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Biomedical applications of fluorescent biosensors

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Abstract. This study aims to systematically summarize the development history of fluorescent biosensors and their applications in biomedicine, and propose optimization strategies to promote the development of fluorescent biosensor technology according to the current challenges. This paper collects the literature of relevant research at home and abroad, and organizes and analyzes the basic principles, development history and biomedical applications of fluorescent biosensors. In addition, in view of the problems existing in fluorescent biosensors, some potential optimization strategies are proposed. After sorting out and analyzing the development history of fluorescent biosensors, it can be seen that the technology of fluorescent biosensors has made significant progress. In the field of biomedicine, fluorescent biosensors have been successfully applied to biomolecular detection, disease diagnosis and drug screening, showing great application potential. However, current fluorescent biosensors still face some challenges, such as light stability and sensitivity, which need to be further optimized. In summary, the application of fluorescent biosensors in biomedicine has broad prospects, but some technical challenges still need to be overcome.

Keywords: Fluorescent Biosensors, Development, Biomedical Applications.

1. Introduction

Fluorescence is a visible light phenomenon emitted by substances after being excited, and its application has a wide range of application prospects in biosensors. Fluorescent biosensor is a technology that uses the principle of fluorescent molecules interacting with specific biomolecules to realize the monitoring and analysis of biological processes. The cell sensor is also a common fluorescent biosensor. Cell sensors use cells as signal conversion units for sensors and enable analyte analysis by detecting intracellular or extracellular fluorescence signals. Cell sensors can use characteristics such as the metabolic activity of cells, protein expression levels, and potential of cell membranes to reflect the presence and concentration of analytes.

2. Fluorescent biosensors

In fluorescent biosensors, the working mechanism of biosensors is a key link. Biosensors enable quantitative or qualitative analysis of analytes by detecting the fluorescence signal they produce by interacting with analytes by specific organisms or biological components. Different types of biosensors have different working mechanisms, and the following will introduce the working mechanisms of several common biosensors.

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First, the enzyme sensor is a common fluorescent biosensor. It uses a specific reaction between the enzyme and the molecule of interest to detect the analyte. The enzyme catalyzes under the action of the target molecule to produce a fluorescent signal. By measuring the intensity or change of the fluorescence signal, the concentration or activity of the analyte can be measured indirectly.

Secondly, antibody sensors are another common fluorescent biosensor. It uses the specific binding between the antibody and the molecule of interest to detect the analyte. Antibodies can selectively recognize target molecules that interact with their ligands and bind to them to form specific complexes. By detecting the fluorescence signal of the complex, quantitative detection of the analyte can be achieved.

In addition, nucleic acid sensors are also a commonly used fluorescent biosensor. Nucleic acid sensors use the principle of complementary pairing to pair sequences of target molecules with fluorescently labeled probe molecules to each other. When analyte is present, the target molecule binds to the probe molecule, allowing the fluorescence signal to be excited and released. By detecting the intensity change of the fluorescence signal, the detection and analysis of the analyte can be realized.

The production of fluorescence is closely related to the excitation and emission processes. In fluorescence biosensors, an excitation light source is commonly used to excite fluorescent molecules, which absorb the energy of the excitation light and transition to the excited state, which has two possible conversion channels, fluorescence transition and non-radiative transition. Among them, fluorescence transition refers to the fluorescent molecules emitting fluorescent photons of a specific wavelength when returning from the excited state to the ground state, which spectroscopic instruments can usually detect. Nonradiative transitions are when fluorescent molecules transfer resonance energy with other molecules in an excited state, allowing energy to be dissipated in other ways. The magnitude of the fluorescence intensity corresponds to the concentration of the fluorescent molecule, so the intensity of the fluorescence signal can be used to quantify the biomolecule to be monitored. This is a two-photon action cross sections and emission spectra from a base set of biological molecules, as shown in Figure 1. (a) Action cross sections (absorption cross-section times the fluorescence quantum yield) of six molecules that contribute much of the intracellular 2PE intrinsic fluorescence. Units: 1 GM (Göppert-Mayer) equals 10-50·cm4. The contents of all the compounds were determined by EtOH in the buffer (pH7.2) salt, with the exception of retinol and colecalciferol (vitamin D). The concentrations of riboflavin, colecalciferol and NADH were determined at 100μ m, while the levels of retinol, folate, phylloquinoline, pyridoxine and nicotinamide were determined at 500μ m. (b) The emission spectra (measured with identical solvents) of the substances listed in Annex I.

