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Recent advances in biological detection with magnetic nanoparticles as a useful tool

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Magnetic nanoparticles have emerged as a powerful tool for magnetic resonance imaging, biodetection, drug delivery, and hyperthermia. This review focuses on the biological detection of magnetic nanoparticles as well as their physicochemical properties. Substantial progress in the sensitivity of detection has been made by developing variety of methods. Five applications of magnetic nanoparticles in biological detection are discussed in this review: magnetic separation, magnetic sensing, magnetic manipulation, magnetic catalysis, and signal enhancer for surface plasmon resonance (SPR). Finally, some future trends and perspectives in these research areas are outlined.

magnetic nanoparticles, biological detection, sensitivity, methods

1 Introduction

In recent years, magnetic nanoparticles (MNPs) have demonstrated great promise for magnetic fluids [1], catalysis [2], biotechnology/biomedicine [3], magnetic resonance imaging [4,5], data storage [6], and environmental remediation [7]. Owing to their unique size and structure, nanoparticles (NPs) possess four basic effects: surface effect, quantum size effect, volume effect, and macroscopic quantum tunneling effect. Compared with NPs, MNPs possess not only these four basic effects but also unique magnetic properties such as superparamagnetism, high coercivity, low Curie temperature, high magnetic susceptibility, and others. MNPs for biodetection should have the advantages of small size, good monodispersity, and excellent magnetic properties. Superparamagnetic nanoparticles are the most common tools for biological detection because the particles no longer show magnetic interaction after the external magnetic field is removed. However, most MNPs with good monodispersity and excellent magnetic properties are oil-soluble, which greatly restricts their biological application. Therefore, the appropriate surface functionalization of MNPs is essential [8–10]. MNPs that have tailored surfaces and appropriate physicochemical properties have been widely investigated for various applications such as drug delivery, hyperthermia, magnetic resonance imaging (MRI), tissue engineering and repair, biosensing, biochemical separation, and bioassay [11]. Clinical trials are in progress to investigate the potential of different magnetic nanosystems for biological detection [3].

This review will briefly describe the physicochemical characterization of MNPs, with particular emphasis upon their applications in biological detection such as magnetic separation, sensing, catalysis, manipulation, and signal enhancer for SPR. The progress since 2009 of research on their applications in biological detection is reviewed. There is no doubt that MRI [12–14] is a major application of

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MNPs in biological detection, but no discussion about MRI will appear in this review.

2 Physicochemical properties

2.1 Composition and size

Magnetic nanoparticles are a class of nanoparticles that can be manipulated using a magnetic field. Such NPs mainly consist of magnetic elements such as iron, nickel, and cobalt, and their chemical compounds. NPs are smaller than 1 μm in diameter (typically 5–500 nm). The main factor influencing the physical stability of MNPs is size under any conditions. The moment and response of MNPs under magnetic fields change along with the change of the size [15]. For example, as the size of the particles reduces, their saturation magnetization reduces and their specific surface area becomes larger. The variation of the specific surface area greatly affects the non-crystalline character of the particles and consequently their magnetic moment [16]. Additionally, MNPs below the critical size can exhibit superparamagnetism, which reveals greater magnetization capabilities than paramagnetic materials.

2.2 Surface properties

As the particle size decreases, the special surface area of a nanoparticle increases naturally. The proportion between surface atoms and bulk atoms also increases, which causes surface and interface properties of nanoparticles to become more and more significant. For instance, for face-centered cubic (fcc) cobalt with a diameter of around 1.6 nm, about 60% of the total number of spins are surface spins [17]. As is known to all, the magnetization results from atom spins. Therefore, for nanoparticles, surface spins greatly influence magnetization. Consequently, some surface- and/or interface-related effects occur, for example, surface anisotropy and, under certain conditions, core-surface exchange anisotropy.

Under the condition of no magnetic or inert surface coatings, surface effects can lead to a decrease of the magnetization of small particles such as oxide NPs [18]. For small metallic NPs such as cobalt, enhancement of the magnetic moment was reported as size decreased [19]. Along with decreasing particle size, another effect driven by surface is the enhancement of the magnetic anisotropy K_{eff} [20]. Under the condition of magnetic coatings, the magnetic properties of MNPs usually undergo significant change. The combination of two different magnetic phases will result in new magnetic nanocomposite that may have many possible applications. When two magnetic phases are in close contact, the most striking feature is the exchange bias effect. For example, when the core and the shell are both strongly magnetic, as in $FePt/CoFe_2O_4$ [21], another interesting aspect of magnetic coating emerges. These bimagnetic core-shell NPs allow us to precisely control the magnetic properties by changing the dimension of the core and shell and then selectively control both the anisotropy and the magnetization [22].

2.3 Magnetic properties

Generally speaking, the magnetic properties of MNPs can be characterized by magnetic susceptibility (χ) and magnetic permeability (μ). Magnetic susceptibility χ is ratio of magnetization (M) and magnetic field (H), and magnetic permeability (μ) is the ratio of magnetic induction (B) and magnetic field (H). There is a relationship between χ and μ: $\chi=\mu-1$. MNPs show different magnetic properties when the value of χ is located in different ranges: ferromagnetism, $\chi>>0$; paramagnetism, $\chi>0$; and diamagnetism, $\chi<0$. The hysteresis loop and the magnetization curve are other parameters that characterize MNPs. We can get magnetic remanence, magnetic moment, etc., from the hysteresis loop, and particle size, monodispersity, etc., from the magnetization curve. These parameters are also used to evaluate the contrast efficacy of an iron oxide formulation [23].

Owning to the spins of the unpaired electrons, ferromagnetic NPs can show magnetism even if they are not in a magnetic field. After magnetization, these ferromagnetic NPs show permanent magnetism. Paramagnetic NPs, which are naturally without magnetism, show magnetism in a magnetic field. However, no magnetism remains when the magnetic field is removed. Diamagnetic NPs, in which the magnetic moments of electrons cancel each other, do not show magnetism whether or not they are in a magnetic field [24].

There is a critical size for MNPs. NPs that exceed this critical size exhibit ferromagnetism, and NPs smaller than this critical size exhibit superparamagnetism. Superparamagnetic NPs, which combine the advantages of the paramagnetic NPs and the ferromagnetic NPs, not only have the excellent magnetism similar to the ferromagnetic NPs, but also have excellent colloidal stability. Therefore, they can be tracked in a magnetic field [25]. In fact, the reason that superparamagnetic NPs can form a stable colloidal suspension is attributed to the null or negligible remnant magnetization of superparamagnetic NPs. That is, when the magnetic field is removed, the NPs no longer exhibit magnetic interaction (negligible remanence and coercivity); as a result, agglomeration is avoided [26].

Two more advantages of the superparamagnetic NPs are their heating behavior [27] and powerful enhancement of the proton relaxation times T_1 and T_2. A benefit of this behavior is that such NPs are also used as magnetic storage media or as magnetic inks for jet printing. Unfortunately, the reduced size of superparamagnetic NPs easily results in pronounced surface effects (due to the enhanced surface-to-volume ratio) such as spin-canting, spin-glass-like

behavior, and noncollinear spin, which can influence their magnetic properties [28].

3 Magnetic separation

In the biological field, for the purpose of analysis, detection, or other applications, the biological object often needs to be separated from its environment. Magnetic separation technology that employs biocompatible MNPs is an optimal method to achieve this goal [29] because it takes advantage of the specific interactions between surface ligand (or receptor) and receptor (or ligand) of functionalized MNPs (such as antigen-antibody or avidin-biotin, etc.) to achieve rapid separation of the target biomolecules. The traditional separation technique, which is mainly centrifugation using a density gradient principle, is time-consuming and has poor effect; however, magnetic separation technology is rapid, simple, and can efficiently capture specific proteins or other biological macromolecules [30]. At present, the most-used MNPs are superparamagnetic nanoparticles. They can be magnetized under a magnetic field, but once the magnet is removed they will immediately redisperse in the solution. Magnetic separation technology usually includes two steps: (1) targeting the biological molecular on MNPs; (2) separating the labeled biological molecular using a magnetic liquid-separation device. Magnetic separation methods have been extended to the separation and purification of bacteria, cells, virus, biological macromolecules, small molecules, and so on [31–34].

