Progress in Medical Devices
ISSN: 2957-5478

Zentime

Review Article https://doi.org/10.61189/403384jfzmyx

Application and progress of functionalized magnetic bead-based biosensors for protein detection

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Declaration of conflict of interest: None.

Received September 25, 2024; Accepted November 6, 2024; Published December 31, 2024

Highlights

- In the field of bioanalysis, a new biosensor technology based on functionalized magnetic beads is leading a new direction in protein detection. With its excellent separation efficiency and sensitivity, it provides a powerful tool for early disease diagnosis and biomarker monitoring.
- This article explores the latest advancements in this technology, including innovative magnetic bead designs, diverse detection strategies, and the technical challenges and future development directions. It reveals the potential and application prospects of biosensor technology in biomarker detection.

Abstract

In the field of bioanalysis, the integration of magnetic beads and biosensors provides a protein detection platform with high separation efficiency and sensitivity. The superparamagnetism of magnetic beads, combined with surface functional modifications, forms the basis for selectively capturing and effectively separating target proteins. Additionally, the high sensitivity and specificity of biosensors ensure precise quantitative analysis of captured proteins. This article systematically reviews the synthesis strategies of functionalized magnetic beads, detection methods for proteins and nucleic acids, as well as the current technical challenges and future development directions.

Keywords: Protein, magnetic beads, biosensor

Introduction

Protein biomarkers play a critical role in medical research and clinical diagnostics. They are indicators that can reflect biological states or conditions, capable of detecting biological activities and processes [1-4]. The significance of protein biomarkers is evident in areas such as disease diagnosis, monitoring, and personalized medicine. For example, the abnormal expression of Programmed death ligand-1 (PD-L1) protein is associated with the prognosis and clinical pathological features of non-small cell lung cancer; abnormal deposition of amyloid beta protein, forming amyloid beta plaques, is one of the main pathological features of Alzheimer's disease [5, 6]. Such protein biomarkers can be detected through traditional methods like Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). However, in early disease diagnosis, pathological changes in protein biomarkers may not be significant enough, which can lead to undetectable increases in protein expression. These traditional methods have long detection cycles, complex operations, and limited sensitivity and specificity, which are insufficient for rapid and precise detection [7]. Biosensors, with high sensitivity, selectivity, and the ability for quick detection, can better address these issues.

A biosensor is an analytical tool consisting of a biological recognition element (such as enzymes, antibodies, and nucleic acids) and a signal transducer, capable of converting the recognition of biological molecules into measurable

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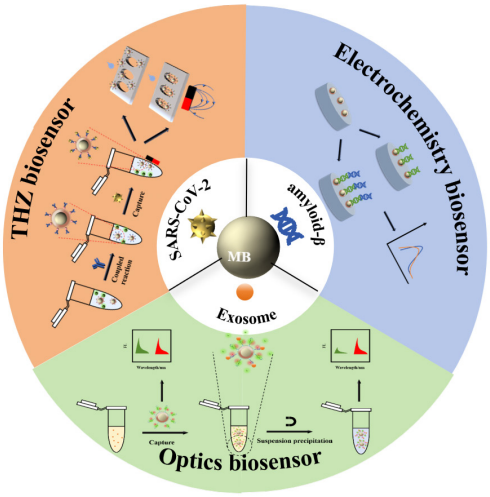

Figure 1. Detection of target proteins using functional magnetic beads combined with various biosensors.

signals. Therefore, biosensors can specifically identify target molecules, improving detection specificity, and, due to the presence of transducers and signal amplifiers, they can detect extremely low concentrations of biomarkers, enhancing sensitivity. Specifically, the working principle of a biosensor is based on the recognition and binding of a specific target molecule by the biometric element. This binding alters the physical or chemical properties of the biosensor, such as conductivity, light absorption, or mass change. These changes are converted by the signal transducer into measurable electrical or optical signals, which are then used to detect the presence and quantity of the target based on signal changes and their intensity [8-11]. Common biosensors include electrochemical biosensors, optical biosensors, terahertz metamaterial biosensors, and giant magnetoresistance biosensors, etc.

Magnetic beads are superparamagnetic microparticles with particle size distribution ranging from micrometers to nanometers. They can play multiple roles and perform various key functions in biosensor systems. For example, due to their excellent molecular dynamics and the inherent magnetism of the magnetic bead material, they can achieve precise magnetic separation, targeted positioning, and delivery through the application of an external magnetic field, allowing for the rapid and efficient separation of biomolecules from other components in a solution and signal modulation for signal transduction via their magnetic properties [12]. The core of the magnetic beads is mainly composed of magnetite (Fe_3O_4), while the outer material may include silica, agarose, or polymers, which minimizes their biological toxicity, making them suitable for binding various biomolecules [13, 14]. Additionally, the small size and large specific surface area of the magnetic beads allow for the binding of more biomolecules, enhancing the sensitivity of the biosensor. This makes them suitable for detecting trace amounts of samples, reducing reagent consumption, and promoting the development of portable and automated detection systems. Surface modification techniques are used to introduce various functional groups, such as carboxyl, amino, biotin, and avidin, to the outer layer of the beads, serving as solid-phase carriers to provide stable interfaces for biomolecules [15, 16].

