Research Advances in the Development and Application of Biosensors in the Field of Detection

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Abstract. Biosensors are immobilized biological components (such as enzymes, proteins, DNA, antibodies, antigens) or organisms themselves (such as cells, microorganisms, tissues, etc.) as sensitive materials, combined with appropriate chemical transducers, Novel devices for rapid detection of physical, chemical, and biomass. In a variety of detection fields, biosensors play an important and difficult to replace role, such as virus detection, trace target substances monitoring in diseases, pesticide residue detection and so on.

Keywords: Biosensor; Virus Detection; Trace Target Substances Monitoring; Pesticide Residue Detection.

1. Introduction

Biosensor, as its name, is a kind of instrument that could transfer the concentration of specific solution into electrical signals to complete the detection. This kind of sensors usually would be consist of three parts. The first is the recognition cell with fixed bio-sensitive materials such as enzymes. The other two parts would be the suitable physical and chemical transducers and amplifiers for the signal detection. With no doubt biosensors will have a much more massive use due to the great characters of sensitivity, selectivity and so on.

Since the 1960s, biosensors have been widely studied and applied abroad, and their development has gone through multiple stages. In 1962, Clark[1] first proposed the idea of "adding enzymes to the sensitive membrane of the chemical electrode to achieve selective analysis of the target" in the collection of papers of the New York Society of Natural Sciences. The first generation of the biosensor was created by Updike et al. [2] in 1967 by combining the oxygen electrode and the glucose oxidase immobilized membrane. After 40 years of continuous development, with the deepening of research, various physical methods have been continuously introduced into biosensors, and today's biosensing technology is changing with each passing day. In 1975, thermal enzyme probe and enzyme thermistor were successfully developed respectively. Since the 1970s, people have been looking for a transducer that can directly capture the combination process between the sensitive source and the target (such as the combination of antibody and antigen). This problem was not solved until 1983, when Leiberg et al[3]. published a report using surface plasmon resonance (SPR) technology to realize real-time monitoring of affinity reactions, and this technology immediately led to the production of immunosensors. In addition, a mediator is often utilized in enzyme electrodes to enhance the movement of electrons within the active site of the enzyme and the electrode's external sides..

In order to enhance the function of the sensor, soluble hexacyanoferrate was initially added to the sensitive membrane in 1976. This mediator acts as an agent for electron transfer between the enzyme and electrode. Now, the mediator has been widely used in the fabrication of amperometric enzyme electrodes. Medisense (a medical company) continued to focus on the research and development of the first generation of enzyme electrodes. In 1984, Turner et al[4]. reported the use of ferrocene and its derivatives as mediators of oxidoreductases body to make cheap enzyme electrode method. Soon on this basis, Medisense developed a screen printing technology capable of large-scale production of enzyme electrodes with high reproducibility, which promoted the development of biosensors. In the early 1990s, the research on biosensors entered the second stage, and the biosensors in this period were the second generation. The characteristics of the second generation biosensor [5] is using

antibodies or receptor proteins as molecular recognition element, the choice of the transducer are more diverse, such as field effect transistor (FET), optical fiber (FOS), piezoelectric crystal (for now,The representative products of the second generation of biosensors are the BIA core and BI2A lite, which were launched by Swedish businessman Pharmacia in 1991., in 1996, the sensor using DNA as a sensitive source was developed by Turner et al. DNA polycation complex was immobilized on the sensor by liquid crystal dispersion technique. All can affect the crosslinking between DNA molecular level of chemical and physical factors can be sensitive to capture and reflect for a "fingerprint" of the structure of the circular dichroism absorption peak. Biosensors developed in the 21st century are the third generation of products. With the development of micro-processor and nano-technology, biosensors are developing towards miniaturization and integration, and portable test instrument is developing rapidly. The focus of biosensor research and development has dramatically altered over the last four years. Many new biotechnologies, such as biochemistry, surface characterization, molecular markers and nanotechnology, promote the application of biosensors in various environments

Today, the application of nanomaterials in biosensors has brought its research into a new stage. Nanoparticles can enhance the sensitivity of enzyme biosensors. The efficacy of the enzyme electrode is contingent upon the catalytic activity conditions of the enzyme and the rate of electron exchange between the enzyme's active center and the surface of the electrode. Nanoparticles possess a significant specific surface area, elevated surface free energy, and a robust adsorption capability, hence enabling a greater number of enzyme molecules to be immobilized on the surface of nanoparticle grains. In addition, due to the small size of nanoparticles, it is possible to interact with the hydrophilic groups inside the enzyme, thereby causing changes in the configuration of the enzyme. With this modification, the enzyme's active core is brought closer to the substrate, which enhances the enzyme's catalytic performance. For example: nano-Au particles are good conductors of electricity and have good biocompatibility, while modified nano-SiO_2 particles have good selective adsorption for biomolecules. Therefore, it is expected to realize the direct electrochemical interaction between the nano-Au and SiO_2 particles, the active center of enzyme molecule and the surface of electrode greatly empower the sensitivity of it.