3.1 Bacteria

Escherichia coli (*E. coli*), which spread rapidly in the natural environment, are a major cause of infection outbreaks with serious consequences. Conventional methods for the detection of *E. coli* are time-consuming and require a long incubation period (24–48 h). Recently, Guven *et al.* [35] developed a new method to enumerate *E. coli* that combines immunomagnetic separation (IMS) and surface-enhanced Raman scattering (SERS). Prepared by immobilizing biotin-labeled anti-*E. coli* antibodies onto avidin-coated MNPs, gold-coated magnetic spherical nanoparticles were used for the separation and concentration of the *E. coli* cells. The limit of detection (LOD) of this method was found to be 8 CFU/mL; additionally, this method is sensitive and rapidly targets objects with a total analysis time of less than 70 min.

Salmonella bacteria are a main cause of food poisoning. Recently, a facile and sensitive analytical method that used gold-coated magnetic nanoparticle clusters (Au/MNCs) and magnetophoretic chromatography with a precision pipette for the detection of salmonella bacteria was developed by Kwon *et al.* [36]. Salmonella bacteria in milk was captured by antibody-conjugated Au/MNCs, and separated from the milk through the application of an external magnetic field. This method can detect amounts of salmonella bacteria as low as 100 CFU/mL in 10 min.

The above-mentioned methods usually detect only one bacterium. Wu *et al.* [37] have developed a highly sensitive and specific multiplex method for the simultaneous detection of three pathogenic bacteria using multicolored upconversion nanoparticles (UCNPs) as luminescence labels coupled with aptamers as the molecular recognition elements. With the help from the magnetic separation and concentration effect of Fe_3O_4 MNPs, the respective detection limits of this method were 25, 10, and 15 CFU/mL for *S. aureus*, *V. parahemolyticus*, and *S. typhimurium*. With the advantages of detecting various pathogenic bacteria based on multicolored UCNPs, this method is promising for food safety and multiplex nanosensors.

3.2 Cells

Cell detection, especially detection of few cells, is increasingly significant for human health. Chen *et al.* [38] have synthesized graphite-coated, highly magnetic FeCo core-shell nanoparticles (NPs) by a chemical vapor deposition method. Through a unique polymer mixture modification, the biocompatibility and stability of the MNPs were significantly improved. When an MNP-chip microarray was used to detect cancer cells, as few as two cancer cells were efficiently and simply detected from 1 mL of whole blood.

The detection of circulating tumor cells (CTCs) has great significance for cancer treatment. The key steps of cancer diagnosis are isolation and enrichment of CTCs, due to their extremely low concentration in peripheral blood. Wen *et al.* [39] fabricated magnetic nanospheres (MNs) by a convenient and highly controllable layer-by-layer assembly method (Figure 1(a)). The MNs proved to be quick-response, stable, and without aggregation or precipitation in whole blood. They used anti-epithelial-cell-adhesion-molecule (EpCAM) antibody-modified MNs to capture extremely rare tumor cells, and achieved an efficiency of more than 94% after only 5 min of incubation, the sensitivity was such that even one CTC in a whole blood sample could be detected.

3.3 Virus

Human pathogenic diseases such as HIV/AIDS and viral hepatitis are a growing threat to human health. For the purpose of preventing the large-scale emergence of a human pandemic, the fast and sensitive detection of human pathogenic diseases has become a significant topic. Luo *et al.* [40] have demonstrated an effective ICP-MS-based multiplex and ultrasensitive assay of viruses with lanthanide-coded oligonucleotide hybridization and rolling circle amplification (RCA) strategies on biofunctional magnetic nanoparticles (MNPs) (Figure 1(b)). Single-stranded capture DNA-functionalized MNPs were used for the separation of target

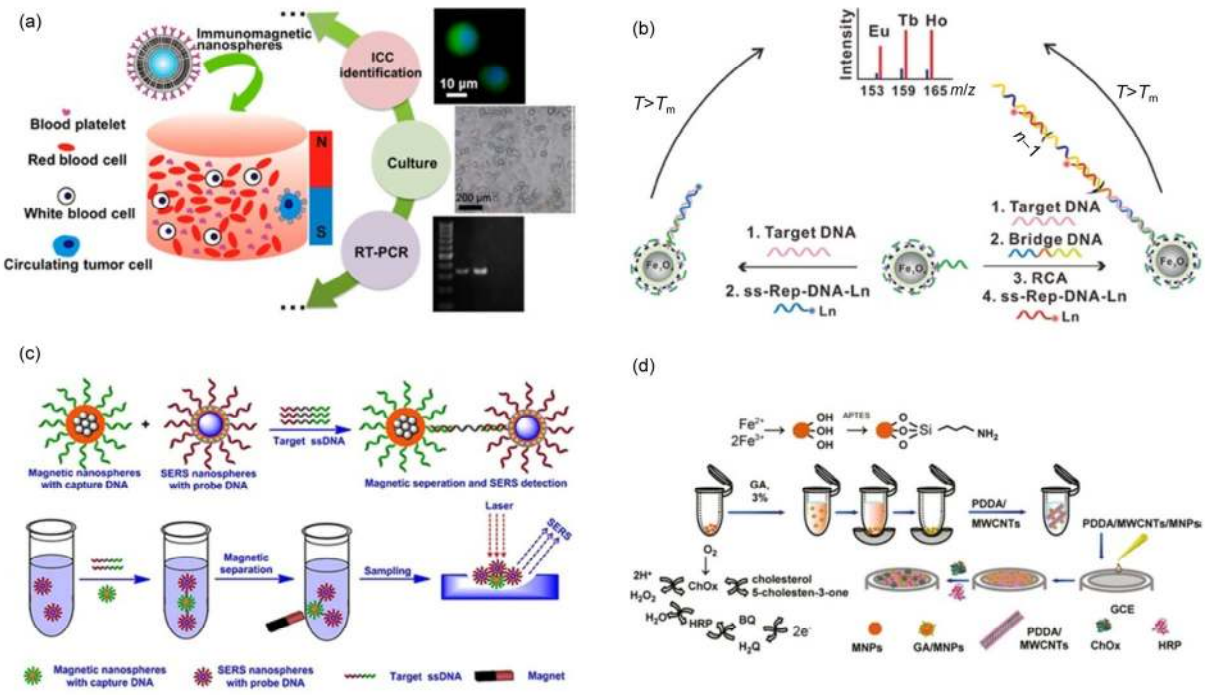

Figure 1 (a) Steps involved in circulating tumor cells' enrichment and detection [39]; (b) ICP-MS-based multiplex DNA assay with lanthanide-coded oligonucleotide hybridization strategy and biofunctional magnetic nanoparticle (MNP) enrichment [40]; (c) the sandwich DNA hybridization assay and the experimental procedure for the detection of target ssDNA strands using a SERS liquid chip (SLC) method [47]; (d) steps involved in the construction and function of the HRP/ChO_x/GA-MNP/PDDA/MWCNT/GCE biosensor [53].

DNAs. This assay exhibited a highly sensitive readout of HIV (28 amol), HAV (48 amol), and HBV (19 amol). We can foresee that this element-coded ICP-MS-based multiplex and ultrasensitive DNA assay will become increasing significant in the field of clinic use, bioanalysis, and virology.

Avian influenza virus (AIV) has emerged to seriously threaten the health of human beings. Therefore, the rapid and sensitive detection of AIV is urgently desired. Zhou *et al.* [41] developed an electrochemical magnetoimmunosensing approach for the sensitive detection of H9N2 avian influenza virus. This method perfectly combined high-efficiency immunomagnetic separation, enzyme catalytic amplification, and the biotion-streptavidin system perfectly to achieve a sensitivity of as low as 10 pg/mL. Based on this foundation, they further developed a magnetic bead-based bienzymatic electrochemical immunosensor for the detection of H9N2 AIV [42]. The significance of this strategy is showing the feasibility of achieving higher sensitivity by bienzymatic or even multienzymatic detection systems.

3.4 Biological macromolecules

Biological macromolecules include protein, nucleic acids, lipids, hydrocarbons and so on. The rapid and sensitive detection of biological macromolecules is a major aspect of disease diagnosis [43–45]. Castilho *et al.* [46] have described for the first time magneto immunoassay-based strategies for the detection by MNPs of plasmodium histidine-rich protein 2 (HRP2) related to malaria. This electrochemical magneto immunosensor exhibited better analytical performance, with a detection limit of 0.36 ng/mL. Owing to the high sensitivity, this novel strategy has shown great potential for rapid, simple, cost-effective, and on-site detection of falciparum malaria disease in patients. It could also screen out at-risk blood samples for the prevention of transfusion-transmitted malaria.