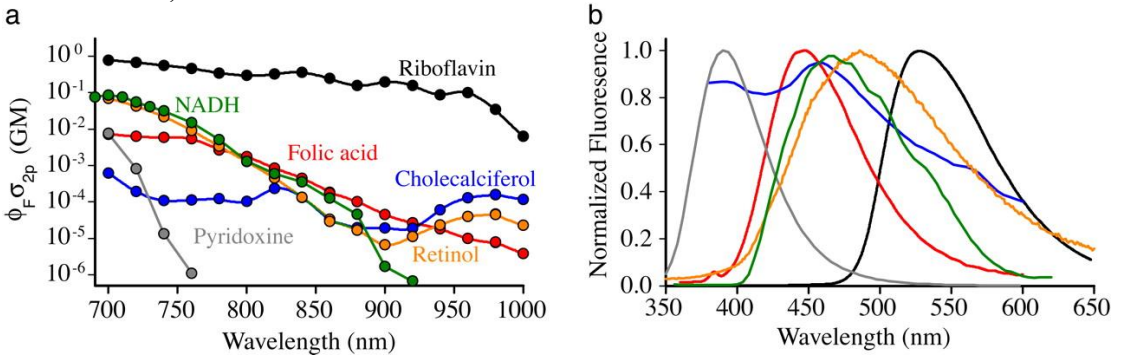

Figure 1. two-photon action cross sections and emission spectra [1].

In the fluorescent biosensors field, various types of biosensors have emerged for different detection objects and application scenarios. These sensors can be classified and distinguished according to their construction and principle of operation.

First, the common type is fluorescent protein sensors. This type of sensor enables detection by utilizing the properties of fluorescent proteins. Fluorescent protein sensors can respond to specific molecules or organisms by changing the structure or environment of fluorescent proteins. Fluorescent

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protein sensors have a wide range of application prospects in biomedical applications, such as detecting pathogens and monitoring intracellular signaling molecules.

Secondly, there are quantum dot-based biosensors. Quantum dots are a new class of nanomaterials with narrow emission spectra and broad absorption spectra. In fluorescence biosensors, quantum dots can be used as fluorescent probes to detect the presence and concentration of target molecules. The fluorescence properties of quantum dots can play an important role in the biomedical field by surface modification to achieve higher sensitivity and selectivity. Quantum dot-based fluorescent biosensors can be widely used in molecular diagnosis, drug molecular screening and other fields. By definition, the fluorescence quantum yield ΦF represents the proportion of excited molecules that deactivate by emitting a fluorescence photon. It is the ratio of the number of emitted photons to the number of absorption photons per time unit: ΦF = Number of Emitted Photons/Number of Absorbed Photons (1) Thus, it can be understood that the Fluorescence Quantum Yield has a direct relation with the Irradiance (Kr) and Non-Radiation (Knr) (2) Measuring the Absolute Quantum Yield is very important and needs a specific device. It is essential that the quantity of excitation light that the specimen receives be precisely known. For calibration of the system, measurements shall usually be made with dispersants and integration balls or actinometers (2). Note that the determination of the absolute fluorescence quantum yields can also be performed with other methods such as calorimetry (4)[2].

