Bio and Nanomaterials Based on Fe₃O₄

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External Editor: Alexandru Mihai Grumezescu

Received: 18 November 2014; in revised form: 16 December 2014 / Accepted: 17 December 2014 / Published: 22 December 2014

Abstract: During the past few years, nanoparticles have been used for various applications including, but not limited to, protein immobilization, bioseparation, environmental treatment, biomedical and bioengineering usage, and food analysis. Among all types of nanoparticles, superparamagnetic iron oxide nanoparticles, especially Fe₃O₄, have attracted a great deal of attention due to their unique magnetic properties and the ability of being easily chemical modified for improved biocompatibility, dispersibility. This review covers recent advances in the fabrication of functional materials based on Fe₃O₄ nanoparticles together with their possibilities and limitations for application in different fields.

Keywords: Fe₃O₄; surface modification; application

1. Introduction

Owing to the unique properties, such as superparamagnetism, high surface area, large surface-to-volume ratio, low toxicity, easy separation under external magnetic fields, Fe₃O₄ nanoparticles have enormous potential in the fields such as immobilization of biomaterials [1–10], bioseparation [11–15], environmental treatment [16–23], biomedical and bioengineering usage [24–36], and food analysis [37–44]. Various fabrication methods have been developed for the synthesis of Fe₃O₄ nanoparticles, including the
physical methods [45–47], wet chemical preparation methods [48–66] and microbial methods [67–69]. Since the bare Fe$_3$O$_4$ nanoparticles often have poor stability and dispersity, various modification methods have been exploited to get the soluble and biocompatible Fe$_3$O$_4$ nanoparticles. The resulting modified Fe$_3$O$_4$ nanoparticles have been extensively used for various applications. In this review, the traditional and modern methods for synthesis of Fe$_3$O$_4$ nanoparticles are summarized; the methods for modification of Fe$_3$O$_4$ nanoparticles are also described. Finally, a variety of practical and potential applications as well as the corresponding limitations of the resulting Fe$_3$O$_4$ nanoparticles are introduced.

2. Methods for Preparation of Fe$_3$O$_4$ Nanoparticles

The outstanding potential of Fe$_3$O$_4$ nanoparticles has stimulated the extensive development of the synthetic technologies, which could be broadly classified into three categories: physical, chemical and biological methods. (i) Physical methods, such as electron beam lithography [45], gas-phase deposition [46], and mechanical techniques [47]. Externally controlled tools like traditional workshop or microfabrication equipment are often involved in physical methods, where are used to process materials into the desired shape and order. Although physical methods are easy to perform, it is rather difficult for them to control the particle size. (ii) Wet chemical preparation methods, such as sol-gel synthesis [48,49], oxidation method [50,51], reduction method [52], chemical coprecipitation [53,54], hydrothermal reactions [55,56], solvothermal method [57], thermal decomposition method [58], flow injection synthesis [59], electrochemical method [60,61], aerosol/vapor phase method [62], sonochemical decomposition reactions [63,64], supercritical fluid method [65,66], synthesis using nonreactors [67]. In the case of wet chemical preparation methods, relatively less energy was consumed compared with that of physical methods. Among wet chemical preparation methods, coprecipitation of Fe$^{3+}$ and Fe$^{2+}$ salts is a most often employed method to prepare water-borne iron oxide nanoparticles. The size and morphology of the nanoparticles can be controlled by selectively choosing the reaction media, the physical parameters of the reaction, such as precursors, reactant concentration, base (NaOH, ammonium hydroxide, and CH$_3$NH$_3$), ionic strength (N(CH$_3$)$_4$+, CH$_3$NH$_3$+, NH$_4$+, Na$^+$, Li$^+$ and K$^+$), reaction temperature, pH of the media, and also some other factors [68]. For instance, an increase of the mixing rate tends to decrease the particle size. Moreover, inlet of nitrogen into the reaction system that protects against critical oxidation of the magnetite also reduces the particle size when compared to methods without oxygen removal. However, coprecipitation protocol leads to reduced control of particle shape, broad distributions of sizes and aggregation of particles. In general, the size distribution of nanoparticles is an important factor to be considered for a particular application. Some of wet chemical methods can yield efficient control of the particle size by carefully adjusting the involved parameters, including sol-gel method, hydrothermal method, flow injection method, electrochemical method, sonochemical decomposition method, supercritical fluid method and synthesis using nanoreactors. (iii) Microbial method. Microbial method is an environment friendly nanoparticle formation processes which can produce 5–90 nm pure magnetite or metal-substituted magnetite without usage of toxic chemicals in their synthesis process [69–72]. Microbial method represents an advantageous manufacturing technology with respect to high yield, good reproducibility, and good scalability, as well as low costs and low energy input, but the fermentation process is rather time-consuming. Table 1 shows the summary of various methods for preparing Fe$_3$O$_4$ nanoparticles.
### Table 1. Comparison between methods for synthesis of magnetic nanoparticles [46,47,73].

