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A biotechnological perspective on the application of iron oxide nanoparticles

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ABSTRACT

In recent decades, magnetic iron nanoparticles (NPs) have attracted much attention due to properties such as superparamagnetism, high surface area, large surface-to-volume ratio, and easy separation under external magnetic fields. Therefore, magnetic iron oxides have potential for use in numerous applications, including magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, drug delivery, hyperthermia, and cell separation. This review provides an updated and integrated focus on the fabrication and characterization of suitable magnetic iron NPs for biotechnological applications. The possible perspective and some challenges in the further development of these NPs are also discussed.

1 Introduction

Nanoscience has emerged as an innovative research field that can be applied in a number of scientific and technological areas, including material science, electronics, biotechnology, and medical sciences [1]. According to the International Union of Pure and Applied Chemistry (IUPAC) definition, nanoparticles (NPs) are microscopic particles with at least one dimension less than 100 nm. Due to their small size in comparison with large biomolecules, NPs can undergo many interactions with biological molecules, which may revolutionize cancer diagnosis and treatment [2]. Nanometer-sized materials are widely used in the fields of biotechnology and biomedicine, for such purposes as enzyme encapsulation, DNA transfection, targeted drug delivery, and hyperthermia in chemotherapy [3].

Among the nanostructured materials, and metallic NPs in particular, magnetic nanoparticles (MNPs)

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have been the focus of intensive research. MNPs are composed of magnetic elements (iron, nickel, or cobalt) and their oxides (magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and cobalt ferrite (Fe_2CoO_4)) [4]. Magnetic materials are classified as five main types. Paramagnetic materials do not retain magnetic moment when the magnetic field is removed. Superparamagnetic particles are nonmagnetic in the absence of an external magnetic field, but they develop a mean magnetic moment in an external magnetic field. This unique property of superparamagnetic particles results in more interest for their application in drug delivery systems [5]. The second group of magnetic materials is ferromagnetic materials with permanent mean moment such as iron, nickel, and cobalt. A ferromagnetic material produces a magnetic field even in the absence of an external magnetic field. When the ferromagnetic material is placed in a magnetic field, the magnetic moments of the domains align along the direction of the applied magnetic field, forming a large net magnetic moment [6].

In diamagnetic materials (such as copper, silver, gold, and most of the known elements), atoms have no unpaired electrons, resulting in zero net magnetic moment. These materials display a very weak response against the applied magnetic field due to realignment of the electron orbits when a magnetic field is applied. They do not retain magnetic moment when the magnetic field is removed. Antiferromagnetic materials (such as MnO, CoO, NiO, and $CuCl_2$) are compounds of two different atoms that occupy different lattice positions. The two atoms have magnetic moments that are equal in magnitude and opposite in direction, which results in zero net magnetic moment [5]. Ferrimagnetic materials (such as Fe_3O_4 and γ-Fe_2O_3) are also compounds of different atoms residing on different lattice sites with antiparallel magnetic moments. However, in these materials, the magnetic moments do not cancel out since they have different magnitudes, which results in a net spontaneous magnetic moment [7].

Magnetic iron oxide nanoparticles (IONPs) have potential applications in various disciplines of science ranging from environmental remediation to biomedical uses, such as magnetic drug targeting, tissue repair, and cell tissue targeting [8]. MNPs possess large surface area to volume ratios due to their nanosize and low surface charge at physiological pH; they aggregate easily in solution because of their inherent magnetic nature. In some cases, an unwanted aggregation may decrease the long-term stability of products, leading to large nanoparticle clusters that are undesirable for medical applications. Additionally, degradation of iron oxide into free ions in physiological environments [9] has been reported to increase free radical production in cells, causing damage which may lead to cell death [10, 11]. Therefore, these particles are commonly coated with organic macromolecules, such as poly(acrylic acid) (PAA) [12], dextran [13], and poly(ethyleneimine) (PEI) [14] or with coatings such as silica [15], carbon [16], or precious metals (e.g., gold or silver) [17]. In light of the unique properties of MNPs, particularly iron oxide NPs, the aim of this review is to discuss the recent developments in the synthesis and application of iron oxide NPs. For this purpose, this paper will highlight the characteristics and application of iron oxide NPs. In particular, the biotechnological applications, such as food analysis, protein/enzyme immobilization, protein purification, and targeted drug delivery will be discussed.

2 IONPs

In recent decades, research in the field of MNPs studying several types of iron oxides has increased. FeO, known in mineralogical terms as wüstite, has only Fe^{2+} ions in its cubic crystal structure, and is thermodynamically unstable and paramagnetic at room temperature. In contrast, Fe_2O_3, or ferric oxide, contains only Fe^{3+} ions and displays polymorphism, that is, the existence of further isochemical phases with different physical properties revealed by their different crystal structures. To date, four Fe_2O_3 polymorphs have been described [18, 19]: (1) α-Fe_2O_3, known mineralogically as hematite, has a rhombohedrally centered hexagonal crystal structure (space group $R\bar{3}c$) and is weakly ferromagnetic or antiferromagnetic; (2) β-Fe_2O_3 has a cubic-body-centered crystal structure of a bixbyite type (space group $Ia\bar{3}$); (3) γ-Fe_2O_3 has a cubic crystal structure of inverse spinel type (space group $Fd3m$) and has ferromagnetic properties; and (4) ε-Fe_2O_3 has an orthorhombic crystal structure (space group $Pna2_1$). The last nonhydrated iron oxide phase, Fe_3O_4, has both Fe^{2+} and Fe^{3+} ions distributed

over a cubic inverse spinel crystal structure and is ferromagnetic or superparamagnetic when the size is less than 15 nm.

Despite the existence of multiple polymorphs, only Fe_3O_4 and γ-Fe_2O_3 have been found to be functional and promising candidates in biomedical and biotechnological applications due to their favorable magnetic properties [20]. They are both strong ferromagnetic materials with two magnetic sublattices mirroring tetrahedral (T) and octahedral (O) sites, the two nonequivalent cation positions in their crystal lattice [18].

IONPs exhibit some attractive properties: They are easily manufactured, can be spatially controlled while inside the human body by external (or internally implanted) magnetic fields that are considered physiologically safe, and their localization can be detected using magnetic resonance imaging. It should be also noted that IONPs are generally considered biocompatible and biodegradable [21], since, following their release, the free iron is integrated in the iron stores of the body, used for metabolic processes, and eventually eliminated from the body [22].

Superparamagnetic iron oxide nanoparticles (SPIONs) are small synthetic α-Fe_2O_3, γ-Fe_2O_3, or Fe_3O_4 particles with a core diameter ranging from 10 to 20 nm. Ferromagnetic iron oxide NPs offer some attractive possibilities in biomedicine. First of all, they have controllable sizes ranging from a few nanometers up to tens of nanometers, making their dimensions smaller than or comparable to those of a virus (20–450 nm), a cell (10–100 μm), a protein (5–50 nm), or a gene (2 nm wide and 10–100 nm long). This indicates that they have the potential to enter a biological entity of interest. These nanomaterials can be coated with biomolecules for binding to or interacting with a biological entity, in order to produce a controllable addressing or tagging tool [23]. Moreover, these MNPs can be influenced by external magnetic fields. Ferromagnetic NPs possess stronger magnetic potential than their SPION counterparts, which is attractive for external magnetic guidance and increased contrast-ability [24–26].

