Multifunctional magnetic iron oxide nanoparticles: an advanced platform for cancer theranostics

Shengzhe Zhao\textsuperscript{a,b,c, \#}, Xujiang Yu\textsuperscript{c, \#}, Yuna Qian\textsuperscript{b}, Wei Chen\textsuperscript{a,*}, Jianliang Shen\textsuperscript{a,b,*}

\textsuperscript{a}State Key Laboratory of Ophthalmology, Optometry and Vision Science, School of Ophthalmology & Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou 325027, China

\textsuperscript{b}Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou 32500, China.

\textsuperscript{c}State Key Lab of Metal Matrix Composites, School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China.

AUTHOR INFORMATION

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\#These authors contributed equally to this work.

*To whom correspondence should be addressed:

Jianliang Shen (shenjl@wibe.ac.cn) or Wei Chen (chenweimd@hotmail.com)
Abstract

Multifunctional magnetic nanoparticles and derivative nanocomposites have aroused great concern for multimode imaging and cancer synergistic therapies in recent years. Among the rest, functional magnetic iron oxide nanoparticles (Fe$_3$O$_4$ NPs) have shown great potential as an advanced platform because of their inherent magnetic resonance imaging (MRI), biocatalytic activity (nanozyme), magnetic hyperthermia treatment (MHT), photo-responsive therapy and drug delivery for chemotherapy and gene therapy. Magnetic Fe$_3$O$_4$ NPs can be synthesized through several methods and easily surface modified with biocompatible materials or active targeting moieties. The MRI capacity could be appropriately modulated to induce a response between $T_1$ and $T_2$ modes by controlling the size distribution of Fe$_3$O$_4$ NPs. Besides, small-size nanoparticles are also desired due to the enhanced permeation and retention (EPR) effect, thus the imaging and therapeutic efficiency of Fe$_3$O$_4$ NP-based platforms can be further improved. Here, we firstly retrospect the typical synthesis and surface modification methods of magnetic Fe$_3$O$_4$ NPs. Then, the latest biomedical application including responsive MRI, multimodal imaging, nanozyme, MHT, photo-responsive therapy and drug delivery, the mechanism of corresponding treatments and cooperation therapeutics of multifunctional Fe$_3$O$_4$ NPs are also be explained. Finally, we also outline a brief discussion and perspective on the possibility of further clinical translations of these multifunctional nanomaterials. This review would provide a comprehensive reference for readers to understand the multifunctional Fe$_3$O$_4$ NPs in cancer diagnosis and treatment.

Keywords: magnetic iron oxide nanoparticles, multifunctional nanoplatform, multimodal imaging, drug delivery, cancer diagnosis and treatment
Typical synthesis methods of magnetic Fe₃O₄ NPs and their applications in cancer diagnosis and treatment.
1. Introduction

Cancer is a class of diseases marked by the malignant proliferation of tumor cells. Despite of the huge advancements achieved during the past decades, cancer still a serious public health issue, which leading the main deaths around the world every year [1]. With the development of nanotechnology, multifunctional nanoparticle-based systems have been emerged as a new approach for efficient cancer diagnosis and treatment due to their inherent advantages in overcoming the deficiencies compared to the traditional cancer diagnostic and therapeutic techniques, such as low efficacy, drug resistance or other side effects [2-5]. Currently, various multifunctional nanoparticle-based systems integrating nanotechnology and molecular biology together have been developed as powerful tools for cancer early-stage diagnosis, real-time imaging and precise therapy [6-10]. The integration of single functional component within a multifunctional therapeutic system has been demonstrated as one effective treatment approach for fighting the cancer [11-13]. The ideal nanoparticle-based diagnosis and treatment systems should not only contain the unique physical, chemical and medical properties of nanoparticles, but also be smart carriers to effectively deliver anticancer agents, thus realizing multi-modal imaging and combined therapeutic effects [14-17]. Moreover, the diverse nanostructures and surface modification of nanomaterials should endow themselves with the tumor-targeting ability by EPR effect or other interactions affiliated to the features of tumor tissues [18-21].

Magnetic iron oxide nanoparticles (Fe₃O₄ NPs) are one representative candidate of multifunctional nanomaterials with increasing utilization in many biomedical fields, including magnetic resonance imaging (MRI), biological catalysis, magnetic hyperthermia, magnetic targeting, magnetic separation, photo-responsive therapy and drug-delivery, and currently have been widely used in tumor diagnosis and treatment [22-28]. Meanwhile, according to the specific treatment demand and characteristics of the tumor microenvironment, decoration of the Fe₃O₄ NPs with different functionalization factors can form the multifunctional iron oxide nanoparticles to achieve better therapeutic effects [29-31]. Compared to other imaging modalities in clinic, MRI exhibits high spatial resolution with rapid in vivo image acquisition [32, 33]. To improve its sensitivity, MRI contrast agents are usually used, which can be divided into two types, namely T₁ and T₂ agents depending on their unique effects to alter the longitudinal or transverse relaxation time of water protons [34-36]. Compared to the widely used T₁-weighted MRI agents of gadolinium...
(Gd)-based contrast agents with shortcoming of unpredictable renal toxicity and blood circulation time, Fe$_3$O$_4$ NPs are contrast agents that can be used for either $T_1$ or $T_2$-weighted imaging with better biosecurity [13, 37-39]. In particular, Fe$_3$O$_4$ NPs is a typical $T_2$ contrast agent, when the size of Fe$_3$O$_4$ less than 5 nm, the decreased magnetic moment will strongly suppressed $T_2$ effect, thus they can also be used for $T_1$-weighted MRI imaging, which provides a possibility to prepare responsive $T_2$-$T_1$ switching MRI contrast agents [40, 41]. Due to the excellent thermal effect under the oscillating magnetic field, magnetic Fe$_3$O$_4$ NPs are widely used in the field of magnetic hyperthermia treatment of tumors [42]. Besides, Fe$_3$O$_4$ NPs also show catalytic activity analogous peroxidase and considered as mimic enzyme for cancer therapy through the well-known Fenton reactions, which could catalyst endogenous hydrogen peroxide (H$_2$O$_2$) into the hydroxyl radical (•OH) with high cytotoxicity and cause the death of tumor cells [43, 44]. This newly defined chemodynamic therapy (CDT) based on the Fenton reaction is a rapidly developing research topic in cancer treatment these years and have endowed magnetic Fe$_3$O$_4$ NPs with a brand-new life [45].