A new approach for the sensitive detection of a specific single-stranded DNA (ssDNA) sequence based on a surface-enhanced Raman spectroscopy (SERS) liquid chip was developed by Li *et al.* [47] (Figure 1(c)). They used MNPs for ssDNA separation, and SERS for ssDNA analysis. With the detection limit of approximately 10 pmol/L and a wide linear correlation range of 10 nmol/L–10 pmol/L between the SERS signal intensity and the target ssDNA quantity, this liquid-phase DNA sequencing method (the SERS liquid chip (SLC) method) shows great potential for the specific detection of trace targets in organisms.

Luminescent labeling and magnetic separation are two important biotechniques [48]. Fluorescent, magnetic, dual-encoded multifunctional bioprobes are a novel strategy for real-time detection and simultaneous separation of multiple targets [49,50]. Benefiting from this idea, Hu *et al.* [51] invented a simple method that can simultaneously detect multiplex DNA sequences. Three kinds of QDs-encoded fluorescent nanosphere (FNS)-labeled probe DNAs and three kinds of corresponding magnetic nanospheres (MNS)-

labeled capture DNAs were put into a reactor containing three kinds of target DNA. After hybridization and magnetic separation, the sandwich-structured adducts were detected by fluorescence spectrophotometry. Owing to the advantages of high sensitivity (~100 pmol/L), convenience, high anti-jamming ability, this method demonstrated great potential for the simultaneous detection and separation of multiple targets at high levels of purity and throughput.

Besides DNAs, multiple types of tumor cells can be detected and isolated by fluorescent-magnetic-biotargeting multifunctional nanobioprobes (FMBMNs) [52].

3.5 Small molecules

Biological small molecules include amino acids, monosaccharides, vitamins, and so on. Eguilaz *et al.* [53] have designed a novel biosensing electrode surface that combines the advantages of magnetic ferrite nanoparticles functionalized with glutaraldehyde (GA) and poly(diallyldimethylammonium chloride) (PDDA)-coated multiwalled carbon nanotubes (MWCNTs) as platforms for the construction of high-performance multienzyme biosensors (Figure 1(d)). A detection limit of 0.85 μmol/L for cholesterol was achieved, which was advantageously compared with the analytical characteristics of other CNT-based cholesterol biosensors.

An inexpensive and simple method using an amine-functionalized MNP-based Ni^{2+}-histidine affinity-pair system for the highly sensitive and selective detection of histidine in human urine by photochemical vapor generation atomic spectrometry was developed by Hu *et al.* [54]. This convenient method achieved a high sensitivity of 1 nmol/L (tens-to-hundreds times better than sensitivity achieved with conventional methods) and shows great potential for practical application.

4 Magnetic sensing

Owing to their unique magnetic properties, magnetic nanoparticles are an important source of labels for biosensing. Modulation of the composition, size, and magnetic properties of magnetic nanoparticles allows their application in a variety of instruments and formats for biosensing [55,56]. New types of instrumentation are promising for the use of MNPs in a variety of applications [57]. Herein, we cover five biosensors that employ MNP labels with different sensing principles and instrumentation: (1) magnetic relaxation switches; (2) magnetic particle relaxation sensors; (3) magnetoresistive sensors; (4) nuclear magnetic resonance (NMR); (5) atomic magnetometer.

4.1 Magnetic relaxation switches (MRSw)

In 2001, Josephson *et al.* [58] developed magnetic relaxation (MR)-based assays called magnetic relaxation switches (MRSws) using the change in T_2 relaxation time produced by MNPs. The principle of MRSw assays is that surface-functionalized MNPs bind specific molecules to produce local inhomogeneities in the magnetic field; these inhomogeneities result in changes in the spin-spin relaxation time (T_2) [58], which are associated with the concentration of analytes [59]. Owing to the advantages of speed and sensitivity, MRSw assays have been widely applied in the detection of carbohydrates, proteins and cancer cells.

Kulkarni *et al.* [60] developed a rapid, sensitive, and simple carbohydrate-based magnetic relaxation-switch assay for the detection of carbohydrate-binding proteins. They have shown that the sensitivity and selectivity of the assay can be improved by using two different recognition elements, and that the magnetic relaxation-switch assays are able to detect toxins in complex media such as stool and environmental samples.

Because lysozyme is a natural anti-infection protein's own bactericidal effect, the detection of lysozyme is necessary for the prevention of bacterial infection. An aptamer-functionalized superparamagnetic nanoparticle-based MRSw sensor for the detection of lysozyme (Lys) protein was developed by Bamrungsap *et al.* [61], who used iron oxide nanoparticles conjugated with either linker DNA or Lys aptamer; these nanoparticles can hybridize with the extended part of the aptamer to form clusters (Figure 2(a)). A detection limit in the nanomolar range was obtained for Lys detection in both human and buffer serum.

MRSw assays can also be used to detect cancer cells.

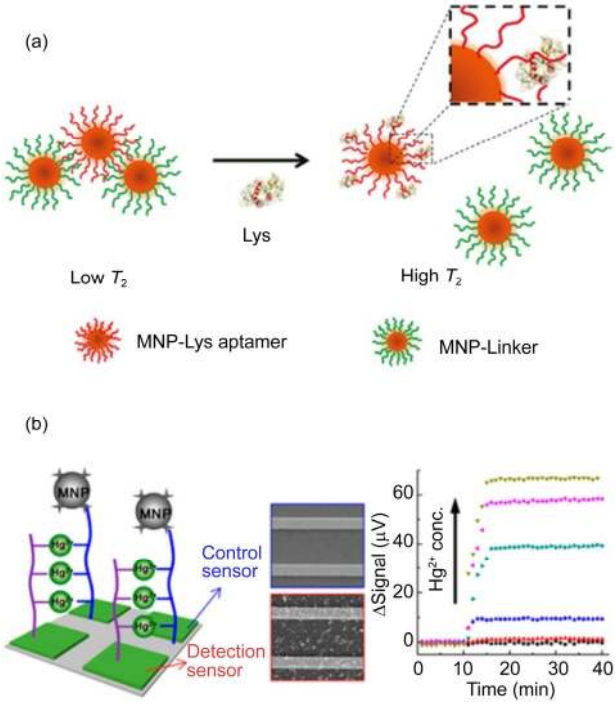

Figure 2 (a) Schematic representation of the magnetic nanosensor for Lys detection based on MRSw [61]; (b) schematic illustration of Hg^{2+} detection using the GMR biosensor [69].

Bamrungsap *et al.* [62] first reported the design of MRSw based on aptamer-conjugated magnetic nanoparticles (ACMNPs). A detection limit as low as 10 cancer cells in 250 μL of sample was achieved. Because the specificity and sensitivity of the ACMNPs is excellent, clinicians can accurately identify cancer cells at the molecular and single-cell level by using an array of ACMNPs.

4.2 Magnetic nanoparticle relaxation-based sensors

Magnetic nanoparticle-based assays are based on the relaxation of the magnetic moments within MNPs. When the magnetic field is turned off, MNPs in a liquid with magnetic moments caused by the applied magnetic field employ two relaxation machanisms: Néel relaxation, which is characterized by the Néel relaxation time τ_N, and Brownian relaxation, which is characterized by the Brownian relaxation time τ_B. The effective relaxation rate is expressed as the sum of the Néel and Brownian relaxation rates:

$$1/\tau = 1/\tau_B + 1/\tau_N \quad (1)$$

As Eq. (1) shows, the effective relaxation process is decided by the faster relaxation time between the two. Target-induced aggregation can decrease the rates of Néel or Brownian relaxations, thereby generating the sensor for molecular targets [9].

Superconducting quantum interference devices (SQUIDs) and AC susceptometers have been used to measure the relaxation of nanoparticles' magnetic moments. Enpuku *et al.* [63] showed the detection of biological targets using magnetic markers and SQUID. The two main advantages of their method are that determination can be operated in the liquid phase and that the bound markers can be detected even in the presence of unbound (free) markers without using the separation process. The feasibility of this method is demonstrated by detecting biological targets such as IgE, biotin-coated polymer beads, and *Candida albicans*.

de la Torre *et al.* [64] for the first time applied the volume-amplified magnetic nanobead detection assay (VAM-NDA) for the detection of rolling circle-amplified (RCA) DNA molecules in a portable, commercial AC susceptometer that requires a total analysis time of about 20 min and operates at ambient temperatures. With a quantitative detection limit of ~4 pmol/L, the VAM-NDA is promising for wide-spread implementations in commercial AC susceptometer setups, which would in turn make it possible to perform magnetic bead-based DNA detection in both point-of-care and outpatient settings.