2.1 Properties

The unique physiochemical properties of MNPs allow them potential roles in biological applications. The particle size, size distribution, shape, and surface area are the special properties of MNPs that play an important role in biological applications [4]. Particle size is the most important characteristic of NPs. Particle size not only influenced the physical property of the particle, such as magnetic moment (responsiveness to applied magnetic field), but also affected biological outcomes after NPs were injected into the human body (blood circulation time and bioavailability of particle *in vivo*). When particle size is less than 10 nm, NPs can be rapidly cleared due to easy exosmosis or renal excretion. If the particle size is more than 200 nm, NPs are easily mechanically filtered by spleen or phagocytized by macrophages in the reticuloendothelial system, leading to a decrease in blood circulation time. NPs with a particle size of 10–100 nm are ideal particles for intravenous injection [27–29]. It was confirmed that these types of NPs had the longest blood circulation time. The volume of particles that are 10–100 nm in size is small enough to escape the phagocytosis of the reticuloendothelial system and penetrate into capillary vessels in body tissues, which ensures an effective distribution in specific tissues [30, 31].

Some of the physical and magnetic properties of iron oxides are summarized in Table 1.

2.2 Synthesis methods

Many of the useful attributes of iron oxides depend on the preparation method used for the nanomaterials. The preparation method plays a key role in determining the particle size and shape, size distribution, and surface chemistry, and therefore, the applications of the material. In addition, the preparation method also determines the degree of structural defects or impurities present in the particles, as well as the distribution of such defects [32]. Many synthesis routes have been developed to achieve proper control of particle size, polydispersity, shape, crystallinity, and magnetic properties [33–36].

Different synthetic routes have been established in the fabrication of SPIONs. For example, physical methods make use of mechanical grinding and biomineralization processes. Chemical methods include coprecipitation, microemulsion, electrochemical, polyol, flame-assisted, and thermal decomposition, as well as

Table 1 General physical, chemical, and magnetic characteristics of iron oxide minerals

Property	Oxide		
	Hematite α-Fe_2O_3	Maghemite γ-Fe_2O_3	Magnetite Fe_3O_4
Crystallographic system	Rhombohedra, hexagonal	Cubic or tetragonal	Cubic
Structural type	Corundum	Defect spinel	Inverse spinel
Space group	*R*3*c* (hexagonal)	$P4_332$ (cubic); $P4_12_12$ (tetragonal)	*Fd*3*m*
Lattice parameter (nm)	$a = 0.50340$, $c = 1.375$ (hexagonal) $a_{Rh} = 0.5427$, $\alpha = 55.3°$ (rhombohedral)	$a = 0.83474$ (cubic); $a = 0.8347$, $c = 2.501$ (tetragonal)	$a = 0.8396$
Usual crystal shape	Hexagonal plates Rhombohedra	Cubes	Cubes
Density ($g \cdot cm^{-3}$)	5.26	4.87	5.18
Color	Red	Reddish-brown	Black
Solubility product[a] (pFe + 3 pOH)	42.2–43.3	40.5	43.4–44.0
Most intense XRD	0.269, 0.251	0.251, 0.295	0.253, 0.297
Spacings (nm)	0.366	0.147	0.1485
Type of magnetism	Weakly ferromagnetic or antiferromagnetic	Ferrimagnetic	Ferrimagnetic
Curie temperature (K)	956	820–986	850
Melting point (°C)	1,350	—	1,583–1,597
Hardness	6.5	5	5.5
M_s at 300 K ($Am^2 \cdot kg^{-1}$)	0.3	60–80	92–100
Standard free energy of formation ΔG_f^0	−742.7	−711.1	−1012.6

[a]Depends on particle size.

hydrothermal and sol-gel syntheses and sonochemical reactions [37, 38]. IONPs are prepared mainly by coprecipitation of Fe^{2+}/Fe^{3+} salt solution under base conditions, since the procedure is simple and requires no harmful chemicals [39]. By using coprecipitation methods, the size of the particle can be controlled by three different factors: temperature, base concentration, and presence of surfactants [40]. To control the size of IONPs, PAA was added to the ion salt solutions, and the size was controllable in the range of 7–14 nm by the concentration of the polymer [41]. The polymer was believed to provide surface coating; however, the addition of the polymer deteriorated the crystallinity and the magnetic property of IONPs [42]. Chemical methods, particularly the solution-based synthetic methods, are generally more suitable than the physical methods and biomineralization processes for producing SPIONs for magnetic resonance imaging (MRI) application. Chemical methods show great advantages in controlling the particle size, size distribution, degree of crystallinity, and phase purity, which are the most fundamental parameters in terms of MRI applications [37, 43].

A recent development in the synthesis of SPIONs is the use of sonochemical routes [44–46]. In this process, a high-energy ultrasonication creates acoustic cavitations that can provide localized heat with a temperature of about 5,000 K. At high temperatures, the formation and growth of nuclei and the implosive collapse of bubbles can take place. Monodisperse NPs of a variety of shapes can be prepared by this method; however, it lacks the potential for large-scale synthesis [47]. Electrochemical deposition under oxidized conditions has also been used to synthesize maghemite and magnetite NPs [48]. In this method, the anode can be oxidized to metal ion species in solution, and

the metal ion is later reduced to metal by the cathode in the presence of stabilizers. This method, however, also lacks application for large-scale synthesis. The hydrothermal method is reported to be the oldest method of synthesis of magnetite, in which iron precursors in aqueous medium can be heated at high temperature at autogenous pressure. Recently, this method has been extended using microwaves for the synthesis of SPIONs [49]. This method produces SPIONs of uniform sizes and can easily be scaled up. Grzeta et al. [50] reported the synthesis of nanocrystalline magnetite by thermal decomposition of iron choline citrate. A similar method was also reported using iron carbonate [51] and iron carboxylate [52]. Recently, Liu et al. [53] prepared magnetic platelets using ethylene diamine as a solvent/reducing agent by the solvothermal route. The solvent free thermal decomposition route was also used recently for the preparation of SPIONs [54].

It has been known for some time that magnetotactic bacteria can perform biomimetic synthesis of SPIONs. Various research groups have used bacteria, fungi, *Mms6* protein, or globular protein for this purpose [55–60]. For instance, Coker et al. [60] exploited the Fe(III)-reducing bacterium *Geobacter sulfurreducens* to synthesize magnetic iron oxide NPs. However, the disadvantage of this method is the lack of large-scale synthesis with well-defined sizes and shapes.

Although most of the above synthesis protocols resulted in either spherical or rhombic morphologies, there are a few reports in which SPIONs were synthesized with other geometries, such as ellipsoid [61] and cubic [62]. Such unique geometries can offer well-defined and reproducible magnetic fields. Peng et al. [63] prepared magnetite nanorods through reduction of beta-FeOOH, whereas large-scale synthesis of single-crystal magnetic (maghemite, magnetite, and hematite) nanorings was achieved by Jia et al. [64] using the hydrothermal route. A list of methods for the preparation of IONPs is shown in Fig. 1.