The modification of functional components onto Fe$_3$O$_4$ NPs can also bring photo-responsive therapy, namely photothermal therapy or photodynamic therapy, and makes Fe$_3$O$_4$ NPs functionalize as advanced nanoplatforms for oncotherapy [46]. For example, the modification of magnetic Fe$_3$O$_4$ NPs with the classic photothermal treatment agent of Au NPs can bring additional photothermal therapeutic capability besides to the MRI and magnetic targeting functionality, thus improving the accuracy of diagnosis and treatment of tumor using Fe$_3$O$_4$ NPs [47]. Similar, Fe$_3$O$_4$ NPs also have other advantages, such as prolonged blood circulation, fast clearance, low side-effects, excellent imaging and therapeutic efficiency. Therefore, several types of magnetic Fe$_3$O$_4$-based NPs systems have been approved to translate from experimental stages to clinical applications by the food and drug administration (FDA), some of them have reached the market for clinic (Table 1) [48, 49]. For example, the contrast agent AMI-25 (Ferumoxide, Feridex IV, Endorem) consists of the dextran coated Fe$_3$O$_4$ nanocrystal has been used in clinic, this agent could rapidly agglomeration in liver and spleen after injection 1 h, and the optimal imaging effects will appear in liver and spleen after injection 2 h and 4 h, respectively.
To improve the efficacy of Fe₃O₄ NPs in tumor imaging and therapeutic, as well as other inorganic nanomaterials, a maximum tumor accumulation is highly desired [50, 51]. Besides the passive EPR effect [52, 53], the more positive method to increase tumor accumulation of nanomaterials is to conjugate tumor-homing or tumor microenvironment (TME) responsive ligands onto the Fe₃O₄ NPs to purposively improve their final amount in tumor tissues through selective recognition and internalization by tumor cells [54-57]. Synthesized Fe₃O₄ NPs through common chemical

| Name            | Short name | Size (nm) | Coating                  | Blood half in patients | r₁ (mmol¹/s⁻¹) | r₂ (mmol¹/s⁻¹) | Type of contrast | Clinical dose Fe/kg | Application               |
|-----------------|------------|-----------|--------------------------|------------------------|----------------|----------------|------------------|---------------------|------------------------|
| Ferumoxide      | AM1-25     | 120-180   | Dextran                  | 10 min                 | 24             | 100-160        | Negative         | 30 µmol               | Liver/spleen imaging    |
| Ferumoxyl       | AM1-7228   | 3         | Carboxymethyl-dextran    | 14 h                   | 15             | 89             | Positive         | 50-400 µmol           | Angiography IV          |
| Ferumoxylil     | AM1-121    | 300       | Silica                   | Not available          | 3              | 72             | Negative         | 105 mg/patient        | GI oral imaging         |
| Ferumoxtran     | AM1-227    | 30        | Dextran                  | 24-30 h                | 22             | 44-85          | Positive/Negative | 45 µmol              | Lymph node imaging      |
| Feruglucose     | NC1001     | 16-20     | Carbohydrate polyethylene glycol | 2h                    | 20             | 35             | Positive         | 36 µmol              | Perfusion angiography    |
| Ferucarbofaxan SHU-555A | SHU-555A  | 66-80     | Carboxyldextran          | 12 min                 | 25             | 164-177        | Negative         | 8-12 µmol            | Liver/spleen IV imaging |
| Ferucarbofaxan SHU-555C | SHU-555C | 20-50     | Carboxyl dextran         | 6-8 h                  | 7              | 57             | Positive         | 40 µmol              | Perfusion lymph node bone marrow IV |
methods are usually coated with the hydrophobic alkyl ligands on the outer surface. Surface modification and further functionalization are necessary to convert them into hydrophilic, improve their biocompatibility and blood circulation time for enhancing tumor accumulation [13, 58], which are also beneficial to reduce the unexpected damage to normal tissues.

Smart drug-delivery systems based on nanoparticles is another vital way to combine chemotherapy with nanotheranostics [59, 60]. Fe₃O₄ NPs can easily be functionalized into a multifunctional platform by modifying with various therapeutic agents, and the common functional components for surface modification can be classified as: (1) small molecule (e.g., carboxylates, oleic acid) [61, 62]; (2) biomolecules (nucleic acids (siRNA), peptides and proteins) [63-65]; (3) inorganic materials (e.g., Au, Ag, MoS₂) [25, 66, 67]; (4) mesoporous materials (e.g., mesoporous silica, metal-organic frameworks) [68, 69]; (5) polymers (e.g., polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), polyethyleneimine (PEI)) [70-72]. Surface modification with these components of nanoparticles usually have a low cytotoxicity, hydrophilic, large pore volume, high surface area, adjustable pore size, in consequence provides the nanoparticles with better biocompatible and biodegradable [73, 74]. Based on these, through surface modification and functionalization increases the ability of Fe₃O₄ NPs in tumor targeting and responsiveness that further enhance their therapeutic effect [75].

This review aims to summarize the recent progress to provide a clear explanation for the rational design, construction and applications of Fe₃O₄ NPs-based multifunctional platform. Firstly, we introduce the common synthesis methods. Secondly, the surface modification and targeting ligand conjugation of Fe₃O₄ NPs are described. Finally, we will focus on the recent development of multifunctional Fe₃O₄ platforms for cancer diagnosis and treatment, including responsive imaging, nanozyme, CDT, magnetic hyperthermia (MHT), photothermal therapy (PTT), photodynamic therapy (PDT) and drug/gene delivery. We believe the summary of recent development will provide a comprehensive understanding of the applications of Fe₃O₄-based NPs multifunctional systems in biomedical research studies and clinic uses.

2. Synthesis of magnetic Fe₃O₄ NPs

The properties of magnetic Fe₃O₄ NPs are initially determined by their size and morphology. Thus, the selection of suitable synthesis method is important. Fe₃O₄ NPs can be synthesized by
physical, biosynthetic and chemical methods [76-78]. Generally, the physical synthesis methods, mainly including the ball grinding method, electron beam lithography, aerosol and gas phase deposition, fail to regulate the size into the nanoscale [79-81]. Biosynthetic methods to synthesize magnetic nanoparticles is an emerging technology, which generates magnetosomes through the regulation of biological macromolecules and iron element by magnetotactic bacteria or cells [82, 83]. However, these methods are always accompanied with the disadvantages of long synthesis time, low yield and broad size distributions. In comparison, the chemical synthesis methods are relatively common because of the advantages of simple operation, low cost and huge production yields. Therefore, we will only focus on the chemical methods in the main text.