This article explores the latest advancements in magnetic bead-based biosensor detection of proteins, including innovative magnetic bead designs, diverse detection strategies, and current challenges and future directions. It reveals the potential and application prospects of biosensor technology in biomarker detection (**Figure 1**).

Functionalized magnetic beads

Functionalized magnetic beads offer unique structures, controllable preparation methods, and diverse functional modifications. By selecting appropriate magnetic core materials, coating layers, and functionalization techniques, efficient capture and detection of target molecules can be achieved, providing a powerful tool for bioanalysis.

Structure

Magnetic beads can be classified into three types based on their physical composition: core-shell structure, hollow structure, and composite structure. Among them, the core-shell structure is the most common and widely used, which uses magnetic nanoparticles as the core, with outer layers typically made of materials such as silica, polymers, or gold. This core-shell structure significantly enhances the chemical stability of the beads and provides a larger specific surface area for further functional modifications. By directly modifying functional groups or biomolecules on the shell, a monolayer surface modification can be achieved. This simple monolayer structure is suitable for the rapid capture and separation of biomolecules. For example, Mao et al. used a supramolecular layer-by-layer self-assembly strategy to construct immunomagnetic beads, which significantly increased the number of functional sites on the beads, improving both capture efficiency and specificity [17].

Hollow structure magnetic beads have high spe-

cific surface areas, light weight, good magnetic responsiveness, and strong drug loading capacity. Their internal cavities can be used to load drugs or other active substances, with adjustable pore sizes. Ding et al. developed a hollow cellulose/carbon nanotube composite microbead with a patterned porous structure [18]. As the concentration of carbon nanotubes and cellulose increases, the diameter of the composite magnetic beads grow, effectively adsorbing methylene blue. Composite structure magnetic beads combine the advantages of different materials, offering multifunctionality and high efficiency. Their design enables the integration of various functions such as magnetism, fluorescence, and catalysis, providing broader application possibilities. The composite structure enhances the stability and biocompatibility of magnetic beads, improving their targeting and specific capture ability. Chen et al. assembled signal probes based on gold nanoparticles onto the surface of magnetic beads by the specific binding between the target and the aptamer, creating functional composite structure magnetic beads for colorimetric detection [19].

In target molecule capture, efficient separation methods are crucial for improving capture efficiency. To achieve separation between the target and biological samples, several magnetic separation methods have been developed based on the inherent magnetism of magnetic beads, all of which have shown promising results. These methods can be categorized into dynamic magnetic separation, static magnetic separation, gradient magnetic separation, automatic magnetic separation, magnetic fluidized bed, and magnetic microsphere aggregation, depending on the application of the magnetic field and the separation mechanism. These methods are selected based on sample size and experimental design, and they have been proven to enhance detection efficiency and accuracy in nucleic acid and protein detection [20-22].

Preparation methods

In recent years, core-shell structure magnetic beads have been most used in biological detection processes, such as nucleic acid and protein assays [23]. Magnetite (Fe_3O_4) is often chosen as the core material due to its simple synthesis, superparamagnetism, controllable magnetization, and low biological toxicity. In protein and nucleic acid detection, the stability and dispersion of magnetic beads are crucial, necessitating the modification and protection of magnetic core material. This can be achieved by using polymer stabilizers or coatings of inert metals. Additionally, functionalized magnetic beads should be equipped with target-specific ligands or functional groups on their surface to ensure stronger binding forces during target capture, improving the success rate and speed of the binding process [24].

The preparation methods for superparamagnetic beads mainly include physical, chemical, and microbial methods. Physical methods often require harsh conditions and are difficult to control the size and shape of the beads [25]. Microbial methods are hindered by long preparation times and low productivity, making them less suitable as high-priority methods for magnetic bead preparation. However, chemical methods can overcome these problems and produce more uniform magnetic beads with consistent size and shape due to their high controllability, making it more suitable for large-scale production [26]. The most common chemical synthesis methods include co-precipitation, microemulsion, and hydrothermal methods.

Co-precipitation is a commonly used method for preparing micron to nanometer-sized magnetic beads. This method involves adding a precipitant to a solution containing soluble salts, forming insoluble hydroxides or oxides, which are then decomposed by heating to produce nanomaterials. This method is simple, cost-effective, and allows for control over the morphology and size of magnetic nanoparticles by adjusting factors such as the solution pH and reaction time. While it is possible to control particle size by regulating conditions, achieving precise control may be more difficult compared to other methods, such as the microemulsion method. Additionally, without proper stabilizers, the particles produced may agglomerate, negatively affecting their performance in biomedical applications [27, 28].