In addition, there are biosensors based on metal nanoparticles. The fluorescent nature of metal nanoparticles can be detected by changing their surface modifications and structure. Metal nanoparticle sensors have high sensitivity, stability, and controllability, so they have a wide range of potential for biomedical applications. These sensors can be applied to the detection of tumor markers and the monitoring of heavy metal ions.

Finally, there are emerging types of biosensors, such as DNA- or RNA-based fluorescent sensors. These sensors take advantage of the properties of nucleic acids to undergo fluorescence signal changes by binding to the molecule of interest under specific reaction conditions. Nucleic acid-based sensors have fast response characteristics, high sensitivity and high specificity, and can be used for genotyping, disease diagnosis, etc.

There are many types of fluorescent molecules in fluorescent biosensors, including organic dyes, quantum dots, and fluorescent proteins. Among them, organic dyes are widely used in fluorescent biosensors with high luminous efficiency and stability. Quantum dots are nanomaterials with special luminescence properties guided by size effects, so they have good application prospects in fluorescent biosensors. Fluorescent protein is a natural fluorescent molecule, and fluorescent protein with specific fluorescence characteristics can be obtained through genetic engineering technology, so it has important research value in identifying and tracking biosensors.

As a new type of biometric identification and detection technology, fluorescent biosensor has been widely used and developed in biomedicine in recent years. The basic principle is to use specific biomolecules in organisms to combine with fluorescent dyes to undergo fluorescence changes, and to achieve quantitative analysis of biomolecules by detecting such fluorescence changes. Fluorescent biosensors have shown great application potential in tumor diagnosis, disease treatment monitoring, and environmental pollution monitoring [3]. The researchers did experiments about new fluorescent biosensor based on inner filter effect and competitive coordination with the europium ion of non-luminescent Eu-MOF nanosheets for the determination of alkaline phosphatase activity in human serum. Abnormality of ALP (ALP) in the bloodstream suggests a medical condition. In this paper, a new fluorescence sensor system, which is composed of Europium (Eu3 +), is proposed to be used in the ALP assay of the human serum, which is a non-luminescence lanthanide metallic organic matrix (NO2-Eu-MOF NS). The fluorescent signal is switched on by an antenna ligand (PEPP), which competes with the Eu3 + ions of the nanometer-sheet, to generate emission species (Eux (PEPP) y (NO2BDC) z) in situ [4]. 4-Nitrophenylphosphate (PNPP), which is an ALP substrate, was hydrolysed to 4-nitrophenol. PNPP shows high absorption in UV-Vis (250 - 400 nm) and includes Eux (PEPP) y (NO2BDC) z excited wavelength (280 nm). Thus, the presence of PNPP results in Eux (PEPP) y (NO2BDC) z being nonemitting and Eux (PEPP) y (NO2BDC) z when hydrolysed by ALP to produce a detectable

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fluorescent signal. Therefore, it is possible to quantitatively analyse the ALP activity from the fluorescent variation. The method has been proved to be effective in determining ALP activity within 5-1000u L-1. The assay limit is 1.1UL-1 (S/N = 3). The results show that the biosensors have good sensitivity and anti-jamming capability. Therefore, it may be used to analyze the ALP activity of the human serum in clinic.

In the current state of fluorescent biosensors, on the one hand, continuous improvements have been made to the sensitivity and selectivity of sensors. By continuously optimizing the structure and design of the sensor, its recognition ability and detection sensitivity of target molecules are improved. For example, researchers have used bimolecular supramolecular self-assembly technology to construct fluorescent biosensors with high selectivity and sensitivity, which can quickly detect and quantify trace substances.

On the other hand, the range of applications of fluorescent biosensors has also been expanded. In addition to the traditional biomedical field, fluorescent biosensors are also used in food safety detection, water quality monitoring, environmental pollution monitoring and other fields. For example, researchers have used fluorescent biosensor technology to develop a rapid detection method for detecting harmful chemicals in food, improving detection efficiency and reducing damage to food samples.