| Methods                      | Advantages                                      | Disadvantages                                      |
|------------------------------|-------------------------------------------------|---------------------------------------------------|
| **Physical methods**         |                                                 |                                                   |
| Electron beam lithography    | well controlled inter-particle spacing          | expensive and highly complex machines requiring   |
| Gas-phase deposition         | easy to perform                                 | difficult to control the particle size             |
| Mechanical techniques        | no chemicals involved                           | highly complex machines requiring and time-consuming |
| Sol-gel synthesis            | precisely controlled in size, aspect ratio, and internal structure | weak bonding, low wear-resistance, high permeability |
| Oxidation method             | uniform size and narrow size distribution       | small-sized ferrite colloids                      |
| Reduction method             | simple                                          | high reaction temperature                         |
| Chemical coprecipitation     | simple and efficient                            | not suitable for the preparation of high pure, accurate stoichiometric phase |
| Hydrothermal reactions       | easy to control particle size and shapes        | high reaction temperature, high pressure          |
| Solvothermal method          | easy to control particle size and shape         | high reaction temperature                         |
| Thermal decomposition method | easy to control particle size and shape          | involve multiple steps                            |
| Flow injection synthesis     | good reproducibility and high mixing homogeneity together with a precise control of the process | need continuous or segmented mixing of reagents under a laminar flow regime in a capillary reactor |
| Electrochemical method       | easy to control particle size                   | bad reproducibility                               |
| Aerosol/vapor phase method   | high yields                                     | extremely high temperatures                       |
| Sonochemical decomposition reactions | narrow particle size distribution            | mechanism not still understood                    |
| Supercritical fluid method   | efficient control of the particle size, no organic solvents involved | critical pressure and temperature                  |
| Synthesis using nanoreactors | precisely control the particle size             | complex condition                                 |
| **Wet chemical preparation methods** |                                                 |                                                   |
| Microbial methods            | Microbial incubation                            | environmental friendly, high yield, good reproducibility, and good scalability, low cost |

3. Modification of Fe₃O₄ Magnetic Nanoparticles

Because of the high surface energy, the naked Fe₃O₄ nanoparticles are generally unstable and aggregate easily, which strongly affects their dispersion into aqueous medium. In addition, Fe₃O₄ nanoparticles are highly susceptible to be oxidized to γ-Fe₂O₃ nanoparticles in the presence of oxygen [74]. To overcome such limitations, various surface modification methods have been developed to modify the surface of naked Fe₃O₄ nanoparticles via loading of other chemicals or biological materials during or after the synthesis process to improve the dispersibility, stability, biocompatibility and biodegradability for specific purposes [75–94]. With proper surface modification, the stability, dispersity and
biocompatibility of Fe₃O₄ nanoparticles could be improved, and the oxidation process from Fe₃O₄ nanoparticles to γ-Fe₂O₃ nanoparticles could be greatly slowed down.