Therefore, the preparation of magnetic beads using the microemulsion method has been a focus in the field of biomaterials. Zhi et al. proposed a new method for the in-situ preparation of magnetic chitosan/Fe_3O_4 composite nanoparticles in microaqueous pools of oil-in-water microemulsions by adding NaOH as an alkaline precipitant and using the oil-in-water microemulsion containing chitosan and ferrous salts as a microreactor [29]. Marija et al. used a microemulsion system with cetyltrimethylammonium bromide as the surfactant, butanol as the auxiliary surfactant, hexanol as the oil phase, and an aqueous solution as the water phase to prepare magnetic beads [30]. The microemulsion method allows for precise control

over the size and shape of magnetic beads, as the size and morphology of the microemulsion droplets directly affect the properties of the resulting nanoparticles. Therefore, magnetic beads prepared using the microemulsion method typically exhibit high monodispersity, and this method allows for direct surface modification during particle formation, which is beneficial for improving the biocompatibility and functionality of the magnetic beads. However, the process is more complex and requires precise control of reaction conditions. Additionally, beads prepared by the microemulsion method may require extra purification steps to remove residual surfactants and other organic solvents, which increases the overall cost. Nonetheless, microemulsion method is a versatile and effective technique for synthesizing monodisperse magnetic beads with controlled size, microstructure, and properties [29].

The hydrothermal method is a more classical method for synthesizing magnetic nanoparticles. Typically carried out in a high-pressure autoclave, this method uses water or alcohol as solvents, transforming iron precursors into magnetic nanoparticles under elevated temperature and pressure. This method is simple to operate, easy for mass production, and produces magnetic nanoparticles with good dispersion, uniform particle size, and complete crystal structure. Hemauer et al. synthesized Fe_3O_4 nanoparticles using the hydrothermal method, employing various crystal growth control agents to control the morphology of iron oxide nanoparticles [31].

Functional modification

Surface functionalization and modification of magnetic beads is a key aspect in various biomedical applications. Magnetic beads have gained attention for their diagnostic and therapeutic capabilities, allowing for simultaneous diagnosis and treatment of diseases [32]. Functionalized magnetic beads typically consist of a magnetic core and a surface functional layer. The magnetic core is primarily made of magnetic materials such as iron oxide, while the surface functional layer is modified through chemical or biological methods to impart specific biological recognition abilities to the beads. For example, antibodies, nucleic acids, enzymes, and other biomolecules can be immobilized on the surface of magnetic beads by covalent bonding, physical adsorption, or bio-affinity. Under the influence of an external magnetic field, the functionalized beads can rapidly bind to and separate target molecules. By employing appropriate functional groups and surface modification techniques, magnetic beads can be customized with various biometric elements or functional groups tailored to specific detection needs, significantly enhancing their capabilities [28]. There are various types of functionalized magnetic beads, commonly categorized based on their surface modifications. For example, carboxyl-functionalized magnetic beads are suitable for chemically cross-linking and immobilizing proteins or other biomolecules; amino-functionalized magnetic beads can undergo various chemical reactions for further functionalization; streptavidin-functionalized magnetic beads specifically bind biotin-labeled molecules; and antibody-functionalized magnetic beads are used to capture corresponding antigens [33-36]. In practical applications, such as in Chen et al.'s research, multifunctional carboxylated magnetic beads were prepared, allowing bovine serum albumin to be immobilized on the particle surface [37]. Overall, the surface functionalization of magnetic beads plays a pivotal role in enhancing their performance in various biomedical applications, ranging from drug delivery systems to diagnostic tools. With the fast and specific binding of streptavidin and biotin, coupled with their strong binding affinity, researchers have conducted extensive studies. For example, Zhao et al. used streptavidin-modified magnetic beads to specifically capture biotin-containing peptides [38]. In the field of antibody magnetic beads, Wang et al. developed a high-sensitivity sandwich assay method using antibody magnetic beads and aptamers to detect C-reactive protein (CRP) [39]. In conclusion, these functionalized magnetic beads, due to their unique surface chemistry and biocompatibility, provide diverse tools and methods for protein detection in biomedical research, with immense potential to drive technological advancements in the field.

Protein detection strategies based on magnetic beads and biosensors

In the ever-evolving field of biochemical diagnostics, magnetic bead-based detection technology has become a promising frontier. These technologies offer a range of advantages that redefine the possibilities for biomarker detection. At the core of their effectiveness are features such as a high surface-to-volume ratio, rapid response, and high throughput, positioning these technologies at the forefront of cancer research. The ability to achieve simultaneous multi-channel detection, automate the processing of large sample volumes, and maintain high sensitivity and specificity has made magnetic bead-based biosensors indispensable

tools in both research and clinical settings. In protein detection, biosensors are of particular interest due to their superior accuracy and sensitivity. In recent years, with the rapid development of nanotechnology, magnetic beads, as excellent biomaterials, have been combined with biosensors to give rise to various efficient protein detection strategies. In the following section, some of the popular methods are introduced.