2.3 Surface modification

Magnetic IONPs with hydrophobic surfaces and large surface area to volume ratio tend to agglomerate in

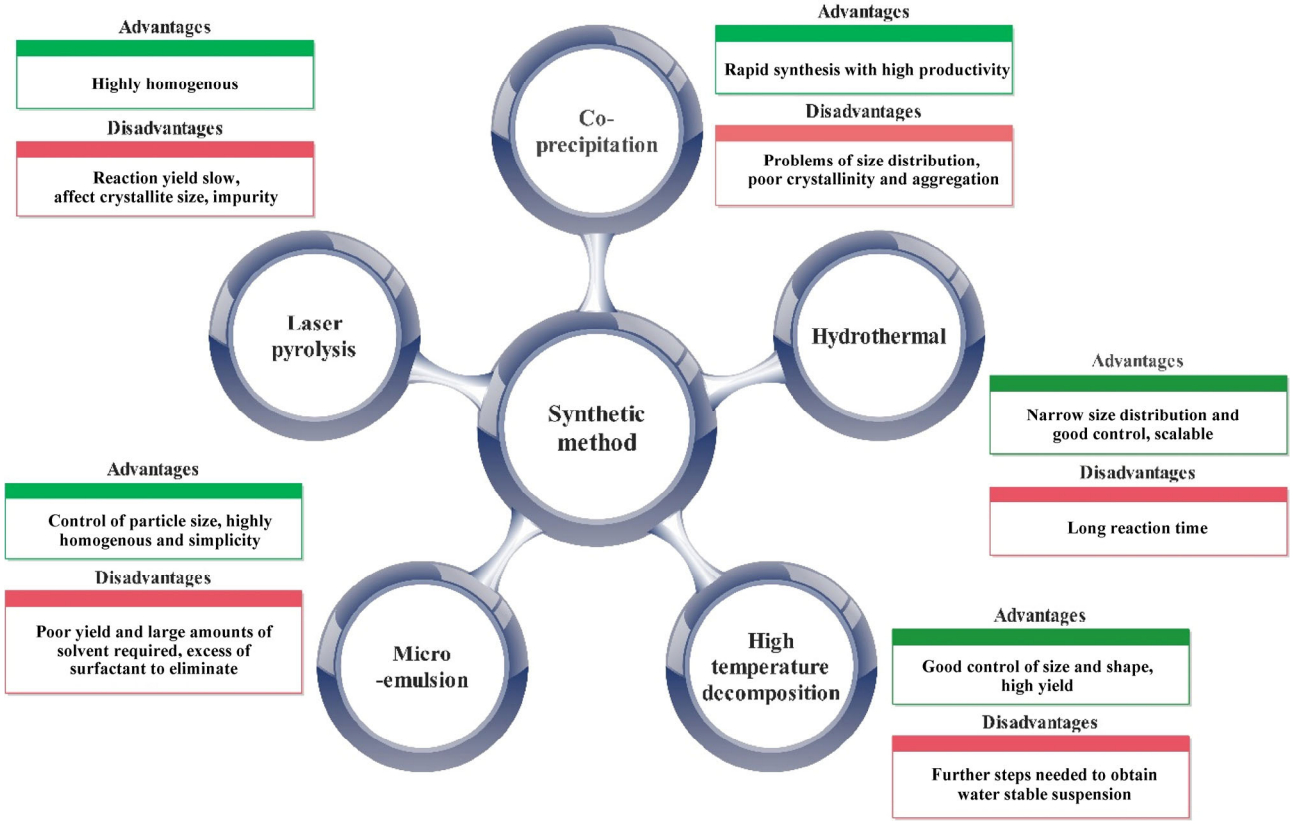

Figure 1 Principal preparation method of IONPs.

both biological medium and magnetic field, creating heterogeneous size distribution patterns. For instance, for *in vivo* applications, agglomeration can result in rapid clearance from the circulation and unexpected responses. In addition to agglomeration problems, some IONPs, particularly Fe_3O_4 and γ-Fe_2O_3 NPs, can easily oxidize in air due to their high chemical activity and lose their magnetic property [65]. In general, there is a need to disperse MNPs into suitable solvents or use surface modification to coat them with certain molecules and polymers to form homogeneous solutions known as ferrofluids [66]. Therefore, the surface modification, which is performed by coating the surfaces of NPs with desirable molecular materials, is indispensable to improve stability, prevent aggregation of NPs, ensure nontoxic status in physiological conditions, and enhance the targeting function.

Perfectly coated material should have the advantages of good affinity to the iron oxide core, good biocompatibility (i.e., non-immunogenicity, no antigenicity, and resistance of plasma protein opsonization), good biodegradation, and high colloid stability [67]. In addition, coated material should also be able to recognize and bind to specific bioactive molecules, including monoclonal antibody, lectin, peptides, hormones, vitamins, nucleotides, or drugs. Since the surfaces of target cells are negatively charged, most MNPs are modified with cationic materials that contain functional groups to conjugate biological molecules and drugs. Rapid uptake of uncoated MNPs by the mononuclear phagocyte system is likely after systemic administration, followed by clearance by the reticuloendothelial system (RES). Opsonization, which involves opsonin binding, i.e., binding of antibody to a receptor on the cell membrane of the pathogen, is a key step in this phagocytosis process. To avoid opsonization of NPs, biodegradable and nonbiodegradable organic and inorganic coatings are used as aids to retard detection and uptake by macrophages [68]. Furthermore, surface modification can reduce these side effects by preventing the leaching of metallic cores and facilitating intact excretion of MNPs through the kidney. Although MNPs are considered biodegradable, the Fe in MNPs can be reused and recycled by cells using normal biochemical Fe metabolic pathways. Thus, such Fe recycling may evoke adverse effects on homeostasis and cause damage to critical cells in the liver, heart, and other metabolically active organs [65].

Generally, coated material can be divided into two types: artificial synthetic material and natural macromolecular material. Polyethylene, polyvinylpyrrolidone, polyethyleneglycol (PEG), and polyvinylalcohol (PVA) are some typical artificial synthetic materials, whereas natural macromolecular materials include gel, dextran microsphere, chitosan, and amylopectin. Currently, most coated materials used in clinical applications are carbohydrates and carbohydrate-derived polymer materials (Fig. 2). In addition to good hydrophilicity, biocompatibility, and biodegradation, their inherent affinity for the core of iron oxide and property of simulating glycoprotein in the biosystem are very important [27].

The ideal molecules used for the stabilization of SPIONs should be biocompatible and biodegradable. However, the most common molecules used are surfactants, such as oleic acid, lauric acid, alkane sulphonic acids, and alkane phosphonic acids [69]. Surfactant molecules are amphiphilic in nature, and they play their role at the interface of SPIONs and the solvent. Most of the surfactant-mediated syntheses are reported in organic solvents, e.g., hexadecane, toluene, n-hexane, as the hydrophobic tail groups (hydrocarbon chain) of the surfactant molecules form as a shell around SPIONs. However, SPIONs in organic suspension cannot be used for biological purposes such as drug delivery [70].

Wang et al. [71] reported a method in which the hydrophobic surface of coated SPIONs was inverted to a hydrophilic surface using alpha-cyclodextrin by host–guest interactions, allowing the NPs to disperse from organic to aqueous solution. Such inversion of hydrophobic surfaces to hydrophilic surfaces was also achieved by using an amphiphilic polymer shell [72]. The synthesis of hydrophilic magnetic NPs has also been reported via a reverse emulsion approach using PEG [73]. Other chemicals used for surface stabilization of SPIONs include Disperbyk 120 [74] and tetramethyl ammonium hydroxide [75]. Furthermore, Park et al. [76] reported using ferrocene in supercritical water to coat magnetite and maghemite NPs with graphitic carbon.