The common reagents employed for modification of Fe₃O₄ nanoparticles includes surfactants (such as oleic acid (OA) [35,75], lauric acid [76], alkane sulfonic acids [77], and alkane phosphonic acids) [78], polymers (such as polyethylene glycol (PEG) [79], polyvinylpyrrolidone (PVP) [80], poly(ethylene-co-vinyl acetate) [81], polylactic-co-glycolic acid (PLGA) [82], polyvinyl alcohol (PVA) [83], polystyrene [84], polyethyleneimine (PEI), and poly(acrylic acid) (PAA) [85]) and natural dispersants (chitosan [86,87], dextran [88], gelatin [89], polylactic acids [90], starch [91], albumin [92], liposomes [93], and ethyl cellulose [94]). The methods of modification of Fe₃O₄ nanoparticles mainly include physical immobilization, covalent conjugation, and biologically mediated specific interaction. The advantages and disadvantages of these three immobilization methods are summarized in Table 2.

**Table 2. Comparison between different immobilization methods.**

| Methods                        | Interactions                        | Advantages                                      | Disadvantages                                      |
|--------------------------------|-------------------------------------|-------------------------------------------------|---------------------------------------------------|
| Physical immobilization        | physical absorption, electrostatic interaction, hydrogen bonds, van der Waals forces, and hydrophobic interactions | easy to perform and recycle, no additional coupling reagents and surface treatment are required | nonspecificity, the binding stability is highly affected by environmental conditions |
| Covalent conjugation           | covalent interaction                | the binding process can be rationally regulated with specific functional groups | nonspecificity, the support can’t be recycled |
| Biologically mediated specific interaction | biologically mediated specific interaction | site-specific | site-selective attachment is desired |

Jadhav et al. prepared oleic acid (OA) functionalized Fe₃O₄ nanoparticles using modified wet method, and sodium carbonate was used to improve the biological applicability (Scheme 1) [75]. In another example, Yang et al. synthesized PEG-coated Fe₃O₄ nanoparticles via traditional chemical coprecipitation method, the influence of vapor pressure, molecular weights and amounts of PEG on the structural and paramagnetic properties of PEG-Fe₃O₄ NPs were investigated [79]. Fe₃O₄ nanoparticles synthesized in sealed environment (S-Fe₃O₄) displayed much high crystalline quality than that synthesized in open environment (O-Fe₃O₄). The calculated average crystalline size of S-Fe₃O₄ and O-Fe₃O₄ is 15.2 nm and 14.5 nm, respectively. Both of the S-Fe₃O₄ and O-Fe₃O₄ nanoparticles showed superparamagnetic properties, and the saturation magnetization for S-Fe₃O₄ and O-Fe₃O₄ nanoparticles is 44 emu/g and 24 emu/g, respectively. The well-dispersed magnetic PEG-Fe₃O₄ nanoparticles with better size distribution can be obtained with adding 4 g PEG1000 while sealing the beaker. There were no significant size change caused by the PEG coating. However, the saturation magnetization of PEG-Fe₃O₄ nanoparticles showed an apparent decrease compared to that of bulk material (92 emu/g), which could be attributed to the surface disorder or spin canting at the surface of nanoparticles. Qu et al. prepared Fe₃O₄–chitosan nanoparticles with core-shell structure [87]. Oleic acid (OA) modified Fe₃O₄ nanoparticles (MN) were firstly prepared by coprecipitation, chitosan was then added to coat on the surface of the Fe₃O₄ nanoparticles by physical absorption, and glutaraldehyde was used to crosslink the amino groups on the chitosan. The saturation magnetization of the Fe₃O₄–chitosan nanoparticles (30.7 emu/g)
was lower than the pristine Fe₃O₄ nanoparticles (74.3 emu/g), which could be partly attributed to the existence of the large amount of diamagnetic chitosan in the Fe₃O₄–chitosan nanoparticles.