In addition, with the development of nanotechnology, the application of nanomaterials also provides new ideas for developing fluorescent biosensors. Researchers can further improve the sensor's performance by introducing nanomaterials such as nanoparticles, nanowires or nanorods.Combining nanomaterial (NMM) and aptamers with aptasensors allows for high specificity and sensitivity in detecting a wide variety of contaminants. It has been widely accepted that aptasensors can detect a wide range of new organic pollutants (EOPs) in a variety of environments and biospheres. Apart from being highly sensitive and selective, NM based aptasensors also have a number of other benefits, including portable, miniaturized, easy to use and affordable.

Based on their sensor mechanisms, the aptastic systems are divided into electrochemistry, colorimetry, PEC, fluorescent, SERS and ECL. Particular emphasis is placed on manufacturing technology, analysis reliability, and sensing mechanism of NM based aptasensors. Furthermore, the effectiveness of the aptasensing methods was evaluated according to their underlying performance measures (such as detection thresholds, sensor ranges, and response times) [5]. The large specific surface area and special optical properties of the surface of nanomaterials enable fluorescent biosensors to analyze and monitor biomolecules more accurately. New nanoscale semiconductor light-emitting particles (quantum dots) have also been successfully used for fluorescent labeling of organisms. Luminescent quantum dots have a series of advantages such as long fluorescence lifetime, high quantum yield, tunable emission wavelength, narrow emission line width, etc. Using them as fluorescent markers can not only efficiently eliminate back-to-bottom fluorescence interference through time-resolved spectroscopy, but also use the same excitation light. The source detects multiple channels at the same time [6].

3. Applications of fluorescent biosensors

In current fluorescence biosensor research, some problems need to be solved. First, although fluorescent biosensors have a wide range of application prospects in the biomedical field, their sensitivity and selectivity still need to be further improved. Current fluorescence probes have low detection sensitivity for target molecules and are sometimes affected by sample complexity, resulting in loud signal noise. In addition, some sensors are sensitive to interference from other substances and lack sufficient selectivity.

Second, the response speed of current fluorescent biosensors needs to be further improved. For certain biological processes that require real-time monitoring, such as cell activity, metabolic reactions, etc., the sensor's responsiveness is particularly important for accurate results. However, most of today's sensors are slow to respond and struggle to meet real-time monitoring needs at the cellular and molecular levels.

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In addition, the stability of the sensor is also an urgent problem. In both the internal and external environment, fluorescent biosensors can be affected by temperature, light, chemicals, etc., resulting in signal drift and attenuation. More stable fluorescent probes and materials need to be developed to maintain sensor stability and long-term reliability.

The preparation cost is also an issue in large-scale applications of fluorescent biosensors. The preparation process of many fluorescent biosensors is complex and the material cost is high. Simple and cost-effective preparation methods need to be developed to advance the practical application of fluorescent biosensors, reducing preparation costs and improving sensor scalability. At present, most of the commercialized products are photoexcitation source unlabeled structures, but the economic cost of these sensors is relatively high, and the sensitivity is also a certain gap compared with indirect sensors. Although all kinds of indirect sensors with markers can obtain high test sensitivity, their stability is generally poor, which seriously restricts the pace of their practical application [7].

In studying fluorescent biosensors, technical optimization is the key to solving existing problems and improving device performance. This section will explore technology optimization strategies from different perspectives and explain their significance for fluorescent biosensors in biomedical applications.

First of all, material selection is at the heart of technical optimization. For fluorescent biosensors, suitable materials are the basis for ensuring high sensitivity and stability. Currently, widely used sensor materials include quantum dots, organic fluorescent materials and metal-organic framework materials. These materials have unique advantages in luminescence properties, such as quantum dots with narrow bandgap and high brightness, while organic fluorescent materials have a wide range of luminescence wavelengths and adjustable luminescence properties. Therefore, selecting the right material becomes a top priority in technical optimisation.