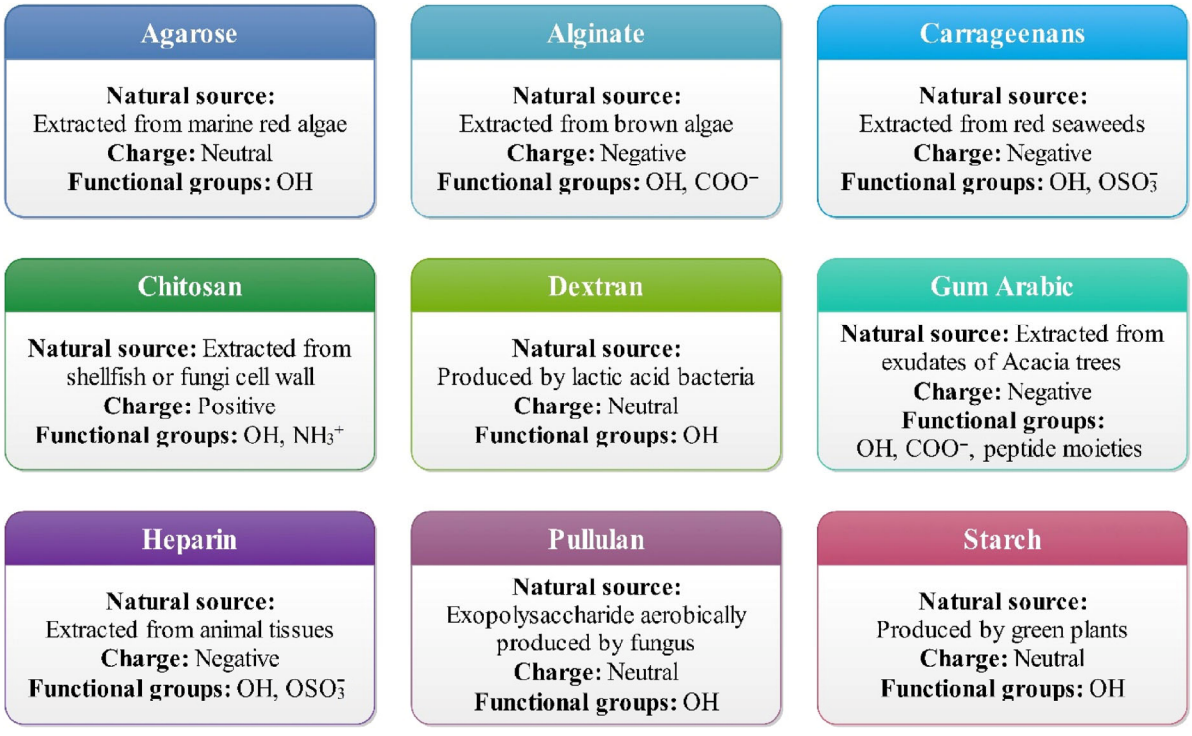

Figure 2 Properties of polysaccharides commonly used for MNPs coating or encapsulation.

Atomic transfer radical polymerization (ATRP) is another common method developed by Wang et al. [77] for coating SPIONs. Li et al. [78] used the ATRP method for coating iron oxide with polystyrene using divinyl benzene as a crosslinker. Several other polymers, e.g., PVA, poly(glycerol monoacrylate), poly(glycerol monomethyl acrylate), and tri-block copolymers, have also been used as coating materials [79, 80]. Inverse emulsion is another method for coating iron oxide NPs with PEG [73]. However, it should be noted that the use of nonmagnetic materials for coating SPIONs may result in a decrease in saturation magnetization [81]. Indeed, Voit et al. [82] found a similar effect when polymers (i.e., PVA and starch) were used as coating materials. Sen et al. [83] also reported a decrease in the saturation magnetization value from 90 to 15 emu·g^{-1} due to the presence of amorphous silica in a biphasic mixture of silica and magnetite NPs.

Due to the interaction of SPIONs with biological fluids, the formation of free hydroxyl radicals and reactive oxygen species can be significantly increased [83]. To protect the *in vivo* environment from these toxic byproducts, biocompatible and rigid coatings such as gold have been employed [84].

2.4 Applications

The magnetic properties of iron oxides have been exploited in a broad range of applications, including use in magnetic seals and inks, magnetic recording media, catalysts, and ferrofluids, as well as in contrast agents for MRI and therapeutic agents for cancer treatment [85]. These applications demand nanomaterials of specific sizes, shapes, surface characteristics, and magnetic properties [33].

Currently, the largest commercial application of iron NPs is in magnetic recording media [32]. Iron NPs are responsible for the very high capacity of many advanced magnetic tapes, such as those used in computer backup tapes and camcorders. Of course, the iron NPs used in these applications is very different from the superparamagnetic particles discussed earlier. These particles are larger and elongated. While iron is known to be a very soft material, these particles

behave as very hard, permanent magnets [86]. This is due to the shape anisotropy. Among different forms of iron oxides, maghemite is useful in recording and data storage applications because of its chemical and physical stability. It is often doped or coated with 1%–5% cobalt to improve its storage capacity [87].

Magnetite and hematite have been used as catalysts for a number of industrially important reactions, including the synthesis of NH_3 (the Haber process), the high temperature water-gas shift reaction, and the desulfurization of natural gas. Other reactions include the dehydrogenation of ethyl benzene to styrene, the Fisher–Tropsch synthesis for hydrocarbons, the oxidation of alcohols, and the large-scale manufacturing of butadiene [88].

All three forms of magnetic iron oxide are commonly used in synthetic pigments in paints, ceramics, and porcelain [89]. They display a range of colors with pure hues and high tinting strength that are also extremely stable and highly resistant to acids and alkalis. Pigments based on hematite are red, those based on maghemite are brown, and magnetite-based pigments are black [90]. These pigments are widely used in water-repellent stains for wood, magnetic ink character recognition devices, and superparamagnetic magnetite particles used in metallography for detecting flaws in engines [88].

The use of magnetite in ferrofluids was originally proposed for high-performance seals in space applications. Ferrofluids contain nanometer-sized superparamagnetic particles dispersed in aqueous or organic media. A ferrofluid has no net magnetic moment except when it is under the influence of an applied field. An external magnet is therefore able to trap the fluid in a specific location to act as a seal. Ferrofluids have interesting properties, such as magnetic field-dependent optical anisotropy, that could prove useful in optical switches and tunable diffraction gratings. They are currently employed in sealing computer disk units and in vibrating environments in place of conventional seals [87]. The use of MNPs has received considerable attention in bioscience for applications [91] in the development of immunoassays, MRI contrast agents, and targeted drug delivery vehicles, as well as in magnetic hyperthermia, which will be discussed in later sections.