**Scheme 1.** Schematic representation for interaction of oleic acid (OA) modified Fe₃O₄ nanoparticles with sodium carbonate. OA is chemically bound to Fe₃O₄ nanoparticles by the carboxyl head group (-COOH) and the hydrophobic tail group is free, making it non-dispersible in aqueous medium. The hydrophobic tail in turn interacts with the free OA via hydrophobic interactions. The formulation is stabilized in aqueous medium by ionization of the carboxyl head group of free OA by sodium carbonate, wherein Na⁺ interact by ionic interactions with COO⁻ group of free OA [75].

### 4. Applications of Fe₃O₄ Nanoparticles

Due to the unique properties, Fe₃O₄ nanoparticles appear to be very promising for their applications in protein immobilization, bioseparation, environments treatment, biomedical and bioengineering usage, and food analysis.

#### 4.1. Protein Immobilization

Protein immobilization serves as a very effective tool to solve the difficulties encountered in the catalytic application of free enzymes, such as poor stability and hard recovery. It is of vital importance to select proper immobilization basis for protein immobilization. Fe₃O₄ nanoparticles have been intensively utilized to realize this objective due to its unique magnetic performance, and various practical and economical biocatalysts with improved stability and reusability have been fabricated based on Fe₃O₄ nanoparticles, which could be easily separated from the reaction medium in the presence of external magnetic field [1–10,95–114]. Proteins could be immobilized onto Fe₃O₄ nanoparticles in the manner of physical absorption [95–97], covalent bonding [98–104], and bioconjagation [105–107]. Coupling reagents, such as glutaraldehyde [99–102,108–110], 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) [103,104,111–113] and sodium tripolyphosphate (TPP) [114], are often utilized to achieve much more stable immobilization via covalent bonding because their functional groups can interact with both functional groups of the modified magnetic nanoparticles and proteins. For example, Huang et al. covalently bound glucose oxidase to Fe₃O₄/silicon dioxide nanoparticles using glutaraldehyde, resulting in an activity of immobilized glucose oxidase of 4570 U/g at pH 7 and 50 °C [102]. The immobilized glucose oxidase retained 80% of its initial activity after 6 h at 45 °C compared to only 20% for the free enzyme. After six cycles of repeated use, the immobilized glucose oxidase still
maintained 60% of its initial activity; 75% of its initial activity remained after 1 month at 4 °C compared to 62% for the free enzyme. Hong et al. obtained amine-functionalized magnetic nanogel by Hoffman degradation of the polyacrylamide (PAM)-coated Fe₃O₄ nanoparticles. α-Chymotrypsin (CT) covalently bound to the magnetic nanogel with reactive amino groups by using EDC as coupling reagent [104]. The binding capacity was determined to be 61 mg enzyme/g nanogel by BCA protein assay. Specific activity of the immobilized CT was measured to be 0.93 U/(mg min), 59.3% as that of free CT. The immobilized CT still had a remaining activity of 60% when the reaction temperature rose to 60 °C while free CT lost all-initial activity. Wu et al. prepared magnetic Fe₃O₄-chitosan nanoparticles by cross-linking with TPP, precipitation with NaOH and oxidation with O₂ in hydrochloric acid aqueous phase containing chitosan and Fe(OH)₂ [114]. The adsorption capacity of the prepared Fe₃O₄-chitosan nanoparticles to lipase was 129 mg/g; and the maximal enzyme activity was 20.02 μmol·min⁻¹·mg⁻¹ (protein), and 55.6% activity was retained at a certain loading amount.

