence technique (IFT) is a method that combines immunological detection techniques with fluorescent labeling technology. It involves labeling a known antigen or antibody with fluorescein to create a fluorescent antibody (or antigen). This fluorescent antibody (or antigen) is then used as a probe to detect the corresponding antigen (or antibody) in tissues or cells. This detection method is characterized by its high sensitivity, specificity, and rapid detection capabilities. But there are problems of non-specific coloring, and the results of the lack of objectivity and others.

2.1.4. Latex agglutination, LA

Latex agglutination (LA) contains two types: the active agglutination and the passive agglutination. The active agglutination is commonly used in detect soluble antigens, while the passive agglutination is commonly used in detect trains in food or pure culture [6]. It has a better diagnosis value for the bacteria detection and rapidly detect in about a minute [6][7].

2.2. Molecular Biology Detection Technology

2.2.1. Polymerase chain reaction, PCR

Polymerase chain reaction (PCR) has been the golden standard for the SARS-CoV-2 diagnosis. PCR is a technique that allows for the detection of a single bacterium at a time [8]. Within the realm of basic PCR, various forms exist, such as Multiplex PCR and Real-time PCR.

Multiplex PCR (mPCR) is a technique that involves the simultaneous addition of numerous pairs of primers to the reaction system in order to amplify specific target fragments. This enables the concurrent identification of multiple harmful bacteria [9].

Real-time quantitative PCR (RT-qPCR) is a molecular technique that integrates reverse transcription PCR and real-time quantitative PCR methodologies to enable the quantitative analysis of RNA molecules.

Compared with conventional PCR, RT-qPCR amplification and detection are completed in one step without opening the cap, which avoids the contamination of PCR products to the laboratory; the degree of automation is high, the quantitative results are accurate and reproducible; and the detection speed is fast, which is capable of simultaneously detecting a substantial quantity of samples. However, the detection cost of this method is high; the enzyme activity is susceptible to interference and inhibition by different food matrix components; the selection and design of specific target gene

sequences for primers and probes are demanding, and improper selection and design may reduce the detection sensitivity and specificity. In addition, neither RT-qPCR nor conventional PCR can distinguish between dead and live bacteria.

2.2.2. Isothermal amplification

This method detects nucleic acid target sequences in a simplified exponential fashion, independent of thermal cycling. Isothermal amplification includes testing, strand displacement amplification, helicase-dependent amplification, simultaneous amplification and nicking enzyme mediated amplification, rolling circle amplification, nucleic acid sequence-based amplification, transcription mediated amplification, loop-mediated isothermal amplification, etc.

Advantages of the LAMP method are: simple operation, and it only need to complete the amplification reaction at a constant temperature. LAMP do not need to go through the cycle of temperature increase and decrease. Hence, it can be observed that the Loop-Mediated Isothermal Amplification (LAMP) technique exhibits a notable level of specificity and sensitivity, surpassing traditional Polymerase Chain Reaction (PCR) by around one to two orders of magnitude. The drawbacks of this approach include the need for meticulous primer design and the associated challenges, such as the limitation of amplification sequence length to 300 base pairs. Additionally, the potential for non-specific binding between ring primers might result in false positive results. Furthermore, there is a susceptibility to contamination during the detection process, which can lead to erroneous positive outcomes.

3. Biosensor detection technology

Based on their different principles and materials, there are many different types of biosensors such as optical, thermal, piezoelectric, electrochemistry (EC), electrochemical immune (ELC) biosensors. Optical biosensors and electrochemical biosensors are more commonly used for food pathogens.

The principle of a biosensor is to detect or specifically interacts with environmental chemicals inside or outside a living organism to produce a response. The components are roughly divided into three parts, including the biomaterial to be detected.

The components are divided into three parts, including the biomaterial to be detected, the transducer and the signal output. The biomaterial may consist of several components, such as cell receptors, animal and plant tissues, organelles, enzymes, antibodies, organisms, and nucleic acids, among others. The transducer is a device that facilitates the conversion of energy from immobilized biomolecules into various types of signals, including acoustic, optical, electrical, and magnetic signals. This conversion occurs subsequent to the biochemical reaction with the biological samples under examination. The resulting signals are then processed in conjunction with an electronic system. The utilization of immobilized biomolecules is employed in the identification of biological samples for analysis, necessitating the presence of specificity and a strong affinity for bonding. In general, the macromolecules that are most frequently utilized include antibodies, antigens, enzymes, nucleic acids, tissues, and individual cells [10].

3.1. Nano-Electronic Chemical Immune Biosensor

Electrochemical biosensors integrate the analytical capabilities of electrochemical techniques with the specificity offered by bio-recognition processes. The fundamental premise underlying electrochemical biosensors is the occurrence of chemical interactions between immobilized biomolecules and target analytes, resulting in the generation or consumption of ions or electrons. Consequently, this process influences the observable electrical property of the solution, such as current or potential. In the fields of biochemistry and electrochemistry, a reaction results in the generation of a quantifiable electric current, a measurable buildup of potential or charge, or alterations in the impedance of the medium situated between the electrodes, encompassing both resistance and reaction impedance [11].Nano-electronic biosensors have active conductive channels consisting of nano-structured semiconductor (NS-SC) materials whose flow is restricted to the surface. Because of

this, the materials become sensitive to electrical perturbations that occur during bioelectrical bio-activation or during the interaction of biomolecules in the vicinity of the surface. The nanoscale dimensions of the NS-SC materials can easily interact with biomolecules because of their similarity in size [12].