3 Applications of IONPs in bioscience

3.1 Biotechnological applications

Research in biotechnology, environmental sciences, and green (environmentally friendly) chemistry has shown that the high magnetic response of IONPs under an external magnetic field allows them to act as efficient carriers in the magnetic separation of various biosubstances or environmentally important species (e.g., proteins, low molecular weight agents, ions, and inorganic or organic pollutants). IONPs can also be tailored to work as separable and renewable nanocarriers for catalysis [92] and used as magnetically drivable platforms for enzyme immobilization [93]. In this section, some of the biotechnological applications of IONPs will be discussed.

3.1.1 Food analysis

For food-related applications, the most important concern of MNPs relates to their safety or potential toxicity. Hence, the MNPs that are frequently utilized in food systems are iron oxides, among which superparamagnetic Fe_3O_4 NPs are the most prevalent materials, because they have no toxicity, good biocompatibility, and do not retain residual magnetism after the removal of an external magnetic field [5, 94].

IONPs are also of interest for food analysis because they exhibit unique properties, such as large specific surface area, high capacity for charge transfer, and easy separation from a complex mixture with an external magnetic field. The introduction of IONPs into conventional detection techniques can make them simple, rapid, highly selective and sensitive. IONPs can significantly enhance the electron transfer between the analytes and the electrode when using as an electrode modifier due to their very high capacity for charge transfer. Hence, the sensitivity of electrodes or electrochemical biosensors is often improved by modification with MNPs [95].

3.1.2 Protein/enzyme immobilization

Enzymes, such as carbohydrases, proteases, lipases, lysozymes, and oxidoreductases, are widely used in the food industry due to their excellent catalytic activity [96]. However, free enzymes usually have

poor stability, depending on pH, heat, or other factors, and are difficult to recover and reuse. Therefore, there is high demand to improve enzyme stability and reusability [94]. Enzyme immobilization, enzyme modification, protein engineering, and medium engineering are techniques that may improve enzyme stability and reusability. Of these, enzyme immobilization is the most frequently used method to improve enzyme features [97, 98].

IONPs have been used in protein/enzyme immobilization because they possess superparamagnetism, high surface area, large surface-to-volume ratio, and easy separation under external magnetic fields [99–102]. To take full advantage of NPs such as IONPs, the ideal regulation of the orientation of the proteins/enzymes on the supports must be determined. Compared to porous supports, such nonporous NPs have no external diffusion problems, making them more competitive, especially for large-scale industrial usage in solid–liquid systems (e.g., precipitated protein). However, unlike porous supports, proteins/enzymes immobilized on nonporous NPs may suffer inactivation for soluble proteins/enzymes, especially through interaction with gas bubbles generated by strong stirring or bubbling of oxygen [103]. Such inactivation by interfaces might proceed and finally result in the irreversible activity loss due to the sustained effects, such as destabilization of electrostatic, hydrophobic, and hydrogen bonds [104]. Superparamagnetic Fe_3O_4 NPs are the most frequently utilized iron oxides because they have low toxicity and good biocompatibility [105]. The bare iron MNPs often have high reactivity and easily undergo degradation upon direct exposure to certain environments, leading to poor stability and dispersity [106, 107]. Various modification methods have been developed to obtain soluble and biocompatible iron MNPs for protein immobilization [21].

3.1.3 *Protein purification*

Proteins are of interest for their nutrition and health benefits to humans. In biotechnology, affinity protein purification using antibody-based separation or a matrix with specific tags for binding target protein are commonly used methods [108]. The challenge is to use a common matrix for purification of different proteins. The commonly used techniques for protein purification are chromatography, precipitation, ultrafiltration, centrifugation, and dialysis; however, these have some limitations, such as the time-consuming pretreatments, expensive instrumentation, or need for a skilled operator [109, 110].

An emerging technique possesses several advantages compared with the traditional ones in the use of MNPs. It is time-saving, scalable, gentle, easily automated, and can be directly used to remove target compounds from crude samples [111]. The use of MNPs such as SPIONs for purification and immobilization of biomolecules, including proteins/peptides, have the following advantages: (1) ability to obtain high specific surface area to bind large amounts of protein samples; (2) selective targeting of biomolecules with desired surface characteristics and easy separation from the reaction mixture with a magnetic field; and (3) elimination of several steps in the purification process such as centrifugation and filtration. Using magnetic separation offers a gentle and fast alternative; targets are captured on the surface of the desired magnetic particle and a rapid separation is achieved from the sample by the use of an external magnetic field. With most conventional or commercially available protein purification/immobilization systems, the cost of protein purification on a large scale is still a major challenge [112].

A promising approach is the use of magnetic IONPs prepared from microemulsion (ME) for the purification or immobilization of proteins. This technique is based on microemulsion synthesis, which allows NPs to be tailor-made to the desired size [113, 114]. Microemulsions are optically transparent, thermodynamically stable solutions consisting of spherical aqueous nanodroplets, so-called reverse micelles, stabilized by surfactant molecules. In the aqueous core of the reverse micelle, an iron metal precursor can be solubilized and then precipitated to form IONPs [113]. Microemulsions consisting of magnetic iron oxide nanoparticles (ME-IONPs) are single domain magnetic dipoles that show no preferred directional ordering in the absence of an applied external magnetic field due to weak dipole–dipole interactions. Upon application of a sufficiently high magnetic field gradient, the NPs exhibit a preferential ordering in the direction of this external field [115].

3.1.4 *Water treatment*

Surface water and ground water are being used as a source for water treatment for human consumption. Potable water should be free from biological contaminants (bacteria, viruses, and parasites), trace minerals (e.g., boron, calcium, chloride, copper, molybdenum, potassium, zinc, magnesium, and manganese); and nutrients (phosphate, ammonia, and nitrate) [116]. The microbial population in freshwater is mostly dependent upon biomass, pH, nutrient composition, temperature, and water flow, which often leads to the distribution of bacterial taxa [117]. Therefore, the water treatment process is critical and varies depending on the quality of water that enters the treatment plant [118].

In surface water, most particles, including microbes, have a negative charge; hence, they repel each other rather than forming aggregates [119]. Aluminum or ferric ions are usually employed as coagulant agents in the water treatment process [120]. However, the presence of aluminum residues in treated water has health and environmental concerns, including neurotoxicity and possibly Alzheimer's disease [121].

Iron oxide nanomaterials have received much attention due to their unique properties; they are extremely small and have high surface-area-to-volume ratio, surface modifiability, excellent magnetic properties, and great biocompatibility [99]. The ability of iron oxide NMs to remove contaminants has been demonstrated in both laboratory and field scale tests [122, 123]. Current applications of iron oxide NMs in contaminated water treatment can be divided into two groups: (1) technologies that use iron oxide NMs as a kind of nanosorbent or immobilization carrier for enhancing the efficiency of removal (referred to here as adsorptive/immobilization technologies), and (2) those which use iron oxide NMs as photocatalysts to break down or to convert contaminants into a less toxic form (i.e., photocatalytic technologies). However, it should be noted that many technologies may utilize both processes [99]. In addition, some studies investigated the use of IONPs as an adsorbent for heavy metal removal from wastewater. It was found that the adsorption capacities of adsorbents rely largely on the available surface areas, and the increase of the surface area is normally obtained by the decrease of the particle size of adsorbents. As a result, there is a need to synthesize such absorbents with proper particle sizes for the removal of heavy metals from industrial wastewater via application of IONPs [124].