![Figure 1. Typical synthesis methods of magnetic Fe$_3$O$_4$ NPs and their applications in cancer diagnosis and treatment.](image)

In general, the key to synthesize monodisperse Fe$_3$O$_4$ NPs in solution is to control the processes of nucleation and growth [84, 85]. Different synthetic strategies produce Fe$_3$O$_4$ NPs usual with different morphology, sizes, crystallinity and surface properties [86, 87]. The common chemical
synthesis methods include thermal decomposition [88, 89], solvothermal reaction [90], polyol synthesis [91], hydrothermal reaction [92], coprecipitation synthesis [93, 94], microemulsion synthesis [95], sol-gel synthesis [96], and template synthesis [97] (Figure 1). Here we will detailly introduce the recent advances using the above methods for the synthesis of Fe₃O₄ NPs (Table 2).

Figure 2. (A) TEM images of monodisperse Fe₃O₄ nanocrystals synthesized by the thermal decomposition in solvent with an increasing boiling point: (a) 5 nm, 1-hexadecene (b.p. 274 °C); (b) 9 nm, octyl ether (b.p. 287 °C); (c) 12 nm, 1-octadecene (b.p. 314 °C); (d) 16 nm, 1-eicosene (b.p. 330 °C) and (e) 22 nm, trioctylamine (b.p. 365 °C). Adapted with permission from [88], copyright 2004 Nature Materials. (B) SEM images of Fe₃O₄ NPs synthesized by solvothermal reaction. Adapted with permission from [90], copyright 2005 Angewandte Chemie. (C) TEM images of Fe₃O₄ NPs synthesized by hydrothermal reaction. Adapted with permission from [92], copyright 2013 Biomaterials. (D) TEM images of Fe₃O₄ NPs synthesized by microemulsion synthesis. Adapted with permission from [95], copyright 2006 Advanced Functional Materials. (E) TEM images of mesoporous Fe₃O₄ NPs synthesis by hard templates. Adapted with permission from [97], copyright 2008 Advanced Materials.
Table 2. Comparison of the different chemical synthesis methods of Fe3O4 NPs.

| Method                  | Solvent          | Reaction conditions | Process temperature | Process time | Shape control | Size distribution | Degree of crystallinity | Yield | Reference |
|-------------------------|------------------|--------------------|---------------------|--------------|---------------|-------------------|-------------------------|-------|-----------|
| Thermal decomposition   | Organic solvent  | N₂ atmosphere      | High                | Day          | Very good     | Narrow            | High                    | High  | [88, 89]  |
| Solvothermal reaction    | Organic solvent  | High pressure      | High                | Hours        | Bad           | Narrow            | High                    | High  | [90]      |
| Hydrothermal reaction    | Water            | High pressure      | High                | Hours        | Bad           | Relatively narrow  | High                    | High  | [92]      |
| Coprecipitation synthesis| Water            | N₂ atmosphere      | Low                 | Minutes      | Bad           | Broad             | Low                     | Medium | [93, 94]  |
| Microemulsion synthesis  | Oil/Water/Oil    | N₂ atmosphere      | Low                 | Hours        | Good          | Relatively narrow  | Low                     | Low   | [95]      |
| Sol gel synthesis        | Organic solvent  | N₂ atmosphere      | Low                 | Hours        | Good          | Relatively narrow  | Low                     | Medium | [96]      |
| Template synthesis       | Water            | Normal pressures   | High                | Hours        | Bad           | Relatively narrow  | Low                     | Medium | [97]      |
presence of organic ligands, the aggregation and overgrowth of nanoparticles are restricted and the
diameter of nanoparticles is closely related to the reaction time and the boiling point of organic
solvent [98]. Generally, the metal complex precursors are rapidly injected into hot organic solvents or
directly heated up with the solvents [99, 100]. In the hot injection process, after the metal precursors solution is rapidly injected into the solvents at the reaction temperature, an instant
nucleation immediately happens with a controlled growth process. In the heating-up procedure, the
nucleation increases gradually with the raise of the reaction temperatures [101-103]. Oleic acid
(OA), oleylamine (OAm) and 1-octadecene (ODE) are the commonly used solvents as well as
surfactants to stabilize nanoparticles and control their sizes and morphologies [104]. It is worth
mentioning that the surface of nanoparticles prepared by this method is hydrophobic, and
subsequent surface modification is needed to improve the hydrophilicity for the utilization in
biological environment [105]. Hyeon et al. first prepared uniform and small-sized monodisperse
Fe$_3$O$_4$ nanocrystals (4-12 nm) through this method [88, 89]. The size of the Fe$_3$O$_4$ nanocrystals were
controlled by the boiling points (b.p.) of the organic solvent. As shown in Figure 2A, the size of
Fe$_3$O$_4$ nanocrystals increases with the rise of solvent boiling point. This method has been widely
used for synthesis uniform small-size Fe$_3$O$_4$ NPs with good crystallinity and be widely used in the
application of MRI contrast agent, drug carrier, further assembly forms cluster structure use for
photothermal or magnetic hyperthermia treatment of tumors, which shows great significance to the
biological application of Fe$_3$O$_4$.

2.2 Solvothermal reaction/Polyol method

In order to prevent the self-aggregation and over-growth of monodisperse Fe$_3$O$_4$ nanocrystalline
during the synthesis process, the decomposition of metal salts in organic solution-phase have been
widely used [88, 89]. Based on this principle, the solvothermal method is extensively used to
synthesize Fe$_3$O$_4$ NPs with low cost, simple operation, and excellent crystallinity. Li et al. [90]
reported the method for the synthesis of hydrophilic, monodisperse, and single-crystalline magnetic
ferrite (MFe$_2$O$_4$; M=Fe, Mn, Zn, or Co) microspheres by a solvothermal reduction method, and the
monodispersed ferrite spheres were controlled with the diameters from 200 to 800 nm (Figure 2B).
This solvothermal method provides an important method to get monodisperse nanostructures
without demanding a narrow size distribution. However, the final nanocrystals from oleic
acid/amine during the solvothermal reaction usually coated with long alkyl chain oleic acids or amines with hydrophobic surface properties, which greatly inhibits their applications in biotechnological areas and others. In order to receive monodisperse and hydrophilic nanocrystals, ethylene glycol could be used as the solvent in the solvothermal synthesis of magnetic ferrite nanocrystals with hexanediamine or polyethylene glycol as protecting reagents.

The polyol method is similarly developed to fabricated Fe₃O₄ NPs by using polyols (such as, diethylene glycol, ethylene glycol and triethylene glycol) to reduce the metal compounds to obtain the corresponding nanoparticles. In this method, the solvent of polyols with high boiling points can be used as both the reducing agents and the stabilizing agents to control the growth process of nanoparticles and prevent possible agglomeration [91]. Wan et al. [106] prepared monodisperse Fe₃O₄ NPs with a uniform size of 10 nm through the polyol method using the triglyceride glycol and iron acetylacetone, the obtained Fe₃O₄ NPs showed well dispersity in aqueous or other polar media due to hydrophilic polyol ligands coated in the synthesis process.