Stromberg *et al.* [65] demonstrated sensitive (1.5 pmol/L) singleplex detection of bacterial DNA sequence in a portable AC susceptometer by a magnetic nanobead-based bioassay principle. This was the first demonstration of biplex volume-amplified magnetic nanobead detection assay (VAM-NDA) in the susceptometer.

Engstrom *et al.* [66] have developed a molecular method for detection of rifampicin-resisitant *M. tuberculosis* based on padlock probes and magnetic nanobeads.

4.3 Magnetoresistive sensor

Based on the binding of MNPs to a sensor surface and the electrical current changes within the sensor, which result from interactions between sensors and the magnetic fields of the nanoparticles, giant magnetoresistance (GMR) spin valves (SV) and magnetic tunnel junction (MTJ) sensors have been successfully employed to sense MNPs [67].

Chan *et al.* [68] developed a magnetoresistive biosensing platform based on a single magnetic tunnel junction (MTJ)-scanning probe and DNA microarrays labeled with magnetic particles; this approach provides an inexpensive, sensitive, and reliable detection of DNA. When they demonstrated this biosensing platform on a DNA microarray assay for quantifying methyl tertiary butyl ether-degrading bacteria, it was possible to detect concentrations as low as 10 pmol/L of methyl tertiary butyl ether-degrading bacteria.

Mercuric ion (Hg^{2+}) has high exposures, may result in acrodynia (heavy-metal poisoning, also known as "Pink disease") and does great damage to the nervous system and kidneys. Therefore, it is highly necessary to develop rapid and sensitive approaches to detect Hg^{2+} in environmental monitoring. A novel sensing strategy employing DNA chemistry and a GMR biosensor for the detection of mercuric ion (Hg^{2+}) was demonstrated by Wang *et al.* [69] (Figure 2(b)). Taking advantage of real-time signal readout and high sensitivity of GMR biosensor and high selectivity of thymine-thymine (T-T) pairs for Hg^{2+}, a detection limit of 10 nmol/L was obtained in both natural and buffer water; this level is the maximum amount of mercury permitted in drinking water by the US Environmental Protection Agency (EPA). This was the first time that GMR-sensing technology as employed in a pollutant monitoring area, which indicates that the GMR sensor will become a useful tool in the areas of food safety testing and environmental monitoring.

4.4 Nuclear magnetic resonance (NMR) sensor

Recently, nuclear magnetic resonance (NMR) has been widely applied for biological detection. When MNPs was used as labels, the principles of biological detection can be illustrated by the T_2-shortening effect of MNPs in NMR measurements. When placed in static, polarizing magnetic fields, MNPs produce local dipole fields with strong spatial dependence that can efficiently destroy the coherence in the spin-spin relaxation of water protons. Consequently, MNP-labeled biological objects produce faster decay of NMR signals, or shorter transverse relaxation time T_2, compared to non-targeted ones [70].

Lee *et al.* [71] developed a diagnostic magnetic resonance (DMR) sensor that combines a miniaturized NMR probe with targeted MNPs for the detection and molecular

profiling of cancer cells. A detection limit as low as 2 cancer cells in 1 μL sample volumes was achieved.

Lee *et al.* [72] developed a new, simple, magnetic nanoparticle-based platform for rapid detection of pathogens. In their methods, bacteria was first labeled by MNPs and then concentrated into a microfluidic chamber, last the bacteria were detected by nuclear magnetic resonance (NMR). Clinical use of this diagnostic platform was explored by detecting tuberculosis (TB). With unprecedented detection speed (less than 30 min) and sensitivity (as few as 20 CFU/mL), the new detection platform will become an ideal point-of-care diagnostic tool, especially in resource-limited settings.

Traditional technologies for bacteria detection often include isolation and amplification of the pathogenic bacteria, which are complicated and time-consuming processes. To meet this challenge, a sensitive NMR-based detection method to identify bacteria via bacteria-induced self-assembly of MNPs was developed by Liang *et al.* [73]. With a detection limit of as few as 8 bacteria cells per mL in experimental samples within 1 h, the method showed great potential for rapid detection of *M. tuberculosis* in clinical samples.

Another inexpensive and rapid diagnostic method for clinical pathogen detection was developed by Chung *et al.* [74]. They presented a nanoparticle hybridization assay that involved ubiquitous and specific probes that target bacterial 16S rDNAs for the detection of amplified DNAs using a miniaturized NMR device. Capable of rapid and specific profiling of pathogens directly in clinical samples, the novel magneto-DNA platform is promising for both universal and specific detections of various clinically relevant bacterial species, with sensitivity down to single bacteria.

4.5 Atomic magnetometers (AM)

Magnetometers are a kind of apparatus used for measuring the strength and direction of a magnetic field. Atomic magnetometers (AM) detect magnetism by measuring the Larmor precession of spin-polarized atoms in a magnetic field [75]. The detecting process consists of two steps: (1) polarizing the atoms by a polarized pump laser; (2) detecting their precession in the magnetic field by measuring the optical rotation or absorption of a probe laser (which may be the same laser used for pumping). Two main methods have been used for the detection of magnetic nanoparticles by AM: continuous flow carried by water and scanning imaging scheme [76].

AMs have been widely used for the detection of MNPs. However, one disadvantage of AM is that it is difficult to determine spatial information without prior knowledge of the amount of the sample. To meet this challenge, Yao *et al.* [77] developed a scanning imaging method using a novel atomic magnetometer to generate a map of the magnetic field (Figure 3(a)). They demonstrated that the spatial information and the amount of the magnetic sample can be detected simultaneously by the full magnetic field profile, instead of a single-point measurement. When they performed a series of scans to obtain a 2D magnetic image of the sample, they were able to accurately measure both the spatial information and the amount of the magnetic sample. By modifying MNPs with antibodies, they successfully used scanning imaging of MNPs for quantitative molecular imaging [78]. Besides antibodies, MNPs can also be modified with cells by specific interactions between the target molecules on cells and the ligand molecules conjugated on MNPs [79].

MicroRNAs (miRNAs) are short RNA strands that contain 18–25 nucleotides; they play important roles in gene expression, cell differentiation, and disease development. Specific and sensitive detection of miRNAs is indispensible for understanding their functions in gene regulation as well as their expressions as biomarkers for cancer diagnostics. Yao *et al.* [80] reported a technique based on exchange-induced remnant magnetization (EXIRM) for the detection of miRNA (Figure 3(b)). When label-free miRNA and magnetically labeled RNA with one mismatched base produce sequence-specific exchange reactions, the decrease in magnetization quantitatively represents the target miRNA. With no washing or amplification steps, the limit of detection achieved at the zeptomole level. It has been demonstrated that this technique is suitable for precise miRNA profiling in early diagnosis of cancers [80].

Specific noncovalent binding between antibody and antigen molecules is the foundation for molecular recognition in many processes in chemistry and biology, including enzyme catalysis, cancer diagnosis, and drug delivery. Quantitative investigation of the binding forces is helpful for potential mechanical manipulation of these processes and for molecular specific analysis. Recently, a force-induced remnant magnetization spectroscopy (FIRMS) technique was reported by Yao *et al.* (Figure 3(c)) [81], who used FIRMS to reveal a well-defined binding force for the bonds between magnetically labeled α-mouse immunoglobulin G and mouse immunoglobulin G. The force was calibrated as 120±15 pN. In comparison, the binding force for physisorption and for biotin-streptavidin bonds is only 17±3 pN and over 120 pN, respectively. This well-defined and molecule-specific binding force provided a new method for distinguishing among noncovalent bonds in biochemical processes. Besides the antibody-antigen binding force, FIRMS has also been used to detect well-defined binding forces of DNA duplexes, with a narrow force distribution of 1.8 pN [82]. These two studies used centrifugal force, which is difficult to implement for direct bond manipulation. Another method, developed by De Silva *et al.*, used acoustic radiation force (ARF) to mechanically resolve noncovalent bonds between protein A and three mouse IgG subclasses. This method allows for the study of molecular interactions under conditions such as *in vivo*, and represents a new branch of mechanochemistry [83].