In the field of biosensor research, there are few reports on the integration of nanotechnology, biotechnology and self-assembly methods to achieve three-dimensional ordered assembly with high enzyme activity. According to some existing reports, the sensitivity of biosensors prepared by adding nanoparticles has been greatly improved. The shortening of the response time and the increase of the linear range of detection all indicate the improvement of the performance of the biosensor. Morrin et al. fabricated biosensors based on processable conductive polyaniline nanoparticles. Scholars at home and abroad have also carried out a lot of research on nanoparticle-enhanced glucose oxidase (GOD) biosensors. The results show that the glucose biosensor has the characteristics of high selectivity, simple and fast test, and is the most commonly used method to detect the concentration of glucose. human blood and body fluids. It contains many interfering substances, and the anti-interference performance of the glucose sensor can also be improved by introducing nanoparticles. In the next section the applications of the biosensors will be discussed.

2. The Application Status of Biosensors

The future of biosensors would be promising and bright, but biosensors are still in a stage of exploration and development. Till now the applications of biosensors mainly focus on the various detection fields due to their excellent selectivity. With the development of knowledge economy in this century, biosensor technology will undoubtedly become a new growth point. This paper will focus on virus detection, monitoring of trace target substances in diseases, pesticide residues.

2.1. Virus Detection

Viruses are infectious, specialized intracellular parasites that can only be observed through electron microscopy as small microorganisms (with a diameter of 20-400 nm), and their genomes are composed of DNA or RNA. In the process of evolution, viruses develop multiple mechanisms which poses a huge threat to global human and animal security and immeasurable economic losses, the best example would be the COVID-19 epidemic that lasted for three years, still having severe influence recently. Therefore, a rapid, accurate, highly sensitive and convenient detection technique is urgently needed to identify the virus causing the epidemic.

Among them, electrochemical the electrochemical biosensor has high selectivity and sensitivity and low cost, and are currently one of the most widely studied and applied biosensors. The detection principle of electrochemical biosensors is to fix bio-active substances as molecular recognition substances on the electrode surface. As recognition element and the specificity of the measured molecular recognition, signal converter converts target molecules and the response signal to current and potential signals, so as to realize the target under test material qualitative or quantitative detection. Their advantages such as high sensitivity, fast signal generation and readout speed, fewer samples required, low cost of virus detection, and the need for relatively simple operating instruments make them widely used in high incidence and highly infectious virus detection. Since December 2019, the globe has been struggling with the major global health catastrophe known as COVID-19, which is caused by the SARS-CoV-2 virus. Within a few months, COVID-19 has transitioned from a relatively understudied viral group to a global public health concern. For the prompt care and isolation of confirmed patients to stop future transmission, laboratory detection of Covid-19 is crucial because to the lack of clear specific antiviral medications and the limited accessibility of symptomatic treatment. Büyüksünetçi et al[6]. established an electrochemical detection method for COVID-19. The sensor immobilizes angiotensin converting enzyme 2 which is abbreviated as ACE2 and CD147 (host specific receptor binding to S protein) on different gold screen printed electrodes (AuSPE), and modifies them with an EDC/NHS mixture. To test the S protein's affinity for the ACE2 or CD147 receptors, SARS-CoV-2 S protein is introduced to the modified working electrode region. The results indicate that the linear range of ACE2 receptor is 7×10^{-5}~1.5×10^{-6} ng·L^{-1}, with a detection limit (LOD) of 2.99×10^{-5} ng·L^{-1}; The linear range of CD147 receptor is 5×10^{-5}~5×10^{-6} ng·L^{-1}, with a LOD value of 3.9×10^{-4} ng·L^{-1}. Previously, micro electrochemical biosensors based on DNA hybridization have been developed to detect other viral bacterial or vira; infections. Tripathy et al.[7] modified and customized this biosensor to make recommendations to detect the type of severe acute respiratory syndrome coronavirus. They modified the titanium working electrode by electrodepositing AuNP. The single-stranded DNA probe is then fixed via a gold mercaptan bond, which complicates SARS-COV RNA type 2 or its corresponding c-DNA. The probe needs to complement specific SARS-COV -2 sequences to achieve highly selective detection. A few SARS-CoV type 2-specific genes, including the gyrase, E protein, and RNA-dependent RNA polymerase genes, have been identified by humans using prior RT-PCR techniques. The latter two do not interact with respiratory viruses, other coronaviruses, or other coronaviruses that infect humans. The drawback is that samples must be processed and extracted before testing, though. This adds a step before diagnosis, which could impair diagnosis accuracy and lengthen the time needed for analysis. For quick and reliable diagnosis, a direct detection approach for SARS-COV-2 is crucial. The detection of SARS-COV-2 surface protein (spike protein, for example) without a specific diagnosis can be made sample preparation with the necessity for the aforementioned RNA extraction and the amplification of nucleic acid diagnosis (RT-PCR). Using spike protein antibody as a detection probe, Seo et al.[8] created a FET-based biosensing device to identify coronavirus type 2 that causes serious acute respiratory illness.The coupling agent 1-pyrene butyrate N-hydroxyhypoimine (PBASE), which served as a detection connection, was used to immobilize the FETs on the graphene sheets after they had been coated with an anti-spike protein antibody (SpAb).To evaluate the effectiveness of the FET, antigen protein, virus, and nasopharyngeal swab samples from 19 COVID patients are being used.The FET biosensor can detect spike protein antigens at concentrations of 1 fM and 100 fM in phosphate