2.3 Hydrothermal reaction

Fe₃O₄ NPs can also be synthesized by the hydrothermal reaction under the condition of suitable temperature (100-250 °C) and relatively high pressure (0.3-4 MPa) [107]. Hydrothermal synthesis involves no organic solvents or metal organic precursors, and the high crystallinity and good hydrophilicity of yielded Fe₃O₄ NPs omits the subsequent surface modification processes [92]. Shi et al. [92] reports the facile preparation of Fe₃O₄ NPs coated with branched PEI (abbreviated as: Fe₃O₄-PEI NPs) via the hydrothermal method (Figure 2C). The surface of Fe₃O₄-PEI NPs with primary amine groups, which could be further modified with PEG, succinic anhydride and acetic anhydride, and induce Fe₃O₄-PEI NPs continue surface functionalities. Hydrothermal reaction provides a choice for the simple synthesis Fe₃O₄-based multifunctional nanomaterial for biomedical applications.

2.4 Coprecipitation synthesis/Sol-gel synthesis

Coprecipitation of Fe²⁺ and Fe³⁺ ions in solution also a classical method applied to manufacture Fe₃O₄ NPs by precipitating a specific proportion of the inorganic salts in aqueous media [79, 108]. Compared with the thermal decomposition synthesis, the coprecipitation can avoid the problem that
surface decoration (template molecules, surface organic ligands, surfactant) are difficult to be removed [93, 94]. The size, quantity and morphology of the obtained Fe$_3$O$_4$ NPs could be controlled by experimental condition, such as power of hydrogen (pH), ion concentration, reaction temperature, precursor and so on. Stroeve et al. [109] synthesized the Fe$_3$O$_4$ NPs in aqueous solutions without any surfactants. The coprecipitation process of Fe$^{2+}$/Fe$^{3+}$ was achieved by changing the pH value of solution, and the achieved Fe$_3$O$_4$ NPs with a narrow size distribution and the average diameter less than 10 nm [98].

Sol-gel method can be considered as the further development from this strategy, which stirred the metal salt with a gelling agent to form a homogeneous gel, then gelled the sol by chemical reaction or solvent removal to get a 3D iron oxide network [96]. To obtain the pure Fe$_3$O$_4$ NPs, the formed gels usually requires an additional crushing step after drying and calcination. The structure and properties of the obtained Fe$_3$O$_4$ NPs are usually influenced by the concentration, reaction temperature, pH values, and solvents. The yield of nanoparticles using the sol-gel method usually is high, thus this method can be used for massively producing large-sized nanoparticles.

2.5 Microemulsion synthesis

Microemulsion method is also widely investigated as a classic method to prepare nanocrystals [110]. Microemulsion is a thermodynamically stable dispersions, which can be obtained by mixing immiscible water/oil phase that stabilized by the arrangement of co-surfactant or surfactant molecules at the interface. The microemulsion system especially the water-in-oil (W/O) phase, which is consist of the dispersion of water nanodroplets in the oil phase to form a stabilized spherical reverse micelle, can be considered as “nanoreactor” for the synthesis of nanoparticles. Since the nucleation and growth of nanoparticles are limited in the nanoreactor, that the size of nanoparticles can be controlled [111]. Li et al. [95] reported the synthesis of the magnetic Fe$_3$O$_4$ NPs by the W/O microemulsions, and investigated the relationship between the experiment condition (concentration, water/ethanol/organic solvent ratio, kinds of surfactants, temperature, reaction time) and the properties (morphology, crystal phase, and size distribution) of the obtained Fe$_3$O$_4$ NPs (Figure 2D).

2.6 Template synthesis

Recently, many researches have designed and prepared of hollow or porous Fe$_3$O$_4$ nanostructures
by the template synthesis method, which can be divided into the hard template method (e.g., silica, carbon spheres, and polymer nanoparticles) and the soft template method (e.g., vesicles, micelles, emulsion droplets, gas bubbles and others) according with the process of package, calcination and separation in obtaining the hollow nanostructure [112]. Among the hard templates, polymer latex particles, especially polystyrene (PS) beads, have been demonstrated to be effective templates for the preparation the hollow spherical inorganic materials of Fe₃O₄ NPs [113], and this method also be applied to other nanoparticles, such as Gd₂O₃ [105], TiO₂ [114], ZnS [115], SiO₂ [116] and so on. Similarly, mesoporous silica can also be used as the hard templates for the preparation of mesoporous Fe₃O₄ NPs, followed by the process of heating treatment and silica removal (Figure 2E) [97]. Compared to the hard template method, the morphology of Fe₃O₄ NPs synthesized by the soft template method is usually more uncontrollable. The obtained Fe₃O₄ NPs usually present nanowires structure and are rarely used in cancer diagnosis and treatment.

3. Surface Modification of Magnetic Fe₃O₄ NPs

Fe₃O₄ NPs prepared with hydrophobic ligands (oleic acid or stearic acid) needed to be converted into hydrophilic ligands for further biomedical applications [104]. Meanwhile, appropriate surface modification can also import the Fe₃O₄ NPs with better biocompatibility, long blood circulation and further functionalization, such as active targeting ability [105, 117]. Currently, the surface modification methods of Fe₃O₄ NPs fall into two categories of ligand replacement and encapsulation [118, 119]. Ligand replacement refers to an exchange of native hydrophobic ligands (oleic acid) on the surface of nanoparticles with strong anchoring groups (such as phosphonates, catechols, thiols, sulfonates, and carboxylic acids), which act as hydrophilic ligands to improve their dispersity in biological environment [120-123]. However, the actual ligand exchange process is often underperformed and always causes the leakage of surface ligand defectiveness. In contrast, the surface modification of Fe₃O₄ NPs through the encapsulation method shows better efficient, which can effectively coating the surface of Fe₃O₄ and ensure their uniformity [124, 125]. The usual strategy of encapsulation process is to utilize inorganic or organic shells to embellish iron oxide nanoparticles into core-shell structured nanocarriers [126-128], and the surface functionalized decorations mainly include noble metals (e.g., gold, silver, gadolinium) [129-132] or oxides (e.g., silica, graphene, titanium dioxide) [133, 134], biodegradable organic polymers (e.g., polyethylene...
glycol (PEG), poly (lactic-coglycolicacid) (PLGA)) [135-137], proteins (e.g., antibodies, monoclonal antibody and their fragments) [138], nucleic acids (e.g., DNA, siRNA, aptamers) [134, 139-141], amino acids (e.g., phenylalanine, tyrosine, arginine, lysine and cysteine) [142], small molecules (e.g., photosensitizer, folic acid, doxorubicin), and other species (vitamins, carbohydrates) [143, 144]. Here we detailly introduce the recent advances in the surface modification Fe₃O₄ NPs (Table 3).