3.2 Bioengineering applications

3.2.1 *Biosensor*

A sensor is a device that measures a physical quantity and converts it into a signal that can be read by an observer or an instrument [125]. Biosensing is based on immobilized biomolecules for detection and determination of target analytes. In general, a biosensor is an analytical device with two components: a bioreceptor and a transducer. First, the bioreceptor recognizes the target analyte, and, second, the transducer converts the recognition event into a measurable signal. The substrate materials (e.g., electrodes, mesoporous materials, NPs, nanotubes, and graphene) for biomolecule immobilization must be modified to introduce functional groups that are attached to biomolecules with high bonding strength, excellent long-term stability, biocompatibility, and high activity [126].

The NPs could be immobilized on the surface of the transducers (e.g., physical adsorption, chemical-covalent bonding, or electrodeposition) for electrochemical-signal generation and amplification [127]. Iron-based MNPs provide a large surface area to immobilize as many biomolecules as possible, resulting in a lower limit of detection. Moreover, iron-based MNPs can play roles in concentration and purification. Iron-based MNPs are particularly efficient in detecting analytes in complex sample matrices, which may exhibit either poor mass transport to the biosensor or physical blockage of the biosensor surface by nonspecific adsorption [128]. Iron-based MNPs can remove the need for sample pretreatment by centrifugation or chromatography, thus shortening the handling time [129]. In addition, most iron-based MNPs, especially iron oxides, are biocompatible and nongenotoxic; they can either be applied for simple adsorption of biomolecules, or functionalized or encapsulated in polymers, metal or silica NPs, or carbon materials to enhance the biocompatibility and increase the functionalities [130]. Thus, iron-based MNPs provide a promising experimental platform for

developing both types of electrochemical biosensors [131].

3.2.2 *Tissue engineering*

Tissue engineering is a promising technology for overcoming the limitations of organ transplantation related to organ donor shortage. It consists of appropriately using cells, materials, and physics/biochemical processes to restore, maintain, or improve tissue function [132].

Tissue repair using IONPs is accomplished via either welding (apposing two tissue surfaces, then heating the tissues sufficiently to join them) or soldering (placing protein or synthetic polymer-coated NPs between two tissue surfaces to enhance joining of the tissues). Temperatures greater than 50 °C induce tissue union. This is believed to be induced by the denaturation of proteins and the subsequent entanglement of adjacent protein chains [133]. NPs that strongly absorb light corresponding to the output of a laser are also useful for tissue-repairing procedures. Specifically, gold or silica-coated IONPs have been designed to strongly absorb light. NPs are coated onto the surfaces of two pieces of tissue at the site where joining is desired. This technique affords methods to minimize tissue damage by using the least harmful wavelengths of light and/or lower powered light sources [134].

3.3 Biomedical applications

Nanomedical research has tremendous potential for application in modern biomedical research, disease diagnosis, and therapy. Magnetic materials are longstanding functional materials with very extensive applications. Notably, their size is compatible with cells (10–100 μm), viruses (20–450 nm), proteins (5–50 nm), and genes (2 nm wide by 10–100 nm long). They are small enough to move inside the body without disrupting normal functions and can access places that are inaccessible to other materials [135]. Cells react in the presence of nanomaterials, and these reactions can induce changes in cells, including cell growth or death [136].

Biocompatible SPIONs, such as magnetite, have been widely used for *in vivo* biomedical applications. These include MRI contrast enhancement [137], tissue specific release of therapeutic agents [138], hyperthermia [139], and magnetic field assisted radionuclide therapy [140].

The application of SPIONs in biology, medical diagnosis, and therapy require that they be stable in water at neutral pH and physiological salinity. Such colloidal stability depends on the dimensions of the particles, which should be sufficiently small so that precipitation due to gravitation forces can be avoided. Another important factor is the charge and surface chemistry, which give rise to both steric and Coulombic repulsions [141]. To control the surface properties of SPIONs, they are coated with a biocompatible polymer during or after the synthesis process, which prevents the formation of large aggregates, changes from the original structure, and biodegradation when exposed to the biological system. In addition, polymer coating can allow binding of drugs by covalent attachment, adsorption, or entrapment on the particles [142].

In the following sections, different biomedical applications of SPIONs are discussed.

3.3.1 *Targeted drug delivery*

Targeted delivery of drugs by NPs can overcome difficulties associated with conventional chemotherapeutic agents, including insolubility under aqueous conditions, rapid clearance, and a lack of selectivity, resulting in nonspecific toxicity toward normal cells [143, 144]. The key parameters in the behavior of MNPs are related to surface chemistry, size (magnetic core, hydrodynamic volume, and size distribution), and magnetic properties (magnetic moment, remanence, and coercivity) [145]. Magnetite conjugated with specific drugs has some limitations, e.g., difficult drug release control and low drug loading capacity. Therefore, for drug delivery applications, MNPs must be precoated with substances that assure their stability, biodegradability, and nontoxicity in the physiological medium to achieve combined properties of high magnetic saturation, biocompatibility, and interactive functions on the surface. The polymer coating not only leads to the creation of more hydrophilic nanostructures, but also provides a variety of surface functional groups to bind drug molecules, inhibit aggregation, and increase stability [146–149].

NPs, nanospheres, liposomes, and microspheres

are SPIONs that are regularly employed in drug delivery. In these systems, the drugs are bound to the surface of the SPIONs (especially in the case of NPs) or encapsulated in magnetic liposomes and microspheres [150]. The recent applications of SPIONs in diagnosis and therapy are presented in Fig. 3. More specifically, SPIONs-assisted drug delivery systems have been designed to deliver peptides, DNA molecules, and chemotherapeutic, radioactive, and hyperthermic drugs [151]. The most recent delivery systems are focused on drugs that are anti-infective, blood clot-dissolving, anti-inflammatory, anti-arthritic, and paralysis-inducing; these systems also focus on photodynamic therapy and stem cell differentiating/tracking [70].

The surface-engineered SPIONs (e.g., with targeting ligand/molecules attached to their surfaces) used together with the aid of an external magnetic field are recognized as a modern technology to introduce particles to the desired site where the drug is released locally. Such a system has the potential to minimize the side effects and the required dosage of the drugs. However, once the surface-derivatized NPs are inside the cells, the coating is likely digested, leaving the bare particles exposed to other cellular components and organelles, thereby potentially influencing the overall integrity of the cells [152].

Figure 3 Recent therapeutic applications of SPIONs.

Recently, efforts have been made to improve the distribution of anticancer drugs in the human body and decrease their toxic effect [153, 154]. Drug delivery systems can not only increase the concentration of drugs in the target area, but also decrease the damage of normal tissues simultaneously. In numerous drug delivery systems, magnetic targeting of drug delivery was considered to be the most efficient and popular system [27].

3.3.2 *Hyperthermia*

As a medical treatment, hyperthermia relies upon locally heating tissue to greater than 42 ℃ for approximately 30 min to destroy the tissue, particularly tumors. The difficulty of selectively heating diseased tissue makes it challenging to apply this technique therapeutically. The heating of magnetic particles has been investigated for decades as a possible approach to selectively heating cancerous tumors [155].