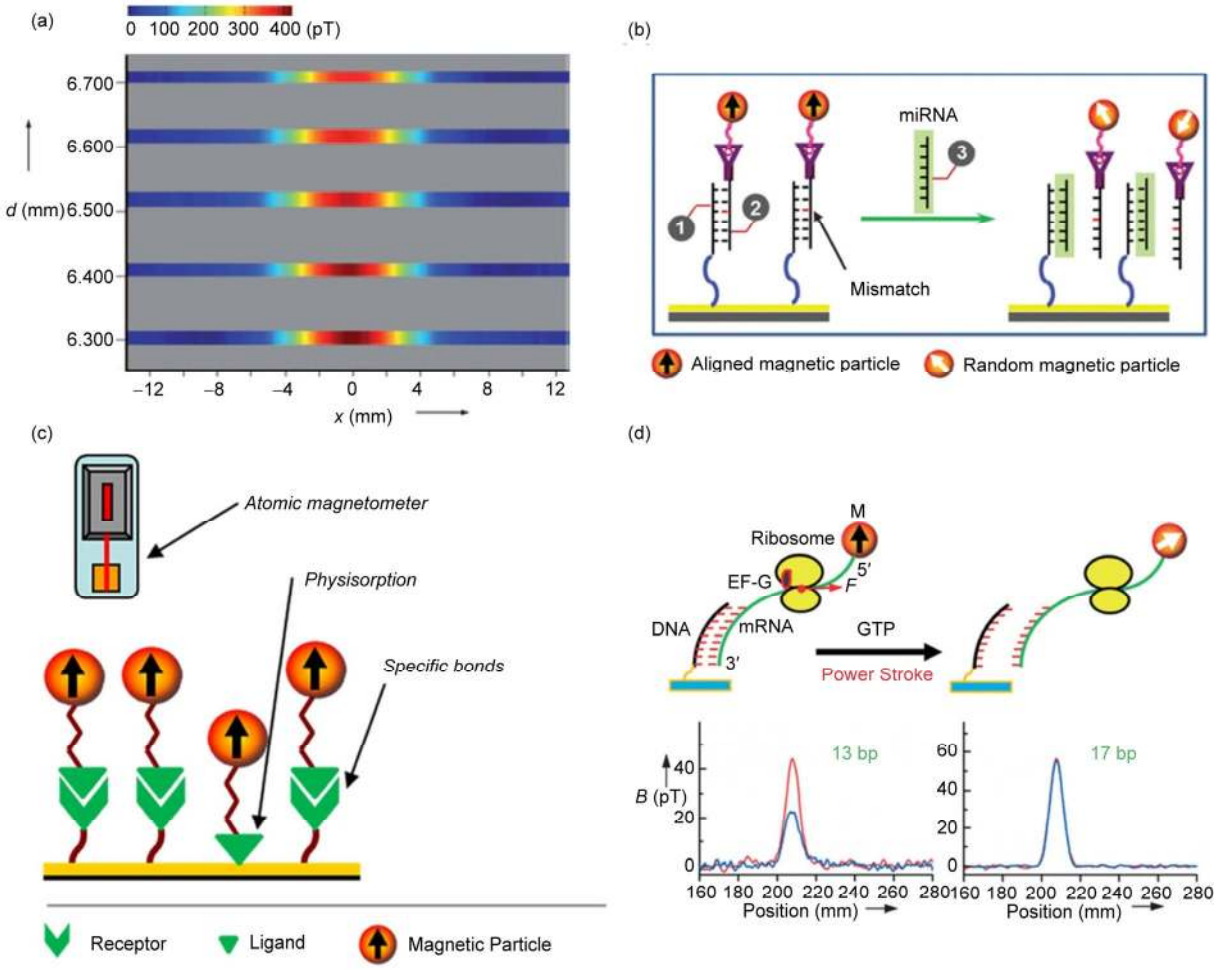

Figure 3 (a) A 2D scanning magnetic image of magnetic samples [77]; (b) principles of the EXIRM technique [80]; (c) schematic of the FIRMS experimental setup [81]; (d) measuring the EF-G power stroke with internal force references [84].

With the development of this technique, a method that uses internal force references to precisely detect the intrinsic force generated by the elongation factor G (EF-G) in the process of ribosome translocation finally arrived (Figure 3(d)) [84]. This method employed the binding forces of a series of DNA-RNA duplexes as internal force references, and applied the mechanical force produced by the motor protein EF-G to dissociate the duplexes. As a result, the power stroke of motor proteins can be obtained during their biological functioning. This method is suitable for a wide range of other motor proteins.

5 Magnetic manipulation

In the biological detection field, manipulation of a biological object (e.g., capture, immobilization, or motion) is often necessary. A magnetron technique using MNPs is useful for the manipulation of biomolecules. Compared to other manipulation technologies, magnetron technique possesses a number of advantages; it is rapid, simple, sensitive, and does not require contact. Owing to these merits, magnetron technique has been widely applied in biological detection [85].

The spread of cancer cells between organs is the main reason that cancer is so seldom completely cured. In addition, conventional diagnosis methods are time-consuming and require large volumes of blood samples. Galanzha *et al.* [86] developed a way to magnetically capture circulating tumor cells (CTCs) in the bloodstream of mice and thus were able to perform rapid photoacoustic detection of CTCs. Functionalized MNPs were used to bind and capture CTCs under a magnet. Using multicolor recognition, magnetic enrichment, and signal amplification, their method concentrated CTCs from a large volume of blood in the vessels of tumor-bearing mice. This approach has great potential for the prevention of metastasis in humans and the early diagnosis of cancer.

Generally, for the detection of bacteria, the most well-known strategy is the two-step method that consists of first capturing bacteria with immunomagnetic nanospheres (IMNS) and then identifying bacteria with immunofluores-

cent nanospheres (IFNS). Pang *et al.* [87], however, developed a convenient and simple one-step strategy that mixed IMNS and IFNS with bacteria. After capture and magnetic separation, these "sandwich" immune complexes (IMNS-bacteria-IFNS) were detected under a fluorescence microscope with a detection limit as low as ca. 10 CFU/mL. This method is simple, sensitive, has excellent selection and anti-interference abilities, and has been applied in synthetic samples (milk, fetal bovine serum, and urine); thus it shows great practical potential.

Biofunctionalized Fe_3O_4/Au-nitrilotriacetic acid(NTA)-Ni^{2+} composite nanoparticles were successfully prepared by Xie *et al.* [88]. Research demonstrated that Fe_3O_4/Au-NTA-Ni^{2+} can be used to rapidly, efficiently, and specifically enrich and separate the histidine-tagged (His-Tag) maltose-binding protein (MBP). A detection limit of lower than 5.5×10^{-8} mol/L was achieved using the facile sodium dodecyl (SDS)-polyacrylamide gelelectrophoresis (PAGE). This method is easy, feasible, rapid, and low-cost; moreover, the significance of this work is allowing new ways of thinking about bioseparation and analysis based on nanomaterials.

A class of novel surface-enhanced Raman scattering (SERS) nanotags with highly uniform and reproducible signals has been developed by Liu *et al.* [89] via the layer-by-layer (LBL) assembly of small silver nanoparticles (AgNPs) at the surface of silica (SiO_2) particles, using poly(ethyleneimine) (PEI). In the detection process, the antibody-conjugated Fe_3O_4@SiO_2 particles, certain tumor markers and the SERS nanotags formed sandwich immunocomplexes that could be easily captured and aggregated by magnet. The sensitivity and reliability of the SERS nanotag-based immunoassay were demonstrated by the detection of the tumor marker carcinoembryonic antigen (CEA) in human serum. A detection limit of 0.1 pg/mL was achieved. Owing to the advantages of high uniformity, reproducibility, quantitativeness and stability, the nanotags show great potential in clinical cancer diagnosis.

Magnetically immobilization is another significant aspect of magnetic manipulation. Lee *et al.* [90] developed a novel 3D immunomagnetic flow assay for the rapid detection of pathogenic bacteria in a large-volume food sample (Figure 4(a)). Antibody-modified magnetic nanoparticle clusters (AbMNCs) were immobilized on the surface of a 3D-printed cylindrical microchannel by an external applied magnetic field. The AbMNCs and AbMNCs-Salmonella complexes were perfectly captured under a high flow rate by stacking permanent magnets with spacers inside the cylindrical separator to maximize the magnetic force. A detection limit of 10 CFU/mL was obtained for a 10 mL sample in less than 3 min, using ATP luminescence measurement.