Electrochemical biosensors

The principle of this method involves transferring magnetic beads that are bound to the target protein onto the surface of an electrochemical sensor. Electrochemical techniques, such as cyclic voltammetry and differential pulse voltammetry, are used to detect the presence and concentration of the target protein. The intensity of the electrochemical signal is proportional to the concentration of the target protein, allowing for quantitative analysis through calibration curves. Compared to traditional methods, electrochemical sensor-based methods offer advantages in terms of detection speed, sensitivity, specificity, and ease of operation. Specifically, methods based on magnetic beads and electrochemical biosensors enable rapid detection, often completing the process within minutes to hours. This is because magnetic beads can quickly capture the target protein, and electrochemical biosensors can monitor the reaction signals in real-time. In contrast, traditional detection methods such as ELISA and Western blot typically require longer times for sample preparation, reaction, and detection, often taking hours to days.

In terms of sensitivity and specificity, the method based on magnetic beads and electrochemical biosensors offers high sensitivity and specificity, allowing for the detection of low concentrations of target proteins. The high specific surface area of the functionalized magnetic beads enhances the binding efficiency to the target protein, while electrochemical biosensors can detect even the slightest changes in electrochemical signals, enabling the detection of trace proteins. On the other hand, traditional methods often have lower sensitivity and specificity, particularly when detecting low-concentration proteins, which may require more complex sample processing and signal amplification steps, thus increasing detection difficulty.

Methods based on magnetic beads and electrochemical biosensors generally have a higher degree of automation, are easy to operate, and require fewer sample processing steps, with no need for complex equipment. Due to the magnetic properties of the beads, the separation and enrichment of the target protein can be controlled by a magnetic field, reducing the need for time-consuming procedures like centrifugation or filtration. In contrast, traditional methods often involve more complicated steps, especially when multiple sample processing and detection stages are involved, requiring skilled technicians and more reagents and consumables.

Based on these characteristics, Zhang et al. developed an electrochemical biosensor for the sensitive detection of human epidermal growth factor receptor 2 (HER2) protein by utilizing DNA aptamers and peptide nucleic acids as recognition probes, combined with magnetic FeO/α-FeO@Au nanocomposites [40]. The aptamers captured large HER2 protein molecules and released single-stranded DNA, resulting in sensitive changes in the electrochemical signal. Peptide nucleic acids then captured the released single-stranded DNA chain, converting the electrochemical signal changes induced by HER2 into the changes driven by the quantity of short-chain single-stranded DNA, thereby expanding the detection range.

Roy et al. developed a compact, yet highly sensitive electrochemical sensor based on micropores [41]. This sensor features a densely packed microelectrode array, which utilizes the structure to precisely control the position and quantity of magnetic beads on the working and counter electrodes, resulting in different bead distributions on the working electrode. This configuration reduced variations in the biosensor signal, enabling stable and highly sensitive detection of amyloid proteins.

Gutierrez-Galvez focused on the study of exhaled gas electrochemical sensing and developed an electrochemical immunosensor for detecting the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (**Figure 2**) [42]. This figure was cited from [42].The sensor consists of two parts: an exhaled gas collection module and a detection module. This immunosensor demonstrated high selectivity for both the SARS-CoV-2 spike protein and the SARS-CoV-2 nucleocapsid protein.

Optical biosensors

Optical biosensors achieve their detection functionality by interacting an optical field with biological recognition components. They are one of the most widely used biosensors due to their minimal sample requirements, high sensitivity,