4.2. Bioseparation

Magnetic separation is a commonly used technique for polypeptide/protein separation and cell separation. Magnetic separation possesses several advantages such as timesaving, gentle, easily automated, and can be directly used to remove target compounds from crude medium by the simple application of an external magnetic field. To construct Fe₃O₄ based composite nanomaterials for separation, core/shell microspheres are generally fabricated with a Fe₃O₄ as a core and other functional materials as a shell [115–123]. Ma et al. synthesized the Fe₃O₄@mTiO₂ microspheres with a well-defined core/shell structure, the high specific surface area (167.1 m²/g), large pore volume (0.45 cm³/g), appropriate and tunable pore size (8.6–16.4 nm), and high magnetic susceptibility [123]. The composite could selectively enrich phosphopeptides from complex mixtures even at a very low molar ratio of phosphopeptides/non-phosphopeptides (1:1000), large enrichment capacity (as high as 225 mg/g, over 10 times as that of the Fe₃O₄@TiO₂ microspheres), extreme sensitivity (the detection limit was at the fmol level), excellent speed (the enrichment can be completed in less than 5 min), and high recovery of phosphopeptides (as high as 93%).

To realize more effective separation of protein, affinity chromatography utilizing Fe₃O₄ based composites as packing materials is usually taken into account [124]. Several functionalized Fe₃O₄ nanoparticles have been strategically developed for the purification of specific proteins utilizing the affinity interactions. The target protein is firstly captured by modified Fe₃O₄ nanoparticles from crude samples to form a complex, and the captured target protein on the particles is subsequently eluted from metal ion chelated magnetic nanoparticles by applying buffer solution with different pH or ion strength (Scheme 2). These metal ion chelated magnetic nanoparticles were usually regenerated by using EDTA to strip the adsorbed protein and metal ion and then reloading with metal ion for recycling.
4.3. Environmental Treatment

As a result of rapid industrialization and urbanization, various pollutants particularly those entering aquatic systems have attracted worldwide concern. The development of efficient and cost-effective methods for environmental treatment is of primary concern for sustainable economic and social development. Due to the extremely small particle size, high surface-area-to-volume ratio, and more important the magnetism, Fe₃O₄ nanoparticles have been widely used and have shown promising performance in environments treatment, including pollutant removal and toxicity mitigation [16–23,125–129]. Proper surface coating cannot only improve the removal capacity and affinity of the Fe₃O₄ nanoparticles, but also promote the desorption process. Pollutants generally adsorb to the surface of Fe₃O₄ nanoparticles through interactions including physical adsorption, ion-exchange, chemical bonding (complexation and/or chelation), hydrogen bonds, and van der Wall forces. Fe₃O₄/ZrO₂/chitosan composite was synthesized and employed for the removal of amaranth and tartrazine dyes removal, the adsorption capacities of which were 99.6 and 47.3 mg/g for amaranth and tartrazine dyes, respectively [128]. In another report, Hakami et al. prepared Fe₃O₄ nanoparticles functionalized with thiol groups by adding (3-mercaptopropyl) trimethoxysilane on silica-coating to remove Hg, and the sulfur atoms in thiol moieties served as ligands to bind with soft metal cation Hg⁺. Thiourea was added to facilitate desorption of Hg because of the presence of sulfur atoms (Scheme 3) [129].

Scheme 2. Illustration of the separation mechanism of affinity chromatography utilizing Fe₃O₄ based composite as packing material.

Scheme 3. Illustration of the moval and recovery of Hg(II) using thiol-functionalized mesoporous silica-coated magnetite nanoparticles [129].
In the real engineering applications, the strategically utilization of Fe_{3}O_{4} nanoparticles should consider the complex environmental conditions such as background ions, humic substances, temperature, and pH.

4.4. Biomedical Usage

Fe_{3}O_{4} nanoparticles with appropriate surface properties have been widely used for numerous biomedical and bioengineering applications such as targeted drug delivery, biosensor, magnetic resonance imaging, hyperthermia, tissue engineering, magnetofection, etc. All these applications require that these nanoparticles not only possess high magnetization values, but also with narrow particle size distribution and similar surface topography, so that the particles have uniform physical and chemical properties. Moreover, the magnetic nanoparticles for biomedical applications should be non-toxic and biocompatible. In a word, both of the nature and the geometric arrangement of surface coatings on the nanoparticles have apparent influence on bio-kinetics and bio-distribution of nanoparticles in the body.