Studies have also shown that nano-electronic biosensors have the ability to interfere with living cells and detect dynamic cellular activity. Several diseases can be targeted at the cellular level in an accurate and consistent manner with the help of nano-electronic biosensors [11].

3.2. Nano metal material

Nano-materials possess several advantageous properties, including a substantial specific surface area, elevated surface free energy, favorable biocompatibility, and abundant surface functional groups. These attributes enable the utilization of nano-materials in the fabrication of biosensing interfaces. Consequently, nano-materials effectively enhance the electrode's specific surface area and facilitate the augmentation of biomacromolecule loading on the electrode surface. The objective is to increase the rate of electron transfer, improve the conductivity of the modified electrode, and expedite the reaction time of the sensor.

3.3. Carbon Nanotubes

Carbon is a material that has garnered significant attention and is extensively utilized in the fabrication of electrochemical sensors. Within the world of macroscopic materials, carbon exhibits three distinct allotropes, namely graphite, diamond, and amorphous carbon.The initial study conducted by Britto et al. (1996) involved the fabrication of carbon nanotubes as electrodes for the purpose of electrocatalytic oxidation of dopamine, a neurotransmitter.

Carbon nanotubes (CNTs) are cylindrical nanostructures that are created by the rolling of graphite-like planes consisting of carbon six-membered rings. In this arrangement, each carbon atom forms strong covalent bonds with three neighboring carbon atoms by sp^2 hybridization [13].

The advantages of CNTs are (1) long lifespans [14]; (2) stability [15]; (3) quick response [16]. Popov et al (2020) [16] stated that CNTs has more sensitive by detecting the E. coli, and it also has more obvious bactericidal effect in long nanotubes.

3.4. Graphene

Graphene, a carbon allotrope, encompasses several forms such as charcoal, graphite, and carbon nanotubes (CNTs), which is a 2D nanostructure of sp^2 carbons, and only 0.34nm thick. As a building block material for sensors, graphene is versatile. Graphene combined with the redox properties of some bioelectrically active molecules, can detect target molecules by generating corresponding current signals through redox reactions on the electrode surface. On the other hand, the bipolarity of graphene adsorbs both electron-accepting groups and electron-donating groups on Graphene that can lead to chemical gating, so it is easy to be used in resistive sensors monitored. The combination of graphene brings ultra-high specific surface area and special electronic characteristics , it means that any molecule breaking the complete structure of graphene will cause a change in conductivity, which can be easily detected. Therefore, graphene is expected to be highly sensitive to monitor, even to monitor the adsorption of individual molecules to or from the surface of graphene [17].

The researchers have compared the antibacterial activity of ESβL-E. coli by the graphene nano sheets and without graphene nano sheets. The result shows that the antibacterial activity is higher and more active while using the graphene nano sheets [18].

3.5. Nano Metal Oxide

Metal oxide nanomaterials are characterized by high activity, small size effect, transition from structureless broad absorption to characteristic structural absorption i.e. color transition, transition from ferromagnetism to paramagnetism, etc., and dispersion of their nanoparticles in heterogeneous mediums such as air, water, solids, organics, leads to refractive boundaries of the system, dielectric

confinement effect, so that they demonstrate a diverse array of applications across various disciplines such as catalysis, detection, energy storage, electrode materials, field effect transistors, and self-driven nanodevices.

In 2018, Kooti built a nano delivery system that include cobalt ferrite with CIP, which can deliver the E. coli, P. aeruginosa, and S. aureus. Comparing to the traditional CIP, the nano particles have the higher activation rate of killing the bacteria [19].

In the year 2020, Ji conducted a study wherein a thin-film nanoparticle, grafted with yellow-colored silicon oxide (SiO2) branches, was synthesized on a magnesium alloy substrate. This nanoparticle was subsequently loaded with CIP, a commonly used antibiotic, and subjected to testing against two bacterial strains, namely Escherichia coli and Staphylococcus aureus. It also proved that there is a inherited bacteria function and multiple layer modified substrate with enhanced corrosion protection by the addition of silica nanoparticles [20].

4. Summary

During the pandemic, screening or detecting a large number of samples dose need a rapid and definitive diagnosis. All but detection techniques require sample pre-treatment exclude biosensor. Metal nanoparticles include distinct physicochemical characteristics, including enhanced biocompatibility, rendering them suitable for expedited pathogen identification. At the same time, they have the effect of resisting infection by pathogens. Metallic nanoparticles have a broad application prospect in anti-infection and provide new ideas to solve the increasingly serious problem of drug resistance.

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