Microfluidic chips are a kind of practical device for multiplex detection. Yu *et al.* [91] developed a sandwich immunoassay method for rapid detection of dual cancer biomarkers in serum on a magnetic field-controllable microfluidic chip (MFCM-Chip). Biofunctionalized superparamagnetic beads (SPMBs) were immobilized in the microfluidic channels and used to specifically capture targets modified by QDs. This rapid and sensitive method shows great potential in the high throughput detection of cancer biomarkers.

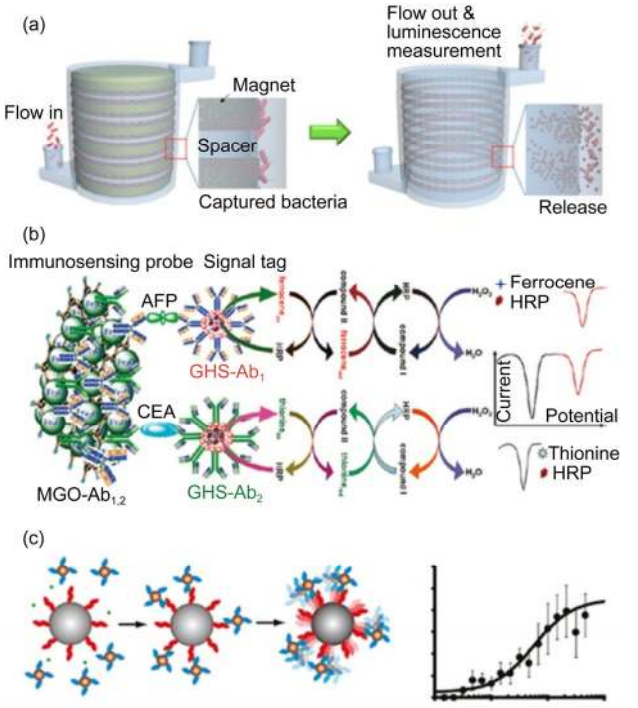

Figure 4 (a) Schematic illustrations of 3D immunomagnetic flow assay [90]; (b) schematic illustration of the multiplexed electrochemical immunoassay protocol and the measurement principles of the sandwich immunoassay [95]; (c) schematic of LAM with thrombin as the analyte and dose-response curve for the detection of thrombin by LAM [96].

A similar portable experiment setup equipped with an optical fiber spectrometer and a microfluidic device was developed by Zhang *et al.* [92] for the simple and rapid detection of avian influenza virus (AIV). With high portability, less assay time, less sample consumption, high specificity, high sensitivity, high reproducibility, this strategy may provide a powerful and point-of-care detection of AIV.

So far, almost all of the methods for biomolecule detection use paramagnetic nanoparticles. However, Fu *et al.* [93] developed a simple surface-functionalization method for conjugating lithographically fabricated antiferromagnetic nanoparticles to a model protein, streptavidin. The streptavidin-functionalized synthetic antiferromagnetic nanoparticles (SAFs) could bind specifically to biotin, and exhibited tunable response to a small external magnetic-field gradient (10 T/m). For the first time, SAF nanoparticles were proved to possess better detection limits than conventional superparamagnetic materials; a detection limit of 10 pmol/L for the biomolecule was obtained.

Recently, a magnetic modulation-biosensing (MMB) system for rapid and homogeneous detection of Ibaraki

virus NS3 cDNA was developed by Danielli *et al.* [94]. The motion of streptavidin-coupled superparamagnetic beads was controlled by an alternating magnetic field. A periodic fluorescent signal produced by their movement was detected by a novel fluorescent resonance energy-transfer (FRFT)-based probe. A detection limit of 1.9 pmol/L for the Ibaraki virus NS3 cDNA was obtained within 18 min, without separation or washing.

Tumor markers are closely associated with cancer, which means that identification and determination of tumor markers are useful in patient diagnosis and clinical therapy. Tang *et al.* [95] developed a novel flow-through multiplex immunoassay protocol for simultaneous electrochemical detection of carcinoembryonic (CEA) and alpha-fetoprotein (AFP) in biological fluids, using biofunctionalized magnetic grapheme nanosheets (MGO) as immunosensing probes and multifunctional nanogold hollow microspheres (GHS) as distinguishable signal tags (Figure 4(b)). Their experimental results demonstrated that the multiplex electrochemical immunoassay could simultaneously detect AFP and CEA in a single run and achieve low detection limits (LODs) for both analytes at 1.0 pg/mL, with wide working ranges of 0.01–200 ng/mL for AFP and 0.01–80 ng/mL for CEA.

A new transduction method, called label-acquired magnetorotation (LAM), was developed by Hecht *et al.* [96] for the detection of protein thrombin in solution, using aptamers with a limit of detection of 300 pmol/L (Figure 4(c)). LAM includes a 10 μm nonmagnetic "mother" sphere as the capture component and 1 μm magnetic "daughter" beads as labels. The daughter beads and mother sphere form a rotating sandwich complex by protein-mediated attachment. The number of attached magnetic beads is proportional to the concentration of the protein in solution, and the rotational frequency of a sandwich complex in a rotating magnetic field is proportional to the number of attached magnetic beads. This is the first study to detect protein using LAM.

6 Magnetic catalyst

Recently, as Yan *et al.* [97] discovered, Fe_3O_4 magnetic nanoparticles (MNPs) (an inorganic nanoparticles) possess an intrinsic peroxidase activity. As peroxidase mimetics, MNPs own the following advantages: (1) most natural enzymes are proteins and they can be easily denatured by changes of environment, for example, temperature, pH and so on, however, MNPs do not, they are more stable than natural enzymes; (2) the intrinsic magnetic separation and enrichment effect of MNPs make biological experiment more easy; (3) the preparation of MNPs is easy and cheap, in comparison, the preparation and purification of the natural enzymes are usually time-consuming and expensive [98]. Therefore, MNPs as enzyme mimetics will be more widely applied to biological detection [99], such as colorimetric, chemiluminescence, and square-wave voltammetry.

One of the major applications of MNPs has been in colorimetric detection. It was found that Fe_3O_4 MNPs can catalyze the oxidation of various peroxidase substrates such as 3,3,5,5-tetramethylbenzidine (TMB), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), diazo-aminobenzene (DAB), and *o*-phenylenediamine (OPD), to respectively produce blue, green, brown and orange colors when enzymatically oxidized with hydrogen peroxide (H_2O_2) [100,101]. Compared with other analytical methods, colorimetric assay is simple and rapid. Additionally, the color changes of analytes can be observed by the naked eye. The principle of the method is based on the ability of MNPs to catalyze the oxidation of organic substrate to produce a color change [102].

Engineered nanoparticles for the diagnosis and therapy of cancer are usually functionalized with ligands or agents, which greatly decreases detection sensitivity because the excess ligands on the nanoparticle surface leads the nanoparticles to bind nonspecially and aggregate. Yan *et al.* [103] discovered that magnetoferritin nanoparticles made by encapsulating iron oxide nanoparticles into a ferritin protein shell can solve this problem, because the recombinant human heavy-chain ferritin (HFn) proteins particularly bind to the tumor cells and the iron oxide can catalyze the oxidation of peroxidase substrates to produce a color reaction. The examination of 474 clinical specimens simultaneously achieved a sensitivity of 98% and a specificity of 95%, which demonstrated that magnetoferritin nanoparticles will play a major role in the rapid, low-cost and universal assessment of cancerization. They also found that acetylcholine and H_2O can produce H_2O_2 in the presence of the enzymes AChE and CHO. The organophosphorus neurotoxins, can greatly inhibit the enzymatic activity of AChE, however, which leads to less H_2O_2. Taking advantage of this principle, they developed a novel Fe_3O_4 magnetic nanoparticle (MNP) peroxidase mimetic-based colorimetric method to rapidly detect organophosphorus pesticides and nerve agents, which achieved a detection limit of as low as 1 nmol/L Sarin, 10 nmol/L methyl-paraoxon, and 5 μmol/L acephate [104]. With further development, this assay can be configured into an array format for the rapid, low-cost, and large-scale field screening of organophosphate neurotoxins.

A novel colorimetric aptasensor for the detection of thrombin using chitosan-modified Fe_3O_4 MNPs as a peroxidase mimic in the color reaction of H_2O_2/TMB solution was developed by Wang *et al.* [105]. They demonstrated that the absorption values at 652 nm increased with the thrombin concentrations in a linear range of 1–100 nmol/L, and obtained a detection limit of 1 nmol/L thrombin. This method indicates that Fe_3O_4 MNPs not only possess peroxidase activity but also will be widely applied in varieties of simple, robust, and cost-effective analytical methods.