| Variety          | Modification methods | Cost | Shape control | Size distribution | Treatment of type | Reference       |
|------------------|----------------------|------|---------------|-------------------|-------------------|-----------------|
| Mesoporous material | SiO₂·Si              | Low  | Easy          | Narrow            | Drug delivery     | [148-152]       |
| Photocatalysts    | TiO₂, C₆              | Low  | Moderation    | Broad             | PDT               | [125-29]        |
| Noble metal      | Au, Pt, Pd           | High | Easy          | Narrow            | PDT               | [155-160]       |
| Polymer          | PEG, PLGA            | High | Modulation    | Broad             | Drug delivery     | [165-169]       |
| Metal-organic framework | ZIF-8, MIL-100(Fe)  | Low  | Moderate      | Low               | PDT               | [171-173]       |
| Liposome         | Lipidosome           | Low  | Moderate      | Moderate           | PDT               | [181-184]       |
| Biomacromolecules| siRNA, Aptamer       | High | Easy          | Narrow            | Drug delivery     | [1860]125-258   |
| Micromolecule    | FA, DOX              | High | Easy          | Narrow            | Drug delivery     | [242-250]       |

Table 3. Comparison of different modification methods of Fe₃O₄ NPs.
3.1 Inorganic mesoporous materials

In recent years, many inorganic nanomaterials have been extensively studied in biomedical fields, such as silica, aluminum oxide, molybdenum dioxide, graphene, calcium carbonate, calcium phosphate and others [145-147]. Among them, mesoporous silica (mSiO$_2$) materials have been used as delivery carriers for small molecule drugs, quantum dots, siRNA and aptamers due to their uniform pore sizes and high accessible pore volume [148, 149]. Meanwhile, the monodisperse mSiO$_2$ smaller than 100 nm, have also been proved to possess a high stability in blood circulation [150-152]. The modification of mSiO$_2$ onto Fe$_3$O$_4$ NPs to form uniform core-shell nanocomposite have been reported to improve their biocompatible, enhance hydrophilicity and provide anchoring points (Si-OH groups) for further loading of molecular drugs (e.g. paclitaxel, platinum-based drugs, DOX) or other targeted functional groups (e.g. FA, antibodies). The modification of mSiO$_2$ shell onto Fe$_3$O$_4$ NPs usually using the sol-gel method or microemulsion method. Hyeon et al. [153] synthesized the composite structure consist of monodisperse Fe$_3$O$_4$ NPs core and mSiO$_2$ shell (Figure 3). CTAB was used as the surfactant for the transfer of hydrophobic Fe$_3$O$_4$ NPs, and provide the soft template for the grown of mSiO$_2$ shell in the sol-gel process. The size of Fe$_3$O$_4$@mSiO$_2$ NPs could be controlled within 100 nm, and the fluorescein isothiocyanate and rhodamine B isothiocyanate were further modified on the mSiO$_2$ shell for fluorescence imaging in vivo, which could show typical emissions of fluorescein and rhodamine B at 516 nm and 577nm with the corresponding excitation wavelength of 460 nm and 520 nm. Meanwhile, the core-shell Fe$_3$O$_4$@mSiO$_2$ NPs also could be used as the contrast agent for $T_2$-weighted MRI in vivo, providing an idea for construction multimodal imaging platform by modifying the hydrophobic Fe$_3$O$_4$ NPs with the functional mesoporous material shell.
3.2 Noble metals

Magnetic Fe₃O₄ NPs, due to their excellent imaging properties and magnetic targeting ability, are usually conjunction with other functional systems to improve their capability for multimodal imaging and precision targeting [154]. Among them, noble metals are a crucial species of functional components. On one hand, most of noble metals have a higher atomic number than the iodine element, and can be used as the contrast agent for computed tomography (CT) imaging, due to their remarkable X-ray attenuation property [155, 156]. On the other hand, noble metals usually have the strong surface plasmon resonance (SPR) effect, which could absorb the energy of light and convert that into thermal or other energy [157]. In particular, the core/shell nanostructures adopt the plasmonic effects of noble metals into magnetic Fe₃O₄ NPs, can be used in the photothermal therapy...
of cancer [158-160]. Shi et al. [157] developed a multifunctional theranostic nanoplatforms for tumor imaging and therapy based on the star-shaped Fe$_3$O$_4$@Au core/shell nanoparticles, which presented an excellent effect in MRI, CT, thermal imaging and photothermal therapy. After further modification of PEI and HA, the nanostars showed better biocompatibility, stability and targeting for cancer cells.

Figure 4. (A) Schematic of synthesis Fe$_3$O$_4$/Au nanocomposite. (B) SEM images of Fe$_3$O$_4$/Au core-shell structure. (C) Thermal images effect of Fe$_3$O$_4$/Au nanocomposite under 808 nm irradiation. (D) The $T_2$-weighted MRI effect of Fe$_3$O$_4$/Au nanocomposite in vivo. Adapted with permission from [158], copyright 2011 Advanced Materials.

Shi et al. [158] reported a method for preparation the core-shell structured of Fe$_3$O$_4$/Au, and the Fe$_3$O$_4$ NPs core are linked to the Au shell by the hybrid of SiO$_2$ and PS-b-PAA (a kind of amphiphilic block copolymers consisting of polystyrene and polyacrylicacid). The details of synthetic process are shown in Figure 4A. First process is modified SiO$_2$ on the surface of Fe$_3$O$_4$ NPs and self-assembly with the PS-b-PAA. Then reduce AuCl$_4^-$ on the surface of Fe$_3$O$_4$@hybrid to Au NPs form
the shell structure, and ultimate formation the Fe$_3$O$_4$/Au. The SPR effect of Au NPs on the core-shell structured could absorb the near-infrared light (808 nm) and converted that into thermal energy and eventually kill the tumor cells. Meanwhile, due to the MRI performance of the Fe$_3$O$_4$, the noble metal modified Fe$_3$O$_4$ not only have property of photothermal therapy, but also an agent of MRI and thermal imagery, demonstrating a flexible way for the construction of Fe$_3$O$_4$-based multifunctional diagnosis and treatment platforms.