Under the alternating magnetic field, magnetic IONPs can absorb large amounts of magnetic energy by hysteresis loss to generate thermal energy. Cancer cells can be killed when the temperature exceeds 43 ℃ for 30 min, but normal cells can survive at relatively higher temperatures [156]. The heat generated by NPs under the alternating magnetic field was associated with the following factors [157]: (1) magnetic properties and particle size of NPs, (2) amplitude and frequency of *in vitro* magnetic field, and (3) cooling rate of blood in tumor vessels. Due to unique surface and small size effects (single domain effect) of magnetic IONPs with a size of about 10 nm, the energy absorption rate of these NPs under the alternating magnetic field was much higher than that of other materials, and the heating effect of these NPs was more significant [27]. Although the thermal effect of magnetic IONPs can be increased with enhancement of amplitude and frequency of magnetic field *in vitro*, Zeisberger et al. [158] found that the reasonable magnetic field parameters with which magnetic IONPs exerted hyperthermia effect were 400 kHz for frequency and 10 kA·m for amplitude.

3.3.3 *MRI*

MRI is a noninvasive powerful three-dimensional technique for the characterization and detection of human diseases [159]. This technique has several

advantages over others: It is non-ionizing and harmless to patients; has high patient acceptance; allows extreme imaging flexibility and obtainment of high-resolution images with an excellent soft tissue contrast between different tissues; and allows acquisition of unique clinical information [160, 161]. Most clinical MRI contrast agents can be classified into two major classes based on their magnetic properties. First, paramagnetic agents are composed of complexes of chelating agents and naturally occurring metal ions, with the complexes having unpaired electrons [160]. SPIONs such as Fe_3O_4 and Fe_2O_3 are widely used magnetic nanoparticle-based contrast agents due to their chemical stability, biodegradability, and low toxicity [162]. SPIONs primarily act to alter T_2 values of the water protons surrounding the particle. When SPIONs present in tissue are subjected to an external magnetic field, the large magnetic moments of the particles align to create large heterogeneous field gradients through which water protons diffuse. The dipolar coupling between the magnetic moments of water protons and the magnetic moments of particles cause efficient spin dephasing and T_2 relaxation, leading to a decrease in signal intensity. The contrast provided by SPIONs in a T_2-weighted image is termed negative contrast enhancement, since areas with high concentrations of SPIONs appear dark on MRI images. While SPIONs provide efficient shortening of T_2 and generate excellent contrast enhancement in tissue with longer T_2 characteristics, signal loss due to the presence of SPIONs can make it difficult to distinguish contrast enhancement in low signal body regions [160, 161, 163].

Zero-valent iron could be used in similar ways and would represent a much-improved magnetic contrast agent. In MRI scans, strong magnetic fields are used, and superparamagnetic particles would be expected to have their magnetization saturated. The fact that the saturation of metallic iron is roughly double that of its most strongly magnetic oxides offers a clear advantage. The disadvantage is again that metallic iron is not stable under biological conditions, but would require a coating to prevent oxidation and loss of magnetism [86].

Due to nonmonodispersal with irregular morphologies and nonuniform sizes of the commercial SPION-based contrast agents synthesized by the coprecipitation method, Ma et al. proposed a new strategy for exploring SPION-based MRI contrast agents with excellent water-dispersibility and high specificity to cancer cells. In this method, the SPION was synthesized by a polyol method, entrapped into albumin nanospheres, and conjugated by a ligand folic acid to construct a SPION-albumin-folic acid composite. The results showed that the fabricated composite had a spherical shape with uniform size and excellent water-dispersibility. Therefore, due to the excellent water-dispersibility, the high specificity to cancer cells, and the strong MRI imaging efficacy, the prepared SPIONs can be used as a negative MRI contrast agent (Fig. 4) [163].

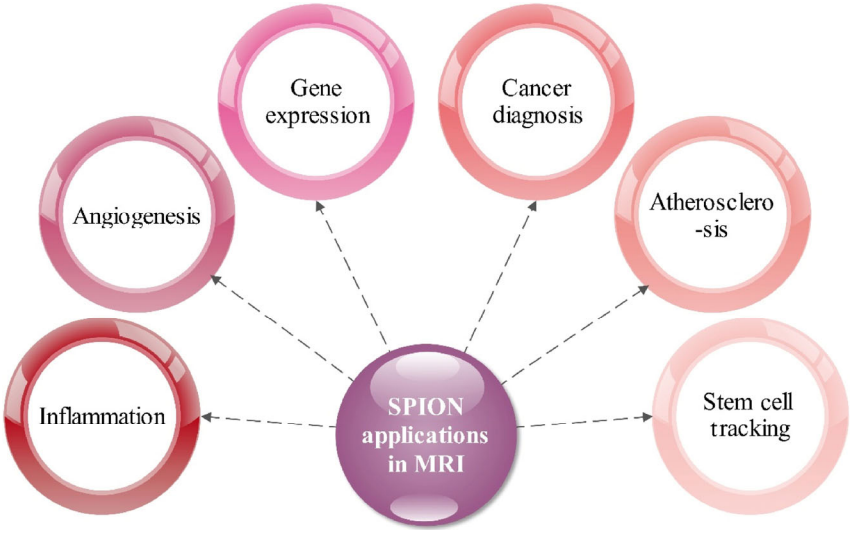

Figure 4 Recent diagnostic applications of SPIONs.

3.3.4 *Photothermal therapy*

As mentioned in section 3.3.2, hyperthermia is the local heating of cancerous cells that undergo irreversible damage due to the denaturation of proteins and the disruption of the cell membrane. Unfortunately, this method also damages the healthy tissues. Recently, incorporation of laser radiation treatment in thermal cancer therapy opened up a photothermal therapy method for the selective treatment of cancers. As a result, laser radiation with fiber-optic waveguides, which is called laser hyperthermia, finds growing applications in cancer therapy. In this procedure, a biocompatible photothermal therapy agent with a large absorption coefficient in the near infrared regions and a near infrared light source [164]. Thus, surface-modified nanomaterials of carbon, metals, and semiconductors with near infrared absorption can be ideal photothermal therapy agents. Noble metal nanostructures are promising candidates in various aspects of chemistry, physics, and biology owing to their unique properties, such as large optical field enhancements due to the strong scattering and absorption of light. The optical and photothermal therapy enhancements of metallic NPs arise from the unique interaction of NPs with light [165, 166]. When illuminated, the valance electrons of the metal NPs undergo a collective coherent oscillation with respect to the lattice. Within the past few years, an interest in magnetoplasmonic nanoparticle compounds has been developing. These are nanoshells with a magnetic core (Fe_2O_3, Fe_3O_4) surrounded by a thin layer of plasmonic metal (Ag or Au). NPs can also have a silicon layer sandwiched between the magnetic core and the metal shell. The plasmonic properties of the metal nanoshells in conjunction with the magnetic core properties, which respond to an external magnetic field, hold potential for guided cancer therapy through a synergistic effect, including the use of laser irradiation guided by MRI [165, 167, 168].