Secondly, the design and optimization of the sensor structure is also an aspect that cannot be ignored. High selectivity and sensitivity to target molecules can be achieved through reasonable structural design. For example, introducing specific functional units, such as acceptor molecules, signal amplifiers, and nanoparticles, can improve the interaction between the sensor and the target molecule and the timely response of the sensing signal. In addition, the optimization of the structure also includes the regulation of the physical form and size of the sensor, so that it can adapt to different detection environments and needs. Therefore, structural design and optimization play a crucial role in technical optimization.

In addition, using reasonable detection methods is also important for technical optimization. Traditional detection methods include fluorescence intensity, fluorescence lifetime, and fluorescence spectroscopy. However, these methods are limited by background interference and fluorescence quenching. Emerging detection techniques such as surface plasmon resonance, Raman spectroscopy, and single-molecule fluorescence imaging have achieved higher detection sensitivity and resolution in recent years. Therefore, in technical optimization, choosing the appropriate detection method is important in improving sensor performance.

Finally, data processing and algorithm optimization are key technical optimisation links. Through reasonable data processing methods and optimized algorithms, errors can be reduced and sensor reliability can be improved. For example, the use of advanced signal processing technologies, such as wavelet analysis, artificial intelligence and machine learning, can achieve more accurate and faster signal interpretation. In addition, optimization algorithms can improve the efficiency of sensor operation and data processing speed. Therefore, in technical optimization, data processing and algorithm optimization are indispensable parts. For example, the BlACore system and lAsys system use carboxylated dextran surface treatment to effectively reduce the non-specific binding n83 of the sensing layer; In the immunoassay based on refractive index detection, the detection sensitivity of low molecular weight substances is also enhanced by the use of a "sandwich" assay with auxiliary nanoparticles. Recently, Nikitin P.I. A new type of marker-free sensor is reported, which not only can obtain high test sensitivity, but also can carry out multi-channel detection simultaneously, and has great development potential [8].

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First of all, with the development of nanotechnology, more and more nanomaterials are used in fluorescent biosensors. These nanomaterials have a large specific surface area and special optical properties, which can effectively enhance the fluorescence signal and improve the sensitivity and detection ability of the sensor. For example, gold nanoparticles can enhance fluorescence signals through surface plasmon resonance phenomena, and carbon quantum dots can be specifically detected through selective modifications.

Second, more and more research is focusing on the versatility of fluorescent biosensors. While traditional fluorescent biosensors can only detect a single target substance, researchers now hope to achieve a sensor that can detect many different molecules simultaneously. For example, simultaneous detection of multiple molecules can be achieved by assembling many different recognition molecules or nanomaterials. This versatile fluorescent biosensor will greatly broaden its range of applications, for example in environmental monitoring, biomedical diagnostics, and food safety.

Third, the automation and convenience of fluorescent biosensors is also future development direction. Traditional fluorescent biosensors require more complex experimental operations and instrumentation, which limits their use in practical applications. Therefore, researchers are working to develop simplified and automated fluorescent biosensor systems, such as using microfluidic chips or integrated organic electronics, to achieve fast, accurate and convenient detection.

Finally, fluorescent biosensors have great potential for biomedical applications. With the growing health concern, there is an increasing demand for fast, sensitive and reliable sensors in biomedicine. Fluorescent biosensors can be used as a non-invasive, real-time monitoring tool to detect physiological indicators, disease markers, and drug analysis in organisms. By combining with bioimaging technology, fluorescent biosensors can also provide more comprehensive and accurate information for biomedical research.

Much progress has been achieved in the recognition of T-cells' role in adaptive immune response, which is due to the development of complex imaging technologies for visualization of T-cells in vivo and in vitro. One of the most difficult tasks in this field is to combine this approach with approaches that will enable researchers to visualize single cellular signaling. Fluorescence biosensors, whether synthesized or genetically coded, have been shown to be an effective approach to understanding the spatial and temporal effects of biological processes. Among them are the minor GTPases of RhoA, Rac and Rap1, the tyrosine kinase Lck, ZAP-70, cAMP, Ca^{2+}, etc. The future development and application of bio-sensors will enable the immune system to combine the enormous amount of available biochemistry on T cell function with the capability of dynamic live cell imaging [9].