4.4.1. Targeted Drug Delivery

Due to the unique capabilities (e.g., superparamagnetism and biocompatibility) and the negligible side effects, magnetic Fe_{3}O_{4} nanoparticles with proper surface modification and conjugated targeting ligands/molecules have become a major research focus for drug delivery applications. Compare to the conventional, non-targeted methods of drug delivery, magnetic nanoparticles are promising drug carriers due to the better specificity to the target site and the reduced adverse effects. Drug carried by magnetic nanoparticles could be concentrated at the desired site to receive much high therapeutic efficiency. Since the drugs simply physically attached to the nanoparticle surface tend to release quickly before reaching the final destination, a core-shell structure consisting of a magnetic core and a shell is preferred in magnetic drug-delivery systems to achieve sufficient drug loading capacity and good transportation effect [130–138]. For example, Chen et al. prepared Fe_{3}O_{4}@SiO_{2} core–shell nanoparticles and grafted a widely used anticancer agent doxorubicin (DOX) to the surface of the core–shell nanoparticles via an amide bond with the aid of a spacer arm. Most of the conjugated DOX can release from the nanoparticles within 12 h and the release process prefers low pH conditions. The saturation magnetization value of the obtained superparamagnetic DOX-grafted Fe_{3}O_{4}@SiO_{2} core-shell structure nanoparticles was 49.3 emu·g^{-1}, indicating its great potential application in the treatment of cancer using magnetic targeting drug-delivery technology [136].

4.4.2. Biosensor

Fe_{3}O_{4} nanoparticles based bioanalytical sensors could be fabricated by coating Fe_{3}O_{4} nanoparticles with materials such as a fluorescent one [139,140], a metal [141,142], silica [143,144], or a polymer [145,146]. Tang et al. developed a practical glucose biosensor by combining the intrinsic peroxidase-like activity of Fe_{3}O_{4} nanoparticles and the anti-interference ability of the nafion film. Glucose oxidase was simply mixed with Fe_{3}O_{4} nanoparticles and cross-linked on the Pt electrode with chitosan medium by glutaraldehyde, and then covered with a thin nafion film. The biosensor showed high sensitivity (11.54 μAcm^{-2}.mM^{-1}), low detection limit (6 × 10^{-6} M), and good storage stability [147]. Liu et al. developed a reusable, single-step system for the detection of specific substrates
using oxidase-functionalized Fe₃O₄ nanoparticles as a bienzyme system and using amplex ultrared (AU) as a fluorogenic substrate. A composite of poly (diallyldimethylammonium chloride)-modified Fe₃O₄ nanoparticles and oxidase was prepared for the quantification of specific substrates through the H₂O₂-mediated oxidation of AU. The reaction process was monitored by checking fluorescence intensity at 587 nm, and the minimum detectable concentrations of glucose, galactose, and choline were found to be 3, 2, and 20 μM utilizing glucose oxidase-Fe₃O₄, galactose oxidase-Fe₃O₄, and choline oxidase-Fe₃O₄ composites, respectively (Scheme 4) [148].

Scheme 4. Illustration of Fe₃O₄ nanoparticles play a peroxidase-like role to detect the specific substrate in the presence of AU [148].

4.4.3. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a commonly used non-invasive medical imaging technique in clinical medicine to visualize the structure and function of tissues, which is based on the behavior, alignment and interaction of protons in the presence of an applied magnetic field [149–153]. For example, Fan et al. present a relatively simple and scalable approach for preparing poly(ethylene glycol) monomethacrylate) (P(PEGMA))-grafted Fe₃O₄ core–shell nanoparticles with well-controlled properties using a solvent-free ATRP [153]. The so-obtained P(PEGMA)-grafted nanoparticles have a uniform hydrodynamic particle size of 36.0 ± 1.2 nm. The morphology and viability of the macrophage cells cultured in a medium containing 0.2 mg/mL of P(PEGMA)-grafted nanoparticles were found similar to those of cells cultured without nanoparticles, indicating an absence of significant cytotoxicity effects. T2-weighted magnetic resonance imaging (MRI) of P(PEGMA)-grafted MNPs showed that the magnetic resonance signal is enhanced significantly with increasing nanoparticle concentration in water. The $R₁$ and $R₂$ values (longitudinal and transverse relaxivities) per millimole Fe, and $R₂/R₁$ value of the P(PEGMA)-grafted MNPs were calculated to be 8.8 mM⁻¹·s⁻¹, 140 mM⁻¹·s⁻¹, and 16, respectively. These results indicate that the P(PEGMA)-grafted nanoparticles have great potential for application in MRI of specific biotargets.