As is known to all, the detection of melamine in dairy products is significant. Ding *et al.* [106] developed a simple and rapid colorimetric method for the determination of

melamine in dairy products by Fe_3O_4 MNPs-H_2O_2-ABTS detection system that does not require any expensive or complex instruments. The detection limit of melamine in real samples is lower than 2.5 ppm (the safety limit in the USA and EU) with the recoveries in a range from 98%–115%, using only a 721-A spectrophotometer without the aid of any instrumentation.

Reduced glutathione (GSH), a tripeptide composed of cysteine, glutamic acid, and glycine, is often involved in many cellular functions such as maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction, and gene regulation. Changes in the concentration of GSH have been proven to be related to many diseases, including HIV/AIDS, cancer, liver damage, psoriasis, and leukocyte loss. Therefore, detection of GSH in biological samples is important in clinical medicine and biochemical studies. Ma *et al.* [107] developed a novel and simple colorimetric method for the detection of reduced glutathione (GSH) in A549 cells using Fe_3O_4 MNPs as a catalyst in the color reaction of H_2O_2 and the peroxidase substrate ABTS, which can be detected by the naked eye. A good linear relationship of 3.0–30.0 μmol/L for GSH and good recoveries of 96.7%–107% were achieved.

Besides the Fe_3O_4 MNPs, $ZnFe_2O_4$ MNPs can be applied in colorimetric detection. Su *et al.* [108] discovered that $ZnFe_2O_4$ MNPs possess intrinsic peroxidase-like activity. They developed a simple, inexpensive, highly sensitive, and selective method for the detection of urine glucose using $ZnFe_2O_4$ MNPs and glucose oxidase (GOx). A detection limit of 3.0×10^{-7} mol/L and a linear range of 1.25×10^{-6}–1.875×10^{-5} mol/L were obtained. The principle for the sensing of urine glucose level is color change observable by the naked eyes, which is based on the oxidation of TMB.

Besides the above-mentioned analytes, colorimetrics can also be applied to detect bacteria. A novel and sensitive magnetic polymeric nanoparticle (MPNP)-polymerase chain reaction-colorimetry (magneto-PCR-colorimetry) technique was developed by Thiramanas *et al.* [109] for the detection of vibrio cholera (*V. cholerae*) (Figure 5(a)). They first made an amplification of *V. cholerae* DNA on the surface of an MPNP, and next they detected the target gene by colorimetry. A detection limit of 10^3 CFU/mL was achieved for *V. cholerae* in a buffer system within 4 h by employing the intrinsic catalytic activity of the MPNP. The specificity and efficiency of this technique were examined by detecting *V. cholera*e in drinking and tap water. Compared with PCR-ELISA (~5 h of analysis time) and PCR-gel electrophoresis (sensitivity ~10^5 CFU/mL), this method has a shorter analysis time and 10^2-fold better sensitivity.

So far, almost all of the reported MNPs-based enzyme mimetics have been investigated by the colorimetric method. However, few parts of studies have evaluated MNPs-based enzyme mimetics by the chemiluminescence (CL) method. CL is generally defined as the emission of light (infrared, visible, or ultraviolet) resulting from a chemical reaction

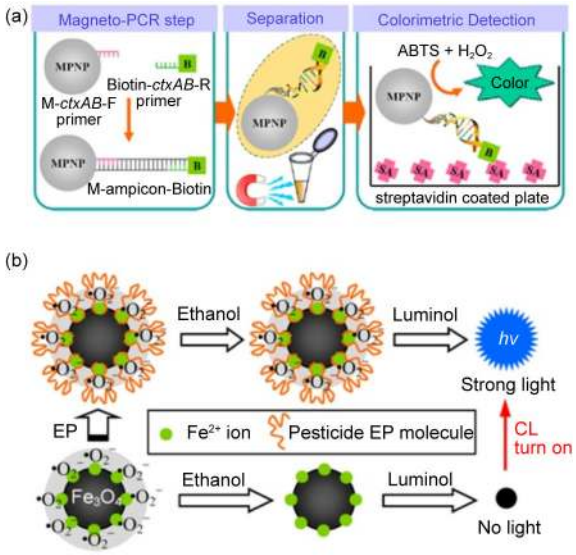

Figure 5 (a) Schematic representation of M-amplicon-biotin detection by the magneto-PCR-colorimetry technique [109]; (b) mechanism of CL switching at the surface of Fe_3O_4 nanoparticles [111].

without using an external light source [110]. Owing to their high sensitivity, simplicity of operation, wide linear range and cost-effectiveness, CL has been widely applied for chemical analyses, biological assays, clinical diagnoses, and environmental detections. However, one disadvantage of the classic CL systems is their low efficiency for transforming chemical energy into light; the improved methods originate in the spontaneous reactions in a redox pair and thus intrinsically lack molecular selectivities to detect specific targets. To meet this challenge, Guan *et al.* [111] reported the novel concept of developing intrinsically selective CL switching at the surface of Fe_3O_4 MNPs for the sensitive detection and simultaneous determination of various pesticides (Figure 5(b)). Fe_3O_4 MNPs with 10 nm size act as catalysis and catalyze the decomposition of dissolved oxygen to produce superoxide anions to amplify the CL intensity of luminol at least 20 times. Using the surface coordinative reaction, a detection limit of 0.1 nmol/L and a wide detection range of 0.1–100 μmol/L were obtained for the sensitive detection of nonredox pesticide ethoprophos. More significantly, the selectivity of CL switching changes followed different surface modifications of Fe_3O_4 nanoparticles, and these Fe_3O_4 nanoparticles with different surface groups that can produce unique CL signals. Therefore, we use these Fe_3O_4 nanoparticles to simultaneously detect various pesticides.

Another method using Fe_3O_4 MNPs as peroxidase mimics for biological detection is the square wave voltammetry by Zhang *et al.* [112]. They developed a sensitive choline biosensor using Fe_3O_4 MNPs and a choline oxidase-modified gold electrode. Choline acts with oxygen and water catalyzed by choline oxidase to produce hydrogen peroxide. Fe_3O_4 MNPs was used to catalyze the reduction of H_2O_2, and produce electron. Using the reduction currents of

square wave voltammertry, which were increased with the logarithm values of the choline chloride concentration, as the detection signals, a detection limit of 0.1 nmol/L was obtained.

7 Signal enhancer for SPR

Surface plasmon resonance (SPR) sensors are useful tools for rapid, sensitive biological detection [113]. Owing to their unique abilities to characterize and quantify biomolecules at low concentration, they have been widely applied in theranostics, pharmaceutics, food safety, environmental monitoring, and homeland security [114]. In fact, SPR sensors are optical refractometers that can detect refractive-index changes of the medium at the SPR-sensing surfaces. Enhanced mechanisms of magnetic nanoparticles (MNPs) for signal amplification of SPR sensors include: (a) large surface-mass loading of MNPs, which can lead to large perturbations on the sensing surface; and (b) catalytic activity of functionalized MNPs, which can further trigger secondary signal amplification [115]. Compared with other types of nanomaterials for enhanced SPR sensing, the main advantages of using MNPs are eliminating the step of initial receptor immobilization onto the SPR-sensing film and lowering the production cost of the MNPs. Additionally, the MNPs can be attracted and controlled by external magnetic fields to form an "aggregate" layer with a strong refractive index, which results in a notable SPR signal when sensing trace amounts of biological and chemical samples.

Recently, a method for rapid purification, concentration, and detection of target analytes from complex matrixes using antibody-modified superparamagnetic nanobeads (immunomagnetic beads, or IMBs) was developed by Soelberg *et al.* [116]. They used the IMBs as detection amplifiers and dramatically increased the SPR detection signal from staphylococcal enterotoxin B (SEB). Ultimately, a detection limit of 100 pg/mL for SEB was achieved both in buffer and stool samples.

Wang *et al.* [117] developed a novel SPR sensor based on an indirect competitive inhibition assay (ICIA) for the detection of adenosine by employing Fe_3O_4 MNPs-anti adenosine aptamer conjugates as the amplification reagent. Their results demonstrated that Fe_3O_4 MNPs can be utilized as a powerful amplification agent to provide a sensitive way of detecting adenosine via SPR within the range of 10–10000 nmol/L, which is much lower than the detection results of a general SPR sensor. More importantly, their detection methodology can be widely applied for the detection of other biomolecules by using the appropriate aptamers in Fe_3O_4 MNP-aptamer conjugates.