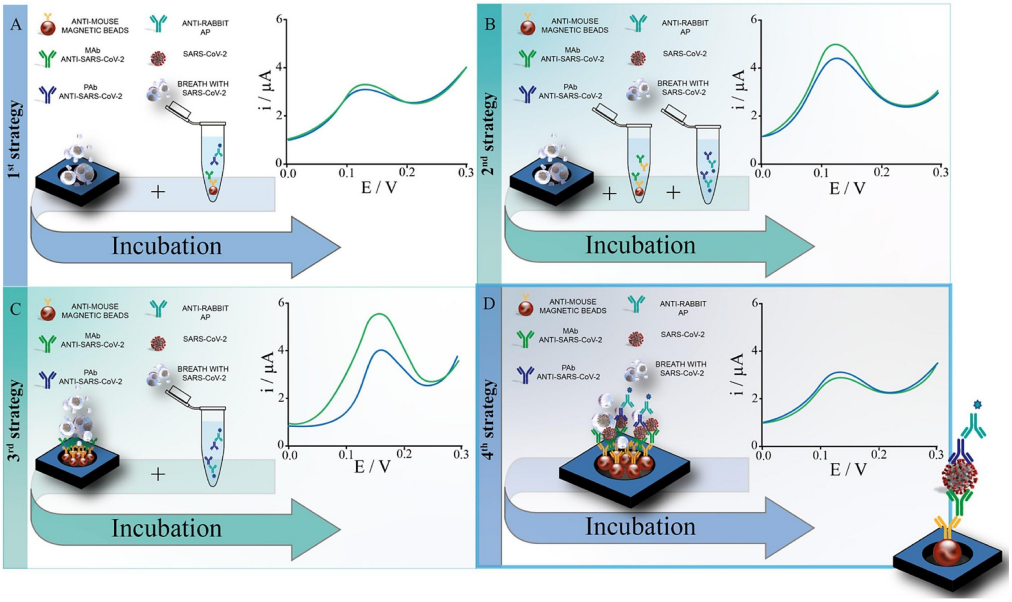

Figure 2. An electrochemical biosensor based on magnetic beads for the detection of SARS-Cov-2. This figure was cited from [42].

and specificity. Generally, optical biosensors can be divided into two categories: label-free and labeled. Label-free sensing means that the signal is directly generated by the interaction between the sample and the sensor. In contrast, labeled methods requires the target analyte to be tagged with a reporter molecule to enable detection via fluorescence, chemiluminescence, or colorimetric signals [43].

Luo et al. developed an integrated magnetic-fluorescent exosome nanosensor for the rapid and sensitive detection of cancer-derived proteins [44]. First, magnetic beads were coated with DNA tetrahedron lipid probes to effectively capture exosomes. A well-designed bifunctional aptamer specifically recognized the exosome PD-L1 protein, initiating catalytic hairpin assembly, which converted the protein signal into a double-strand signal. Finally, the double-strand body activated CRISPR-associated protein 12a, which cleaved substrate reporter molecules, amplifying the fluorescence signal. By integrating magnetic separation and fluorescence signal amplification, this nanosensor exhibited excellent specificity and sensitivity for PD-L1-positive exosomes.

Fluorescence-based optical biosensor detection is another highly efficient strategy. Wu et al. proposed a ratio-fluorescence method for one-step, label-free protein detection [45]. The method uses a fixed protein-reactive dye, Cy5-labeled Cluster of Differentiation 63 hairpin aptamer (Cy5-Apt) on the surface, along with a dual-color streptavidin magnetic bead to recognize the protein. The Cy5-Apt stem is embedded with the green SYBR Green I to detect the protein. After successful capture, the Cy5-Apt undergoes a conformational change and releases the encapsulated SYBR Green I, allowing the exosome to be measured based on the fluorescence ratio of Cy5 and SYBR Green I.

Additionally, surface plasmon resonance (SPR) is an optical phenomenon occurring on a metal surface. When light hits the metal surface, free electrons in the metal interact with photons, generating surface plasmon waves, which are electromagnetic waves propagating along the metal-medium interface. SPR occurs when the propagation speed of the surface plasmon wave matches the speed of the incident light. This phenomenon can also be combined with magnetic beads to construct optical biosensors for protein detection. For example, Sun et al. established an SPR biosensor system for immunoassays, where magnetic microbead conjugates coupled with antibodies were captured firmly on the Au film by magnetic forces [46]. They used magnetic microbeads as solid supports for Heat Shock Protein 70 (Hsp 70) antibodies, with antibody-functionalized magnetic microbeads replacing single antibody assays to detect Hsp 70.

Wang et al. developed a grating-coupled surface plasmon resonance sensor by combining

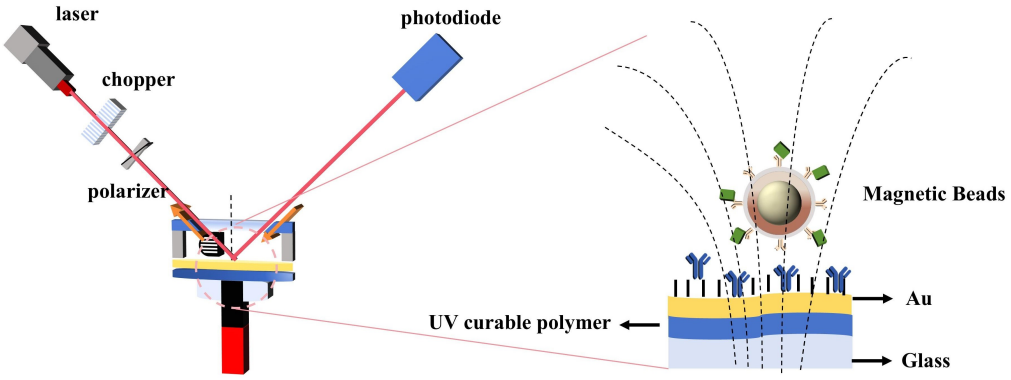

Figure 3. Plasmon resonance biosensor based on magnetic bead. This figure was cited from [47].