3.3 Polymers

In the last decade, biocompatible polymers have been extensively used to improve conventional mode of medication in drug-delivery [161-163]. Meanwhile, the magnetic Fe$_3$O$_4$ NPs are gaining more research interests in the medical fields relying on their unique properties such as imaging capability of MRI and magnetic targeting drug carrier. For improving the hydrophilicity and other physiochemical properties of Fe$_3$O$_4$ NPs, the surface modification with polymers provided an ideal choice, and the commonly used polymers include polyethylene glycol (PEG), poly(L-lysine) (PLL) [164], poly(propyleneimine) (PPI), polyethyleneimine (PEI), polystyrene (PS) [165], poly (vinyl pyrrolidone) (PVP), poly (lactic-co-glycolic acid) (PLGA) and poly (vinyl alcohol) (PVA)), which having hydrophilic segments to improve biodegradability and biocompatibility. Parveen et al. [166] revealed the surface coated Fe$_3$O$_4$ NPs with the hydrophilic PEG could prevent nanoparticles from being removed by the phagocytic activities to further boost the endurance of the Fe$_3$O$_4$. PLGA is a biodegradable polymer and has been frequently used as a drug-delivery platform in the treatment of cancer therapy in recent years, and has also been reported to modify magnetic Fe$_3$O$_4$ NPs for enhance their magnetic targeting delivery ability to tumor tissues under magnetic field conditions [167-169]. Wu et al. [170] prepared the uniform microcapsules with Fe$_3$O$_4$ and PEGylated PLGA (abbreviated as Fe$_3$O$_4$@PEG-PLGA MCs) for MRI and ultrasound bimodal imaging (USI) (Figure 5). The Fe$_3$O$_4$@PEG-PLGA MCs showed better stability in physiological solutions owing to the PEGylation. Meanwhile, the in vivo and in vitro experiment results showed the polymers modified Fe$_3$O$_4$-based microcapsules could be used as an agent for USI and MRI performance, without dramatic cytotoxicity and embolism to mice even at high doses.
Figure 5. (A) The schematic diagram: (1) The traditional method yielded microcapsules with an uneven distribution; (2) Premix membrane emulsification method obtained uniform microcapsules with the help of microporous membrane; (3) The structure diagram of Fe₃O₄@PEG-PLGA MC. (B) SEM images of Fe₃O₄@PEG-PLGA MCs. The imaging of in vivo USI (C) (liver) and MRI (D) of mice. Arrows of yellow, red and blue corresponding to kidneys, spleens and livers. Adapted with permission from [170], copyright 2015 ACS Applied Materials & Interfaces.

3.4 Metal-organic frameworks

Metal-organic frameworks (MOFs) is an emerging species of porous nanomaterials acquire from metal ions or clusters coordinating with bridged ligands, which could be used to load guest nanoparticles to gain new performance due to their good physicochemical stability, larger surface area and tunable functionality [171]. Responsive MOFs coated Fe₃O₄ NPs have been studied for improving the hydrophilicity, increasing the porosity of delivery system and enhancing the responsiveness of the tumor environment. The mechanism of MOFs responsive decomposition may be due to the effects of H⁺ and glutathione (GSH) in the tumor microenvironment on the binding of metal ions and organic ligands [171, 172]. Yang et al. [172] designed a novel MRI contrast agent,
which utilized the pH and GSH responsive ZIF-8 as nanocarrier to deliver small-sized Fe₃O₄ NPs (about 5 nm, $T₁$-weight MRI) into a Fe₃O₄-ZIF-8 nanostructure ($T₂$-weight MRI). The slightly acidic conditions and overexpressed GSH in tumor microenvironment would lead the decomposition of the Fe₃O₄-ZIF-8 nanostructure and release the small-sized Fe₃O₄ (Figure 6A), leading to the MRI effect from $T₂$ to $T₁$ and enhancing the contrast of the tumor tissue (detail in part 4.3), which could distinguish tumor tissue from the normal tissue by the $T₂$ to $T₁$ conversion. Han et al. [173] synthesized a novel Co-ferrocene metal-organic framework (Co-Fc MOF) with high Fenton activity. After combined with the glucose oxidase (GOx), the nanoplatform (Co-Fc@GOx) construct an enzymatic/Fenton catalytic synergistic effect for enhanced tumor treatment effect. GOx delivered by Co-Fc MOF could catalyze endogenous glucose of tumor microenvironment to H₂O₂ and gluconic acid, which further favored the Fenton reaction of Co-Fc MOF and enhanced the generation of ROS. Experimental results demonstrated this synergistic enzymatic/Fenton catalytic activity triggered by Co-Fc@GOx nanoplatform enabled remarkable anticancer properties both in vivo and in vitro.

Figure 6. (A) Schematic of Fe₃O₄-ZIF-8 switch from $T₂$ to $T₁$ weight. (B) TEM images of Fe₃O₄-ZIF-8 assemblies. (C) In vivo $T₁$-weight MRI of tumor after intravenous injection of Fe₃O₄-ZIF-8, and homologous $T₁$ signals (D) extracted from tumor sites. Adapted with permission from [172], copyright 2019 Chemical Communications.
3.5 Cell membranes and derivatives

It is a common method to functionalize the nanoparticles with tumor imaging and therapy with the exogenous ligand. However, several problems are still needed to be solved, such as: (1) the foreign nanoparticles can be easily detected by immune systems and cause severe immune responses; (2) the physiological barriers could eliminate nanomaterials from the blood circulation and restrict the accumulation in target sites; (3) nontargeted nanomaterials that rely on the EPR effect limit the therapeutic effects and increase the damage to normal tissues; (4) the potential toxicity of nanomaterials in vivo [174-180]. Fe3O4 NPs with their unique properties and long blood circulation time are widely researched to overcome these limitations. Compared to exogenous ligands for surface modification, the endogenous cell membranes for nanoparticles wrapping provide a novel method for solving these problems [181-184]. The classic types of biomimetic cell membranes include red blood cell membrane, white blood cell membrane, cancer cell membrane, stem cell membrane and so on [185]. Zhao et al. [183] fabricated the delivery platform based on the platelet mimicking Fe3O4 NPs for enhancing the blood circulation time and targeting ability. As shown in Figure 7, step 1 showed the collections of blood from the mice, step 2 showed the dissociation of membrane and protein from platelet formed the vesicles, step 3 was to coat the vesicles of platelet on the surface of Fe3O4 NPs, step 4 was to injection the vesicles modified Fe3O4 NPs into blood vessel, steps 5 to 7 were the systematic circulation of vesicles modified Fe3O4 NPs, enrichment in tumor tissues by EPR effect and entry into tumor cells with the help of vesicles, steps 8 and 9 showed the performance test of imaging and therapeutic by the MRI and photothermal therapy (PTT). The results showed that the cell membrane modified Fe3O4 NPs have a good tumor targeting ability and could kill the tumor cells by photothermal treatment under the irradiation of near infrared light.
3.6 Aptamers