buffer brine and general transport medium (for floating in clinical diagnostic of nasopharyngeal swabs). The LOD of nasopharyngeal swab samples and cultured viral samples were 16 pfu/mL and 2.42×10^2 copies/mL, respectively. Mahari and others [9] Similar methods were employed to identify SARS-COV-2, or severe acute respiratory sickness.They have developed a new type of internal biosensor eCovSens hyderabad (India national animal biotechnology research institute), at the same time the use of fluorine doped tin oxide (FDTO) electrodes and AuNP for detecting SARS coronavirus spike protein type 2. First, they immobilize the antispike protein antibody (SPAB) to AuNPs via physical adsorption and electrostatic interaction to form AuNPs/SPAB. The preparation method of the device is covered on the glass electrode FDTO, then drip AuNPs/SpAb. Under optimal conditions, measurements were obtained ranging from 1 fM to 1 μM, while the reporters were able to achieve a LOD of 90 fM using labeled saliva samples. In addition, the biosensor can quickly identify spike proteins within 10 to 30 seconds, ensuring clinical applications.

2.2. Trace Target Substance Monitoring in Diseases

In the past few years, it was found that the different biological markers associated with a variety of disease states, to indicates the state of health and the stage of disease various disease biomarker more and more get the attention of people. The identification of these biomarkers has been accomplished using a variety of analytical tools, but electrochemical analysis is among the most reliable due to its high sensitivity, high accuracy, broad range of measurement, relatively low cost, and portable instrument requirements. However, one of the most severe challenges in this field involves electrochemical sensors in the operation of biological media, because when exposed to biological fluid, sensing interface of biological fouling will adversely affect the function of the electrochemical sensor. Cancer, as one of the important and fatal diseases that endanger human health, has no absolute effective treatment methods yet. However, early detection and intervention therapy can have a positive impact on patients' lifespan and even rehabilitation, which is also an important direction for the development of medical devices at present. For instance, Wang et al. [10] produced an ultra-sensitive impedance anti-fouling biosensor, and the surface Self-polymerizing dopamine (PDA) modified electrode Adenosine triphosphate (ATP) was further functionalized by the self-assembly of PEG and ATP aptamers. The apterer sensor was employed for the detection of adenosine triphosphate (ATP) in human plasma, exhibiting a coefficient of variation (%RSD) ranging from 2.71% to 4.18%. The limit of detection (LOD) for ATP in cancer cell extracts was shown to be 0.29 mM with a standard deviation of ± 0.02 mM, based on three replicates. These findings indicate a favorable potential for the accurate quantification of ATP in complex biological samples. The investigation focuses on evaluating the antifouling capabilities for the aptamer sensor. The results show that the interface design using PEG significantly decreased after exposure to human plasma interface charge transfer resistance. This indicates that the self-assembled layer containing PEG is resistant to protein adsorption, which is attributed to the empowered hydrophilicity.