SPR biosensor technology with magnetic bead assays (**Figure 3**) [47]. This figure was cited from [47]. This system utilizes a metal diffraction grating sensor chip, to recognize target analytes after functionalization with specific antibodies. Magnetic beads, coupled with specific antibodies, not only serve as signal amplifying markers to enhance refractive index changes caused by target molecule capture but also act as carriers to rapidly deliver analytes to the sensor surface. The efficient collection of magnetic beads was achieved by applying a magnetic field gradient to the grating-coupled surface plasmon resonance sensor chip. The role of the movement speed of magnetic beads in the presence of the magnetic field in enhancing the sensitivity of grating-coupled surface plasmon resonance biosensors was investigated. Additionally, the validity of the technique was confirmed in an immunoassay modeled on β-Human Chorionic Gonadotropin.

Terahertz metamaterial biosensors

Terahertz (THz) metamaterial biosensors combine the detection principles of biological magnetic beads with the strong localized field enhancement effects of THz metamaterials. By integrating the high specific surface area and magnetic separation characteristics of biological magnetic beads, these biosensors enable high-sensitivity detection of biomolecules. In the detection process, biological magnetic beads, acting as solid-phase carriers, can capture and enrich target biomolecules through functional modifications on their surface, such as the attachment of antibodies or specific molecules. When these magnetic beads are combined with THz metamaterials, the localized field enhancement effect of the THz waves significantly boosts the sensitivity for detecting changes in the biomolecules on the magnetic bead surface [48]. Furthermore, under the influence of an external magnetic field, the magnetic beads can quickly separate from the solution, simplifying the operation process and shortening the reaction time.

For instance, Bi et al. proposed a THz metamaterial biosensor for detecting the SARS-CoV-2 spike protein antigen [49]. By utilizing the positioning of magnetic beads in the sensor (resonant metamaterial unit cell), the target molecules induced response signals within the domain. The sensor successfully detected trace concentrations of the SARS-CoV-2 spike protein antigen, with a detection range of 0.005 ng/mL to 1000 ng/mL and a detection limit of 0.002 ng/mL. However, challenges remain, including technical complexity, sample preparation and processing issues, biocompatibility and stability concerns, as well as data standardization and analysis.

To address these challenges, researchers are working on optimizing design and manufacturing processes to reduce costs and enhance the accessibility of THz metamaterials. They are also applying advanced signal processing algorithms and machine learning techniques to improve the analytical capabilities and accuracy of the signals. Additionally, efforts are focused on standardizing sample processing protocols to reduce variability during sample preparation and improve reproducibility, conducting more biocompatibility studies to ensure the safety of THz metamaterials and magnetic beads in biomedical applications, and developing validated data analysis workflows to improve the accuracy and reliability of the detection results [50].

Giant magnetoresistance biosensors

Giant magnetoresistance (GMR) biosensors combine the detection principles of biological magnetic beads with the giant magnetoresis-

Table 1. Detection performance of various magnetic bead-based biosensors

Sensor type	Magnetic bead type	Test object	LOD
Zhang's Electrochemistry [40]	Gold nanocomposite magnetic beads	HER2	4.1 fg/mL
Roy's Electrochemistry [41]	Antibody magnetic bead	Amyloid proteinβ	<10 fg/mL
Gutierrez-Galvez's Electrochemistry [42]	Antibody magnetic bead	SARS-CoV-2 Spike protein	1 ng/mL
Luo's Optics [44]	DNA tetrahedral functionalized magnetic beads	PD-L1	1.71 × 10^3particle/μL
Wu's Optics [45]	Fluorescent magnetic bead	Exosome	4.0×10^4 particle/μL
Sun's Optics [46]	Antibody magnetic bead	Hsp 70	0.30 μg/mL
Wang's Optics [47]	Antibody magnetic bead	βHuman chorionic gonadotropin	0.45 pM
Bi's Terahertz [49]	Gold nano antibody composite magnetic beads	SARS-CoV-2	0.002 ng/mL
Gupta's GMR [53]	Antibody magnetic bead	ESAT-6	12 pg/mL
Ding's Electrochemistry [54]	DNA functionalized magnetic beads	protamine	80 pM

Note: LOD, limit of detection; HER2, Human Epidermal growth factor Receptor 2; PD-L1, programmed death-ligand 1; Hsp 70, Heat Shock Protein 70; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; ESAT-6, Early Secretory Antigenic Target 6 kDa.

tance effect, where the electrical resistance of a material changes significantly as its magnetization state varies. These sensors consist of immune magnetic beads, a highly magnetic-sensitive GMR sensor, and associated readout circuitry.

In the detection process, a biological probe specific to the target molecule is first equipped on the surface of the GMR sensor. The sample containing the target molecules is then passed over the sensor surface, where the target molecules are captured by the probe. Immune magnetic microspheres are added, which interact with the target molecules to complete the labeling process. An external gradient magnetic field is applied to separate the unmarked excess immune magnetic microspheres, reducing background noise and improving detection accuracy. Finally, an alternating magnetic field is applied to magnetize the magnetic labels. The resulting alternating magnetic field generated by the magnetic labels causes changes in the sensor's magnetoresistance, which are then read out to determine the presence and concentration of the target molecules in the sample [51, 52].