In general, when the Fe₃O₄ NPs circulate in the blood system, they are easily subjected to macrophage uptake and reticuloendothelial system clearance. To improve specificity and recognition of the tumor targeted delivery system, many functionalized substances such as aptamers, peptides and small molecule can also be used for the surface modification of nanoparticles. Aptamer is a single-stranded oligonucleotide generated from an in vitro selection process, which called systematic evolution of ligands by exponential enrichment (SELEX) and they could bind with the targeted small molecules, proteins, and even intact cells and tissues with excellent specificity and high affinity. Aptamers used for surface modification have many advantages, such as easy to operate, good stability, rapid tissue penetration and lack of immunogenicity, thus making them as a suitable candidate. Tan et al. [186] designed a multifunction delivery platform that perform five distinct functions synergistically and effectively. To accomplish this, they prepared the gold-coated rose-
shaped Fe$_3$O$_4$ (Au@Fe$_3$O$_4$) nanoplatform as an agent for photothermal therapy (PTT) and MRI. To enhance the targeting ability, the sgc8 aptamers were easily conjugated with the Au@Fe$_3$O$_4$ via the thiolate bonding, which could specifically recognize CCRF-CEM leukemia cells. Additionally, the chemotherapeutic agent doxorubicin (DOX) could also intercalate into the GC base pairs in the extended part of the aptamers, and with the releasing of DOX, the fluorescence signal in vitro also changed and cause the optical imaging. Therefore, the DOX-loaded Au@Fe$_3$O$_4$ nanoplatform presented five distinct functions for simultaneous imaging and therapy with the help of aptamers.

4. Fe$_3$O$_4$ NPs for Tumor Imaging

4.1 $T_2$-weighted magnetic resonance imaging

MRI is an effective method for tumor diagnosis due to the acquired high contrast images and the precise handling of details of the targeted tissues with non-invasiveness and real-time monitoring [184]. The sensitivity of MRI could be significantly enhanced with the help of contrast agents. The agents of MRI could be classified base on the effect on longitudinal ($T_1$) or transversal ($T_2$) relaxations, and the ability is defined as relaxivity ($r_1$, $r_2$). Therefore, MRI contrast agents could be divided in two types, which are $T_1$-weighted (positive) and $T_2$-weighted (negative), mainly shortens the $T_1$ and $T_2$ contrast media. In general, fast $T_1$-weighted results appear to be bright contrast in the MRI, while the opposite $T_2$-weighted results to be the dark contrast [187]. Due to the superparamagnetic property, Fe$_3$O$_4$ NPs typically decrease the relaxation time of surrounding protons, thus providing possibility to be employed as $T_2$-weighted MRI agent media.

Shi et al. [188] prepared a novel agent for MRI, which was the nanogels consisting of PEI coated Fe$_3$O$_4$ NPs immobilized with alginat (abbreviated as: AG/PEI-Fe$_3$O$_4$ NGs). The synthetic process is shown in Figure 8, and the nanogels presented a dispersion state in water with the size distribution from 153 nm to 219.2 nm. Moreover, the novel Fe$_3$O$_4$-based nanogels were excellent $T_2$-weighted contrast agent for the MRI (the relaxivity of $r_2$ is 170.87 mM$^{-1}$s$^{-1}$) and could be vastly swallowed by the tumor cells.
Figure 8. (A) The schematic of the synthetic process of AG/PEI-Fe$_3$O$_4$ NGs. (B) The imaging of $T_2$-weighted MRI in vitro (a) and signal intensity analysis (b). (C) The images of $T_2$-weighted MRI in vivo. Adapted with permission from [188], copyright 2016 Biomaterials Science.

4.2 $T_1$-weighted magnetic resonance imaging

Superparamagnetic Fe$_3$O$_4$ NPs (SPIONs) are widely used as $T_2$ contrast agents, since the strong magnetic moment could lead the magnetic inhomogeneity. However, some reasons limit their clinical application of $T_2$ contrast agents [189]. Because the intrinsic dark signal of $T_2$-weighted MRI could not accurately distinguish the tumors and other hypointense areas, such as calcification, metal deposition, or bleeding. Meanwhile, the $T_2$-weighted contrast agents usually with the high magnetic moment that perturbation the local magnetic field, which could exaggerate the size of the labeled area and blurs the images, thus causing the so-called “blooming effect”. Therefore, $T_1$ contrast agents shows better desirable than $T_2$ contrast agents for the accurate and high-resolution imaging [190]. It is noteworthy that compared with the familiar $T_1$ contrast agents, Fe$_3$O$_4$ contrast agents show better biocompatibility due to the iron element are rich in human blood, and stored in the body in the form of ferritin [201]. However, the common Fe$_3$O$_4$ NPs are unfit for the $T_1$ contrast agents because of that the ideal $T_1$ contrast agents would have high $r_1$ value and low $r_2/r_1$ ratio to realize the $T_1$ contrast maximize effect [191]. Although iron with five unpaired electrons which could increase the $r_1$ value, the innate high magnetic moment of Fe$_3$O$_4$ NPs lead to the high $r_2$ value and prevents then to be used as $T_1$ contrast agent. However, this problem could be solved by
decreasing the size of the Fe₃O₄ NPs, because the magnetic moment of Fe₃O₄ NPs could rapid decreases with the sizes reduction, which can lead to the reduction in the volume magnetic anisotropy [41, 192-194]. Therefore, a series of research on Fe₃O₄ NPs as contrast agent are widely carried out.

Figure 9. (A) TEM images of Fe₃O₄ NPs; T₁-weighted MRI of Fe₃O₄ NPs with the diameters of 3 nm (B) and 12 nm (C); (D) The MRI intensity with the dynamic time in vivo. Adapted with permission from [189], copyright 2011 Journal of the American Chemical Society.
Hyeon et al. [189] reported the preparation of small-sized \( \text{Fe}_3\text{O}_4 \) NPs (mainly 3 nm) by the thermal decomposition (Figure 9), and the obtained \( \text{Fe}_3\text{O}_4 \) NPs showed extremely low magnetization derived due to the spin canting effect. For improving the water-dispersion and biocompatibility of \( \text{Fe}_3\text{O}_4 \) NPs, the PO-PEG ligands were modified on the surface. In vitro cytotoxicity assay of PO-PEG capped \( \text{Fe}_3\text{O}_4 \) NPs showed no observed toxic response, exhibited a high \( r_1 \) relaxivity (4.78 mM\(^{-1}\)·s\(^{-1}\)) and low \( r_2/r_1 \) ratio (6.12), which demonstrated the magnetic \( \text{Fe}_3\text{O}_4 \) NPs could be used as \( T_1 \) contrast agents. The high \( r_1 \) relaxivity of \( \text{Fe}_3\text{O}_4 \) NPs could be attributed to the large surface number of iron with five unpaired valence electrons. The \( \text{Fe}_3\text{O}_4 \) NPs (around 3 nm) indicated a longer circulation time than the clinically used Gd-based contrast agents. High-resolution blood pool MRI using \( \text{Fe}_3\text{O}_4 \) NPs were able to provide a clear observation of various blood vessels within sizes less to 0.2 mm. All of these results demonstrated the \( \text{Fe}_3\text{O}_4 \) NPs with the potential as \( T_1 \) contrast agents for MRI in clinic.