4.4.4. Hyperthermia

Magnetic induction hyperthermia means the exposition of cancer tissues to an alternating magnetic field, in which heat is generated due to magnetic hysteresis loss. Cancer cells exposed to magnetic
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particles will heat up to a temperature higher than 43 °C, at which the cancer cells are destroyed whereas the normal cells can survive. Much research work using magnetic particles for hyperthermia have manifested a therapeutic effect on several types of tumors [30–33,154–156]. For example, Ghosh et al. synthesized Fe₃O₄ magnetic nanoparticles (MN) capped with either oleic acid (Fe₃O₄-OA-MN) or polyethylene glycol (Fe₃O₄-PEG-MN), which were prepared by a co-precipitation method. The average particle sizes of the obtained Fe₃O₄-MN, Fe₃O₄-OA-MN and Fe₃O₄-PEG-MN were found to be 12, 6 and 8 nm, respectively. A 35% increase of killing effect was observed in human breast cancer cells (MCF7) after Fe₃O₄-OA-MN treatment, which was further enhanced (65%) in the presence of induction heating. However, only 5%–10% killing was achieved while Fe₃O₄-MN or Fe₃O₄-PEG-MN was used to treat MCF7 cells after induction heating. The effect of only OA (0.088 mg·mL⁻¹, a concentration low than that in Fe₃O₄-OA-MN) or PEG (0.1 mg·mL⁻¹) with/without induction heating on cell viability experiments indicated that loss of viability by OA was ~75%, which was higher than 1 mg of Fe₃O₄-OA-MN (35%) alone. However, PEG at this concentration did not show any significant change in cell toxicity. The same control experiments conducted under induction heating showed insignificant change in cell viability. These results displayed the surface characteristics of the modified magnetic nanoparticles (e.g., lipophilicity) greatly influence their hyperthermia applications in cancer therapy [156].

4.4.5. Tissue Engineering

Tissue engineering is a promising technology for overcoming the organ transplantation crisis, and the fabricated tissue equivalents may also be used to screen the effects of drugs and toxins [157,158]. It has been a great challenge for scientists and medical experts to fabricate functional organs of the similar architectures in vitro to the in vivo organs, in which the cells are allocated precisely. To realize this objective, three-dimensional constructs (scaffolds or hydrogels) functioning similarly as under in vivo conditions should be firstly built up [159–161]. The cells generally isolated from a tissue biopsy, cultured in vitro, subsequently seeded into the three dimensional constructs. To achieve an efficient cell seeding and to enable controlled tissue assembly and complex tissue formation, magnetic force-based tissue engineering technique is required to provide magneto-responsive features to the cells [162–164]. The inclusion of magnetic particles has no significant effect on the porosity, stability and wetting properties of the composite scaffolds, making them appropriate for cellular support and cultivation. For instance, Sapir et al. created a stimulating microenvironment by inserting magnetically responsive Fe₃O₄ nanoparticles into a macroporous alginate scaffold, which was suitable for promoting endothelial cell organization into capillary-like structures in vitro [165].

4.4.6. Magnetofection

Magnetofection rely on the delivery of nucleic acids (e.g., DNA, antisense oligodeoxynucleotides (AODN), and small interfering ribonucleic acids (siRNA) into the targeted cells in presence of a magnetic field [46]. The delivery of nucleic acids using viral vectors is called transduction, whereas the delivery using nonviral vectors is termed transfection. The negatively charged nucleic acids generally interact with MNPs chemically modified by cationic substances such as PEI or protamine sulfate polymers [166], which could contribute to the intracellular penetration. The application of an external Fe₃O₄ magnetic
field directs viral or non-viral gene delivery vectors facilitates fast and efficient nucleic acid delivery towards the target cells [167].