For example, Gupta et al. proposed a highly sensitive and specific technique based on the principle of GMR sensors for early tuberculosis diagnosis [53]. This GMR biosensor assay used monoclonal antibodies specific to the early secreted antigenic target-6 (ESAT-6) secreted by M. tuberculosis antigen, with magnetic beads serving as the labels. In the presence of ESAT-6, the magnetic beads bind to the GMR sensor, and the binding is proportional to the concentration of the ESAT-6 protein. This causes a change in the overall resistance of the GMR sensor, and the resistance variation corresponds to the ESAT-6 concentration, achieving the detection goal.

Trends and challenges in protein detection based on magnetic beads and biosensors

As discussed above, the combination of magnetic beads and biosensors for protein detection is an efficient analytical method, which leverages the rapid separation capabilities of magnetic beads and the high-sensitivity detection characteristics of biosensors (**Table 1**). This technology is advancing toward higher sensitivity, faster detection speeds, and improved user-friendliness. Below are some key trends and challenges in this field.

Improving sensitivity and selectivity

Background noise is one of the main factors that affect the sensitivity of protein detection. For instance, when fluorescent probes are used to label target analytes, low concentrations of the target molecule often result in very weak signal, which can be easily obscured by background noise. In fluorescence-based analyses, common sources of background noise include Raman scattering from the solvent, residual fluorescence from unbound fluorescent molecules, and autofluorescence from the capture surface. Although washing steps can help reduce background noise, they add complexity and time to the detection process [55].

Additionally, some magnetic bead and biosen-

sor systems may have limited dynamic detection ranges, which restricts their ability to detect proteins over a wide concentration range. Developing sensors with a broader dynamic range is crucial for enhancing their clinical applicability.

In terms of selectivity, due to the large specific surface area and numerous active sites of magnetic beads, they tend to have some degree of adsorption to biomolecules. The non-specific binding of magnetic beads to biomolecules can lead to false positive signals, affecting the accuracy of detection [56]. To reduce non-specific binding, the surface of the magnetic beads can be appropriately functionalized to enhance the specificity of binding between the bead surface and the target protein, thereby minimizing the occurrence of false positive signals.

Additionally, in practical operations, most researchers use blocking strategies for the active sites to reduce the generation of false positive signals. This is typically done by adding excess target protein aptamers or sufficient bovine serum albumin to block the non-specific binding sites on the magnetic beads.

Multi-target detection capability

In the field of protein detection using magnetic beads and biosensors, despite rapid technological advancements, there are still challenges and limitations in achieving multi-target detection. These challenges are particularly evident in areas such as the selection of biological recognition elements, signal amplification and detection technologies, as well as sample separation and processing techniques [57].

First, the complexity of multi-target detection is a key issue. Interactions between different proteins can lead to cross-reactivity, which may compromise the accuracy of the detection results. For example, the concentration of certain disease biomarkers may be much lower than that of more common proteins, requiring extremely high sensitivity and a broad dynamic range of the sensors. Furthermore, the selectivity of the biological recognition elements is another important consideration. It's essential to choose an appropriate recognition element for each target protein, especially when the target proteins are highly similar, which remains challenging in practical applications [58]. To address these challenges, one approach is to develop and screen highly specific antibodies or aptamers to ensure accurate recognition of the target proteins. Additionally, bioinformatics methods can be employed to predict and design biological recognition elements that specifically bind to the target proteins, enhancing the precision of multi-target detection [49].

Furthermore, sample processing and separation techniques are necessary steps before conducting multi-target detection to reduce interference from sample matrices. Magnetic beads are widely used in sample preparation due to their high separation efficiency, but in multi-target detection, effectively separating and enriching different targets remains a challenge. Additionally, data interpretation and analysis also present difficulties in multi-target detection. Detection generates large amounts of data, which requires sophisticated data processing and analysis methods to distinguish and interpret signals from different targets [59]. To address these challenges, researchers have implemented strategies such as using nanomaterials to enhance signals and developing new signal transduction methods. Specifically, the optical or electrochemical properties of nanomaterials, such as gold nanoparticles and quantum dots, are utilized to amplify signals, while enzyme-catalyzed reactions or the plasmonic resonance effects of nanostructures are employed to further enhance signals [49].

In conclusion, while magnetic beads and biosensor technology show great potential in protein detection, achieving effective multi-target detection still requires overcoming the challenges outlined above. Future research needs to focus on improving the selectivity of biological recognition elements, developing new signal amplification strategies, refining sample processing and separation techniques, and advancing data processing methods to enable more efficient and accurate multi-target detection.