At the same time, we cannot ignore the rapid development of wearable sensors. Wearable biosensors can be used in conjunction with the Internet of Things and big data to detect, transmit, store, and analyze human physiological and biochemical data in-depth. The market potential for this technology is enormous in the cutting-edge domains of chronic disease diagnosis and self-monitoring of personal health. To illustrate this idea, a variety of significant analytes have been employed as targets. In order to improve the management of chronic diseases, continuous real-time monitoring of biomarkers linked to wearer health can shed light on their dynamic biochemical processes and give health information. The epidermis wearable biosensor prevents the blood collection needle from piercing the skin to achieve the convenience of daily-life-use. At present, wearable biosensor has been made in a variety of fundamental, such as gloves, wrist strap, tattoos, etc. Comfort is its greatest advantage. For example, Nyein et al. developed a flexible microfluidics patch system, real-time analysis of sweat samples. The sensor is based on the flexible plastic substrate, integrates the embedding special spiral ion selective sensors the microfluidic channel. The system is connected to a sensing component and can analyze perspiration using printed circuit board (PCB) technology. The

sensor can monitor ion concentration such as sodium ion and sweating rate, which is beneficial to monitor physiological and clinical condition of human body through sweat parameters. In addition, there is still room for improvement in the time resolution of the sensor, which can make fabrication easier and high throughput. Considering the requirement of WB that can mimic the softness and flexibility of the skin surface, a wearable patch platform lab with integrated microfluidic collection system was constructed using polydimethylsiloxane (PDMS) developed by Lee et al. In this system, a cortisol-specific antibody (MX210Ab) is fixed to a stretchable and conformable nanostructure surface through an impedance measurement-based detection. The LOD was 1.0 pg/ml and the detection range was up to 1 μg/ml at the optimal antibody concentration. 3D AU nanostructures can achieve higher sensitivity as working electrodes, although the sensor is limited by the instability of Ag-AB complexes and is not reproducible. To overcome this instability, Palrak et al. reported a copolymer synthesis through the artificial molecularly imprinted polymer (MIP), used for screening of cortisol.[11] MIPS is highly selective, reversible, and reproducible for the cortisol template. The same team has developed a device called SKINTRONICS, which uses electrical currents in the skin to determine skin responses to stress levels. It is a multilayer device, wear time of 7 hours, in accordance with flexible mixed skin function, can capture the real-time data. Currently, wearable patches or sensing platforms with various skin-based interfaces are in the development stage, indicating that the focus is shifting to flexible sensing.

2.3. Pesticide Residue Detection

Agricultural products such as vegetables, fruits and so on in the production process will inevitably appear a variety of diseases and pests, resulting in a decline in yield and quality. As an important means of production, pesticide have a huge impact on agricultural production. However, with the extensive use of pesticides and the increase of years of use, pesticide residues have gradually increased.

The pesticide residues in vegetables are mainly banned organophosphorus pesticides and carbamate pesticides. However, excessive pesticide residues in agricultural products have a great impact on the Chinese people and the export trade of Chinese agricultural products. Therefore, relevant departments in China have formulated strict standards for pesticide detection. The rapid detection methods of pesticide residues mainly include biochemical determination and chromatographic detection. The chromatographic method has the advantages of accurate analysis and high sensitivity, but it relies on large analytical instruments in the laboratory, and the detection cost is high, so it cannot be used for on-site detection in the field and cannot be timely feedback to farmers. For biochemical detection, the enzyme inhibition approach is practical, however it only detects organophosphorus and carbamate pesticide residues. In recent years, as a new detection technology in electrochemical sensing, photoelectric chemical sensing detection technology is a low background and high sensitivity detection technology that combines optical response and electrochemical sensing. The technology uses light as excitation signal and current as output signal, which realizes signal separation in energy form, reduces background signal, improves detection sensitivity, and has the advantages of less sampling, fast and simple. Based on the photoelectric chemical sensing detection technology and coupled with biosensing strategies such as immune recognition and aptamer binding, the application of photoelectric chemical biosensors in pesticide residue detection has attracted wide attention .[12]As early as 2011, Li et al.[13] used poly (3-hexylthiophene) functionalized nano titanium dioxide (TiO_2) to construct a visible light and zero potential response photoelectric chemical sensor to detect chlorpyrifos, an organophosphorus pesticide. The titanium dioxide hole in the photoexcited poly -LRB-3-hexylthiophene) transitions from the valence band of poly -LRB-3-hexylthiophene) to the valence band of Polypoly (3-hexylthiophene), which reacts with water to produce hydroxyl radicals, during the exposure of visible light. These electrons then inject the titanium dioxide's conducting band and transfer to the electrode. The tested substance chlorpyrifos is oxidized to its free radical form and promotes the amplification of photocurrent signal. Till 2020, Tan et al. [14] established a photoelectric chemobiological sensing platform based on aptamer competitive