Although substantial progress has been made with creating proper delivery systems for nucleic acids, our knowledge of the internal operation mechanism inside cells is still unclear, and target delivery of nucleic acids still has not lived up to its potential clinical application. The processes governing nucleic acid uptake and delivery are far from being clarified, as well as their intracellular interactions, intracellular trafficking and the regulation of nucleic acid action inside cells [46].

4.5. Food Analysis

It is of vital importance to accurately analyze food components and food contaminants for ensuring food safety and quality. Although the frequently employed techniques (e.g., gas chromatography (GC), culture and colony counting, immunoassay, high-performance liquid chromatography (HPLC), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)) are of very great usage for food analysis, most of them are laborious, complex, time-consuming, expensive, and show somewhat dissatisfying specificity and detectability to some special targets. Magnetic nanoparticles such as Fe3O4 are of special interest for food analysis not only because the unique properties such as low toxicity, good biocompatibility, large specific surface area, high capacity for charge transfer and convenient separation from a reaction mixture with an external magnetic field, but also for the rapid, highly selective and sensitive detection of food contaminants and food components after the proper surface modification. Fe3O4 nanoparticles are usually involved in detection techniques for food analysis in two ways: electrode modifier and sample pre-concentrator [37–43,168]. Fe3O4 nanoparticles have been widely used in many detection techniques for food analysis, including PCR, immunoassay, HPLC, LC-MS/MS, and optical method. For example, Liu et al. developed a superparamagnetic nano-immunobeads (SPM-NIBs) by conjugation of Fe3O4 nanoparticles with specific antibodies (Scheme 5). The prepared SPM-NIBs showed superior colloidal stability and reversible magnetic response to Vibrio parahaemolyticus, a main foodborne pathogenes from contaminated seafood. About 80% of Vibrio parahaemolyticus cells could be captured when the concentration of the broth was 10^3 CFU/mL [41].

Scheme 5. Illustration of the process of target bacteria separation using superparamagnetic nano-immunobeads (SPM-NIBs) [41].
5. Concluding Remarks and Prospects

Due to the unique properties (e.g., superparamagnetism, high surface area, large surface-to-volume ratio, low toxicity, and easy separation), Fe₃O₄ nanoparticles have emerged as ideal frame materials for generating functional materials of different surface architecture, which have already displayed promising effects in practical applications in protein immobilization, bioseparation, biomedical science, environmental treatment, and food analysis. Various Fe₃O₄ based nanoparticles have already realized their practical applications. However, there are still many Fe₃O₄ based nanoparticles having not been scaled up from the laboratory scale into industry-level, and several crucial scientific, technical, and economical issues still need to be settled. Therefore, more and more efforts are still required to meet the tremendous demands for advanced materials of modern technology, which might call for interdisciplinary cooperation of material, chemistry, physics, medicine, and other related disciplines.

Acknowledgments

The authors thank Qingdao Scientific and Technological Achievements Transformation Program (14-2-4-91-jch), National Natural Science Foundation of China (NSFC) (31200642), Science and Technology Development Plan of Shandong Province (2014GHY115029), Qingdao Municipal Science and Technology Plan Project (12-1-4-12-(2)-jch), and Special Scientific Research Funds for Central Non-profit Institutes, Chinese Academy of Fishery Sciences (2013A1002) for financial support. The authors would also like to thank Xingguo Liang for reviewing this article.

Author Contributions

J.-K.X.: conception, design, and manuscript preparation; F.-F.Z.: manuscript preparation; J.-J.S.: manuscript preparation; J.S.: review of the manuscript; F.W.: review of the manuscript; M.S.: review of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Not available.