Wang *et al.* [118], by combining the spectroscopy of grating-coupled long-range surface plasmons (LRSPs) with MNPs assay, invented a new SPR biosensor for the rapid and highly sensitive detection of bacterial pathogens. This amplification strategy shows great potential for the detection of large analytes that diffuse slowly from the analyzed sample to the sensor surface. *E. coli* O157:H7 at concentrations as low as 50 CFU/mL could be detected, which was an improvement of 4 orders of magnitude on the limit of detection of regular grating-coupled SPR with direct detection format.

The high refractive index and molecular weight of the Fe_3O_4 MNPs means that they can greatly enhance the localized surface plasmon resonance (LSPR) response to biological binding events, and consequently improve the sensitivity, reliability, dynamic range, and calibration linearity of LSPR assays for the detection of small molecules in trace amounts. Tang *et al.* [119] were the first to use a label-free LSPR nanosensor for low-cost, clinical-oriented detection of a disease biomarker in physiological solution (Figure 6). This extremely light, robust, and low-cost nanosensor is promising for installation on a lab-on-a-chip system to provide point-of-care medical diagnostics. To further evaluate the practical application of Fe_3O_4 MNPs in the enhancement of LSPR assay, they used a gold nanorod (GNR) bioprobe to detect cardiac troponin I (cTnI) for myocardial infarction diagnosis. Results demonstrated that spectral responses resulted from MNP-captured cTnI molecules are 6-fold higher than direct cTnI adsorption on the GNR sensor. A detection limit of ca. 30 pmol/L for plasma samples was obtained, which is 3 orders lower than a comparable study.

Besides the abovementioned analytes, Mousavi *et al.* [120] developed an ultra-sensitive, label-free method for the detection of an mRNA biomarker using functionalized MNPs for signal enhancement in conjunction with SPR on gold nanoslits. In their study, along with using MNPs to enhance the SPR signal, MNPs were applied to isolate the target molecule from the sample matrix to prevent nonspecific binding. Without amplification and labeling of the target molecule, a detection limit of less than 30 fmol/L hnRNP B1 mRNA in a 7 μL sample (corresponding to 1.26×10^5 molecules) was achieved.

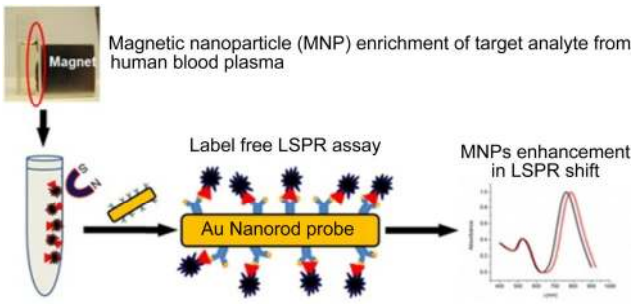

Figure 6 Schematic showing bioseparation of target molecules from blood plasma by functional Fe_3O_4 magnetic nanoparticles (MNPs), followed by the MNP-mediated nanoSPR assay. The application of MNP results in an enhancement of the LSPR shift at peak absorption wavelength [119].

8 Conclusions and perspectives

Combining the advantages of magnetic particles and nanomaterials, magnetic nanoparticles (MNPs) have the characteristics of small size, superparamagnetism, and large surface atoms/bulk atoms ratio. By undergoing surface modification, MNPs can possess better biocompatibility, and consequently can be widely used for biological detection,

Table 1 Sensitivities of magnetic nanoparticles-based biodetection

	Analyte	Method	Sensitivity	Reference
Separation	*E. coli*	SERS	8 CFU/mL	[35]
	salmonella	chromatography	100 CFU/mL	[36]
	S. aureus	luminescence	25 CFU/mL	[37]
	V. parahemolyticus	luminescence	10 CFU/mL	[37]
	S. typhimurium	luminescence	15 CFU/mL	[37]
	HIV	ICP-MS	28 amol	[40]
	HAV	ICP-MS	48 amol	[40]
	HBV	ICP-MS	19 amol	[40]
	H9N2 AIV	electrochemistry	10 pg/mL	[41]
	HRP2	electrochemistry	0.36 ng/mL	[46]
	ssDNA	SERS	10 pmol/L	[47]
	DNA	fluorescence	100 pmol/L	[51]
	cholesterol	electrochemistry	0.85 μmol/L	[53]
	histidin	spectrometry	1 nmol/L	[54]
Sensing	Lys	MRSw	nanomolar	[61]
	cancer cells	MRSw	10 cells/250 μL	[62]
	DNA	AC susceptometer	4 pmol/L	[64]
	DNA	AC susceptometer	1.5 pmol/L	[65]
	DNA	GMR	10 pmol/L	[68]
	Hg^{2+}	GMR	10 nmol/L	[69]
	cancer cells	DMR	2 cells/1 μL	[71]
	TB	NMR	20 CFU/mL	[72]
	bacteria	NMR	8 cells/mL	[73]
	bacteria	NMR	Single bacteria	[74]
	miRNAs	AM	zeptomole	[80]
Manipulation	S. typhimurium	fluorescence	10 CFU/mL	[87]
	protein	SDS-PAGE	5.5×10^{-8} mol/L	[88]
	bacteria	ATP luminescence	10 CFU/mL	[90]
	CEA	fluorescence	3.5 ng/mL	[91]
	AFP	fluorescence	3.9 ng/mL	[91]
	AIV	fluorescence	3.7×10^{4} copy/μL	[92]
	biomolecule	GMR	10 pmol/L	[93]
	DNA	fluorescence	1.9 pmol/L	[94]
	AFP and CEA	electrochemistry	1.0 pg/mL	[95]
	thrombin	LAM	100 pmol/L	[96]
Catalyst	sarin	colorimetric	1 nmol/L	[104]
	methyl-paraoxon	colorimetric	10 nmol/L	[104]
	acephate	colorimetric	5 μmol/L	[104]
	thrombin	colorimetric	1 nmol/L	[105]
	melamine	colorimetric	2.5 ppm	[106]
	GSH	colorimetric	3.0 μmol/L	[107]
	urine glucose	colorimetric	30 nmol/L	[108]
	V. cholera	colorimetric	10^{3} CFU/mL	[109]
	pesticide	CL	0.1 nmol/L	[111]
	choline	voltammetry	0.1 nmol/L	[112]
Signal enhancer	SEB	SPR	100 pg/mL	[116]
	adenosine	SPR	10 nmol/L	[117]
	E. coli	LRSPs	50 CFU/mL	[118]
	cTnI	LSPR	30 pmol/L	[119]
	RNA	SPR	30 RNA/7 μL	[120]

drug delivery, hyperthermia, and tissue engineering. In this review, we have summarized five applications of MNPs in biological detection, including magnetic separation, sensing, manipulation, catalysis, and signal enhancer for SPR. To make it easier for our readers to find the references on different applications of MNPs, we summarize representative works in Table 1. Due to their superparamagnetism, MNPs can be used for the separation and manipulation of biological objects. Biosensors using MNPs as labels are useful tools for biological detection. The intrinsic peroxidase activity of Fe_3O_4 MNPs makes them indispensable in a variety of methods. Moreover, their large surface mass-loading and catalytic activity can greatly enhance the SPR signal of biological detection. The development of biological detection with MNP since 2009 suggests that the sensitivities of MNP-based biodetection can be attributed to three aspects: intrinsic performance of probes, detection strategy of analyte, and sensitivity of instruments. These three points are critical to the enhancement of sensitivity because a relationship should be considered among the concentration of analyte, the response of probe, and the signals produced by the above methods. Other parameters such as selectivity, dynamic range, linearity, and accuracy are also critical for an excellent detection technique; this review, however, has mainly emphasized sensitivity. The relentless pursuit of ultrasensitive methods for biological detection should be placed in the context of practical needs. In addition, it is worth noting that some experimental methods and apparatus are very complicated. Further development should pay more attention to practicability, miniaturization, integration, and low cost.

Great progresses have been made in the sensitivity of MNPs-based biological detection, and a variety of methods using MNPs have been developed to analyze biological objects. However, improvements must be made to aspects of MNPs in both the high field and ultra-low field. Further advances in this area will rely on the development of new techniques, especially *in vivo* detection. There is no doubt that, with our joint efforts, MNPs will eventually be one of the best candidates for biological detection.

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