Also researchers made recent progress and prospects of alkaline phosphatase biosensor based on fluorescence strategy, mostly about ALP. Fluorescent detection strategies, such as "off", "on" and "on", "and" ratio ", were reviewed, and their operating principles, strengths and weaknesses were compared. It can be seen from Table 1 that the majority of fluorescence materials are Cu^{2+}, AA2P and PNPP. as an intermediate in ALP assay, and their use often makes ALP detection more complex. Moreover, the organ metal-based probes and ratiometric probes can prevent the application of these intermediates, making them an effective way to detect ALP. Unfortunately, the process of preparing organic molecules and ratiometric probes was complicated, limiting their wide use. Moreover, as biomedical science develops rapidly, it is very important to accurately diagnose ALP related illnesses. Through a review of ALP sensor techniques, the precision and fast determination of ALP depends heavily on the discovering and applying of novel materials. So it is very important to develop and design a detecting system according to the relation of ALP to detect ALP effectively. Moreover, in order to achieve excellent detecting capability, the use of computerized analogue digital platform will be an attractive and desirable technique. To sum up, in view of the advances in detecting ALP, it is our belief that fluorometric technique will be able to provide a promising prospect in the field of biology and medicine.

In summary, future research directions should focus on improving fluorescent biosensors' performance and application range. Miniaturization, integration and intelligence will be the inevitable trend of its future development, with the improvement of the structure and function of the sensor, its role in production, life and research and other fields will also become increasingly prominent, such as

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the biochip technology developed based on biosensors in recent years has achieved great success in the application. At present, the related research work of supporting disciplines such as material science, optical technology and computer technology has been gradually carried out. For example, the development of new organic fluorescent materials and their application in biomarkers have attracted a large number of material scientists and made great progress, which has laid a solid foundation for the development of photo fluorescent biosensors [10].

4. Conclusion

In the field of fluorescent biosensors, although remarkable research results have been achieved, there are still many potential research directions that are worth exploring in depth. First, the researchers can further improve existing fluorescence sensor designs to improve their sensitivity and selectivity. By introducing new functional materials and rationally optimizing the structure of fluorescent probes, the researchers can make the detection of target molecules by sensors more accurate and efficient. For example, the application of nanomaterials in fluorescent sensors can be explored, using their special physical and chemical properties to enhance the performance of sensors.

In addition, the researchers can explore the wider use of fluorescent biosensors in biomedical applications. Currently, most of the research has focused on ex vivo detection or application in animal models, but the researchers can consider introducing fluorescent sensors into clinical diagnostics. By developing portable, portable sensor devices, the researchers can monitor the level of biomolecules in real time in a clinical setting, thereby improving the early diagnosis and treatment of diseases. At the same time, the researchers can also delve into the application potential of fluorescent biosensors in drug discovery and drug efficacy evaluation, providing more accurate and high-throughput methods for the development of new drugs.

In addition, with the increasing attention of people to environmental pollution and food safety issues, fluorescent biosensors also have broad application prospects in the field of environmental monitoring and food detection. By combining sensors with microfluidic technology, the researchers can enable online monitoring and rapid detection of specific contaminants to safeguard public safety and environmental protection.

In summary, future research directions should focus on improving the performance and application range of fluorescent biosensors. The researchers can make more important contributions to the development of this field by improving sensor design, expanding biomedical applications, and exploring areas such as environmental monitoring. The researchers have reason to believe that in the near future, fluorescent biosensors will play a more important role in biomedical and environmental sciences, and make positive contributions to human health and environmental sustainability.

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