Portability and real-time monitoring

With the development of wearable devices and mobile health technologies, portable and real-time protein detection techniques have garnered increasing attention. This requires magnetic bead and biosensor technologies to not only offer high sensitivity but also be portable and easy to operate, for better meeting the demands of various monitoring environments. For example, wearable devices can monitor proteins in sweat, saliva, or tears in real time, providing dynamic data for disease diagnosis and health management [60]. Furthermore, the ability to monitor protein biomarkers in real time will significantly enhance the level of precision medicine. Current technologies, such as ELISA and Lateral Flow Immunoassays, can

only provide single-time measurements with time delays, limiting timely responses to rapidly changing life-threatening conditions [61].

However, achieving multi-target detection is still challenging. For example, interactions between different proteins may lead to cross-reactivity, affecting the accuracy of the detection results. Additionally, sample preparation and separation are necessary steps before multi-target detection to reduce interference from sample matrices. Magnetic beads are widely used in sample pre-processing due to their high separation efficiency, but in multi-target detection, effectively separating and enriching different targets remains a challenge [62].

Future research needs to focus on improving the selectivity of biosensors, developing new signal amplification strategies, refining sample processing and separation techniques, and advancing data processing methods. With these advancements, magnetic beads and biosensor technologies are expected to exert more profound influences in wearable devices and mobile health, providing more accurate and convenient protein detection solutions for individuals and clinical healthcare.

Conclusion

This review summarizes the research progress and applications of functionalized magnetic beads in protein detection. Functionalized magnetic beads, as a novel tool for bio-separation and detection, exhibit significant advantages in biomolecule capture, separation, and detection due to their unique physical structure and surface modification capabilities. The article first introduces the three main physical structures of magnetic beads: core-shell, hollow, and composite structures, and discusses in detail the characteristics of each structure and their specific applications in protein detection. Core-shell magnetic beads, with their high stability and large specific surface area, are widely used in various bio-detection platforms, while hollow and composite magnetic beads, by providing more functional sites and enhanced capture ability, further improve the sensitivity and specificity of detection.

In practical applications, functionalized magnetic beads, when combined with electrochemical biosensors, demonstrate numerous advantages over traditional detection methods. Through surface functionalization of the magnetic beads, target proteins can be rapidly and efficiently captured, and electrochemical biosensors can be used for real-time detection of reaction signals. This method not only accelerates the detection speed but also enhances sensitivity and specificity, simplifies the operation process, reduces sample processing time, and is well-suited for multiplex detection and on-site applications. Furthermore, the cost-effectiveness of functionalized magnetic beads in high-throughput detection makes them an indispensable part of modern bio-detection technologies.

Overall, this article thoroughly discusses the key technological advancements of functionalized magnetic beads in protein detection, highlighting their broad application prospects in fields such as biomedicine, environmental monitoring, and food safety. Future research will continue to optimize surface functionalization techniques for magnetic beads, further improving their detection performance and promoting their wider practical application.

In future bio-detection, the synergistic application of magnetic beads and biosensors is expected to make significant progress in several areas, particularly in technological innovations, the development of multimodal detection platforms, and the creation of portable detection devices.

Future research is likely to explore novel magnetic materials and surface modification techniques to enhance the magnetic properties and biocompatibility of magnetic beads. For instance, by introducing rare earth elements or transition metals, magnetic nanoparticles with higher magnetic saturation and superior magnetic responsiveness can be synthesized. Additionally, advancements in surface modification techniques, such as dopamine polymerization or silanization reactions, will further improve the stability and functionality of magnetic beads. These innovations will make magnetic beads more efficient in biomolecule capture and separation while reducing non-specific binding and improving the specificity and sensitivity of detection.

Integrated biosensors combining multiple detection modes will be developed for multiparameter analysis of proteins. This multimodal platform will combine electrochemical, optical, and magnetic resonance technologies to provide a more comprehensive analysis of protein characteristics. For example, electrochemical biosensors will allow monitoring of protein redox states, while optical biosensors will detect interactions between proteins and fluorescent markers. The application of magnetic resonance technology will provide detailed informa-

tion about protein structure and dynamics. This integrated detection strategy will significantly enrich our understanding of protein functions and behaviors.

To meet the demand for rapid on-site detection, the development of portable and on-site detection devices will become an important direction. These devices will integrate miniaturization technologies, wireless communication, and user-friendly interfaces, making protein detection more convenient and accessible. With the advancement of microfluidic technologies and nanomanufacturing, future detection devices will be capable of performing complex biochemical analyses on smaller platforms. Additionally, by integrating with smartphones or other mobile devices, these systems will provide instant detection results and data analysis, greatly expanding the application scope of bio-detection technologies.

In conclusion, future research will continue to drive the development of magnetic beads and biosensor technologies to enable faster, more accurate, and cost-effective protein detection. These technological advancements will not only provide powerful tools for biomedical research but also bring revolutionary changes to fields such as clinical diagnostics and environmental monitoring.

Author Contributions: Haoyuan Su organized papers and wrote the manuscript; Yuehua Liao, Shu Wu, Jun Ji, Shuya An have made important contributions to the analysis and manuscript preparation, Dongdong Zeng gave instruction and revision of manuscript writing.

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