recognition strategy and CuS QDs/ Co_3O_4 mediated multiple signal amplification, and achieved highly accurate and sensitive chlorpyrifos detection. Under the optimal conditions, the linear correlation range of chlorpyrifos detection was 0.1 ng/mL ~ 10 mg/mL, and the LOD was 0.34 pg/mL. Li et al. induced a biocatalytic reaction to generate BiOBr/ Bi_2S_3 based on the combination of malathion and aptamer, obtained an enhanced photocurrent response, and realized a sensitive detection of malathion with LOD 0.12 pg/mL as the lowest.

Pyrethroids are a broad-spectrum insecticide that can control various pests and have a strong contact killing effect on insects. Pyrethroids are relatively safe for humans and animals due to their low dosage and concentration, and have minimal environmental pollution. Its main disadvantage is that it is toxic to fish and harmful to some beneficial insects. Long-term repeated use can also lead to pest resistance. The detection of such pesticide residues has become an increasingly serious problem. Based on the urgent need for highly selective pesticide field detection equipment, Wang et al. [15] introduced molecularly imprinted photochemistry into the paper-based microfluidic analysis strategy and designed a paper-based photochemical detection method. CdTe quantum dots and molecularly imprinted polymer (CdTe QDs @ MIPs) are combined in this photochemical method, which is activated by a standard ultraviolet lamp (365 nm). In order to create a highly sensitive paper sensor based on photochemical molecular imprinting, AU NPS were used to fix CdTe QDs@MIPs on the paper-based screen-printing working electrode. Au NPS were also electrodeposited on the working electrode's surface to improve the electron transfer efficiency. The photocurrent produced by the paper-based molecular imprinted photochemical sensor under ultraviolet irradiation dropped as the concentration of the fenvalerate solution rose while using fenvalerate as the model analyte. The LOD of the quenching paper based molecular-imprinted photochemical sensor is 3.2 nmol/L. In this study, a photoelectric chemical sensor with high selectivity and sensitivity for S - cyanovalerate monitoring was successfully developed.

At the same time, among traditional powerful pesticides, organochlorine pesticides have serious damage to the functions of various organs in humans and animals due to their difficult degradation and excretion characteristics. Therefore, it is necessary to optimize rapid detection. Wang et al. [16] developed -halocyclohexane molecular-imprinted polymer films on TiO_2 nanotubes using electropolymerization of o-PD monomer and -halocyclohexane template molecules for photochemical sensing detection of -halocyclohexane molecules. Under visible light, the molecularly imprinted polymer film may produce the photoelectric transition from the highest occupied molecular orbital to the lowest unoccupied molecular orbital, and the excited electrons can then be transported to the conduction band of titanium dioxide nanotubes.At the same time, we suggest that the positive charge (h +) of the molecularly imprinted polymers involved in the oxidation process is depleted to facilitate the amplified photocurrent response. The sensor exhibits high specificity and demonstrates successful applicability in the recognition and detection of γ-halohexane, while also demonstrating a strong linear independence. Meanwhile, the sensor construction strategy has broad future in the treatment of organochlorine pesticides.

3. Conclusion and Discussion

Biosensors, as new bio-integrated technology products and new economic growth points, will promote the rapid development of the biotechnology industry, and can provide indispensable analytical capabilities and rapid detection capabilities for many fields. The development of novel techniques for expeditious analysis, the emergence of novel bioinstrumentation sources, and the convergence of biosensors with advancements in materials science and microelectronics engineering are anticipated to facilitate the creation of an expanded array of biosensors. In the future, the general direction of biosensors may turn to the road combined with computers and artificial AI, they will become more and more automated, scientific, and accurate, which will greatly enhance our detection capabilities, which will greatly promote the analysis of viruses, pesticide residue detection, disease target substances and so on.

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