Iron oxide gold nanoshells have a magnetic core of maghemite (Fe_2O_3) or magnetite (Fe_3O_4) surrounded by a plasmonic gold layer. Larson et al. [169] studied this structure using imaging and other medical applications using particles with an average diameter of 45 nm and a peak absorption at 540 nm. Iron oxide silica gold (Fe_2O_3–Si–Au) nanoshells are three-layered structures that have a magnetic core, metallic outer shell, and silicon layer in between the two. The silica interface is inserted between the iron oxide and gold to allow an easier tuning of plasmonic resonance peaks to the NIR region. Melancon et al. [170] found a high absorbance peak at 650–900 nm for a particle with an average diameter of 90 nm. This study implies that changes in temperature with plasmonic magnetic nanoparticles that are guided through an MRI imaging can be mapped. Melancon and colleagues conveyed that *in vivo* MRI imaging provided enhanced darkening of the tumor through a comparison of a T_2 map before and after administration of SPION gold nanoshells. Furthermore, it showed a decrease in signal with the presence of the multifunctional MNPs.

4 Safety aspects and toxicity

Toxicology is the study of the potentially harmful effects of substances on living organisms, and nanotoxicology refers to the study of the potentially harmful effects of nanomaterials, in particular NPs [171]. Nanomaterials can enter the human body through dermal absorption, respiratory inhalation, or by oral route. Due to their ultrafine size, they are able to move across the olfactory mucosa, alveolar membrane, and capillary endothelium. The ability of nanomaterials to cross the blood-brain barrier enhances their toxicity in the nervous system [172]. There is an urgent need to understand the potential risks associated with IONPs, along with the range of surface coatings utilized for IONP functionality [173, 174].

However, superparamagnetic NPs have minimal toxicity in the human body. A study comparing several metal oxide NPs showed IONPs to be safe and noncytotoxic at concentrations below 100 $mg \cdot mL^{-1}$. However, intravenous administration can lead to accumulation in a targeted organ, potentially leading to iron overload, which can be toxic. High free iron levels can cause an imbalance in homeostasis, leading to DNA damage, oxidative stress, and inflammation (Fig. 5). Based on these reports, our opinion is that these particles can be used safely in humans if the concentrations are maintained below 100 $mg \cdot mL^{-1}$, and accumulation in organs is monitored to prevent

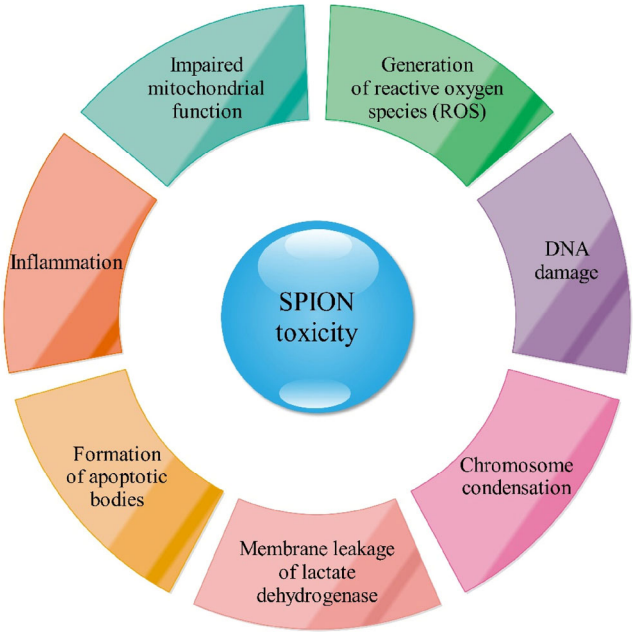

Figure 5 Cellular toxicity induced by SPIONs.

iron overload. Special attention should also be given to leaching of iron (Fe^{3+}) ions and interaction of these particles with H_2O_2, which could generate free radicals, such as hydroxy radicals, due to Fenton chemistry [9, 163, 175].

5 Commercialization consideration

Currently, a variety of MNPs are in early clinical trials, and some formulations have been clinically approved for medical imaging and therapeutic applications. Table 2 lists some of the commercialized SPIONs that are used for different diagnostic and therapeutic applications. These include Lumiren and Gastromark for bowel imaging and Feridex and Endorem for liver and spleen imaging. IONPs were highly used owing to their superparamagnetic effects and acceptable biocompatibility. NanoTherm, produced by Magforce AG, is a liquid of iron oxide that reacts to the presence of a magnetic field. Ferumoxytol has potential for use as a contrast agent in imaging studies of the lymph system, especially involving lymph nodes that have been affected by cancer. Ferumoxytol is taken up by normal lymph nodes, but excluded from cancerous lymph node tissue [176–178].

To enhance transfection efficiency, the commercialized magnetic nanoparticle CombiMag (OZ Biosciences) has been used as an additive for gene transfection. After mixing transfection reagents with therapeutic genes, CombiMag is added to the mixture, which results in 3-fold higher gene transfection in A549 cells

Table 2 Commercialized SPIONs

Brand name	Surface modification	Application
AdenoMAG	Variable polymers	Virus delivery
Combidex	Dextran	Lymph node metastases imaging
CombiMAG	Variable polymers	Gene transfection
Endorem	Dextran	Detection of liver lesions
Feraheme	Polyglucose sorbitol carboxymethylether	Treatment of chronic kidney disease
Feridex	Dextran	Liver and spleen imaging agent
Ferumoxytol	Polyelectrolytes	Contrast agents for MRI tracking, iron replacement therapy
FluidMAG	Chitosan, starch, PVA	Cell labeling
LipoMAG	Lipid	Gene delivery
Lumiren	Silicon	Bowel imaging
PolyMAG	Polyethyleneimine	Plasmid DNA delivery
Resovist	Carboxydextran	Liver imaging agent
Sinerem	Dextran	Lymph node imaging

(human lung carcinoma cells) than conventional liposomal transfection using lipofectamine. The PolyMag™ particles (100–200 nm, OZ Biosciences, France) showed plasmid DNA delivery to NCI-H292 human lung epithelial cells to be 4-fold greater than the commercial lipid-based carrier, lipofectamine. In addition, LipoMag™ particles and AdenoMag have been developed for gene delivery and virus delivery, respectively. The diverse fluidMAG particles (Chemicell) are MNPs coated with various polymers, including chitosan, starch, PVA, and lipids. The particle sizes are in the range of 50–200 nm, which can be taken up by cells via endocytosis. Custom-made magnetic particles coated with heparin and PEI by Chemicell have been investigated [9, 175, 179, 180].

6 Concluding remarks and future trends

Substantial progress has been made in the application of MNPs in nanotechnology and biotechnology. Compared with micron-grade particles, NPs have strong mobility due to their small size. As the size of the NPs decreases, the surface-to-volume ratio (and consequently the fraction of the surface atoms with respect to the bulk ones) increases. The large surface-to-volume ratio of the NPs is the key factor that provides the novel physical, chemical, and mechanical properties not found in the corresponding bulk material. Methods that offer control over the size, size distribution, shape, crystal structure, defect distribution, surface structure, and magnetic properties of NPs have been developed. Magnetic iron oxides are suitable for a range of applications, such as MRI contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, drug delivery, hyperthermia, and cell separation. The major challenge in the application of SPIONs is to design effective surface coatings that provide optimum performance for *in vitro* and *in vivo* biological applications. Additionally, development of large-scale production and confirmation that the surface treatment of these particles is safe and nontoxic are necessary for the effective use of SPIONs. However, the application of SPIONs, particularly in biotechnology, is always uncertain due to our lack of knowledge on the toxicity of iron oxide particles, particularly those in their nanometric form. Nevertheless, many companies have great interest in the use of SPIONs in synthesized drugs for different *in vivo* and *in vitro* applications.

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