![Figure 10. (A) The synthetic process of BSA modified \( \text{Fe}_3\text{O}_4 \) nanoclusters. (B) (a) TEM image of BSA modified \( \text{Fe}_3\text{O}_4 \) nanoclusters, (b) \( T_1 \)-weighted MRI of ultrasmall size \( \text{Fe}_3\text{O}_4 \) NPs (USNP) and BSA modified nanoclusters. (C) \( T_2 \) and \( T_1 \)-weighted MRI after injection for 2 hours, the measured gray values of kidney and brain regions are shown in table. Adapted with permission from [196], copyright 2017 Nanoscale.](image)

However, there are still several problems limit the ultrasmall size \( \text{Fe}_3\text{O}_4 \) NPs used as \( T_1 \) contrast agents [195]. First, ultrasmall size nanoparticles could be fast cleared out by renal metabolism and limit the imaging. Second, due to the high surface energy, the self-aggregation is a major concern of ultrasmall nanoparticles. Once aggregated the small \( \text{Fe}_3\text{O}_4 \) NPs will lose their \( T_1 \) performance.
Third, the surface modification of the ultrasmall size Fe₃O₄ NPs is critical to maintain the $T_1$ performance, because the molecules modified on the surface directly control the paramagnetic [195, 196]. Therefore, it is necessary to modify the ultrasmall size Fe₃O₄ NPs reasonably while ensuring its performance. Bao et al. [196] reported a novel method to form nanoclusters by crosslinking bovine serum albumin (BSA) onto ultrasmall size Fe₃O₄ NPs (Figure 10). Different from traditional studies showing $T_1$ signal decrease or complete loss after polymer encapsulation, the nanoclusters not only maintain the $T_1$ contrast agent’s performance of the Fe₃O₄ NPs, but also significantly enhanced the blood circulation times from 15 minutes to over two hours.

4.3 Responsive $T_1/T_2$ imaging

Due to the fact that magnetic Fe₃O₄ NPs display a remarkable change from the $T_2$ enhanced contrast effect to $T_1$ enhanced contrast effect when the size less than 5 nm, Fe₃O₄ NPs have become an attractive material for the preparation of responsive MRI contrast agents for tumor diagnosis. Yang et al. [172] utilized the ZIF-8 as a carrier for agglomerate small Fe₃O₄ NPs (a $T_1$ contrast agent) into Fe₃O₄-ZIF-8 assembly (a $T_2$ contrast agent). Because ZIF-8 is more stable under the normal physiological conditions and decompose under the acidic microenvironment of tumor tissues or in the presence of competitive ligands (Figure 6A). Therefore, the acidic conditions and GSH of the tumor microenvironment would trigger the disassembly of Fe₃O₄-ZIF-8 and to release the ultrafine size Fe₃O₄ NPs, leading the conversion from $T_2$ to $T_1$ enhancement on the sign of the tumor tissue.

The low delivery efficiency of nanomaterials is still a challenge in tumor imaging and treatment. Generally speaking, the EPR effect is considered as a driving force through either active or passive targeting for nanoparticles to reach and accumulate in the tumor tissues. Previous research has shown that nanoparticles in appropriate sizes (from 50 nm to 200 nm) can enhance the EPR effect through limit nanoparticle intravasate back into the circulation. Recently, more and more researches have been proved the mutual effect between nano system and the biological environment also affect the effect of nanomaterials and the mount of accumulation in tumor, some modified ultrafine size nanoparticles show better permeation and distribution in the tumor tissue than large nanoparticles, which include the size less than 5 nm Fe₃O₄ $T_1$-contrast agent. Mao et al. [197] reported a research using the Fe₃O₄ NPs (3.5 nm) modified with oligosaccharide (uIONPs), which could penetrate the tumor tissue and self-assemble in the acidic microenvironment of tumor tissues (Figure 11). The
improvement on the delivery and tumor retention of Fe₃O₄ NPs were achieved by combining the reduced intravasation and enhanced extravasation. Moreover, in vivo MRI revealed that ultrafine Fe₃O₄ NPs showed “bright” $T_1$ contrast when injected into the tumor vasculature, and then turn into “dark” $T_2$ contrast after 24 h. The switch of $T_1$-$T_2$ contrast demonstrated the ultrafine Fe₃O₄ NPs with $T_1$ contrast were dispersed state when inject into blood, and may aggregate formed large size Fe₃O₄ clusters with $T_2$ contrast after penetrated into the tumor tissues. Therefore, this property of Fe₃O₄ NPs showed an inspiration on the design of responsive $T_1/T_2$ conversion MRI contrast agents.

4.4 Multimodal imaging

In the process of the diagnosis and treatment, single imaging modality often cannot provide complete information about the tissues and organs [194]. Therefore, the combination of Fe₃O₄ NPs with two or more components to construct multimodal imaging system has become one of the main...
research directions [108]. Liu et al. [198] have designed the core-shell structure of Fe₃O₄@Cu₂₋ₓS (<10 nm), which showed excellent superparamagnetic and photothermal conversion capability. Therefore, the Fe₃O₄@Cu₂₋ₓS nanoparticles could be used as the agent of MRI, thermal imaging and photothermal therapy. Lee et al. [199] designed a MRI agent by the construction of Fe₃O₄/MnO nanoparticles to implement the dual modes of T₂ and T₁ contrast enhancement of each compound. The in vitro and in vivo results that the dumbbell-shaped Fe₃O₄/MnO nanoparticles with negative T₂ contrast effect in the full state, but in low pH environment, the positive effect of T₁-weighted MRI was raised due to the releasing of Mn²⁺. Yang et al. [200] have designed a multifunctional nanoplatform by assembly the upconversion nanoparticles (UCNPs: β-NaGdF₄:Yb/Tm@β-NaGdF₄) on the surface of graphitic-phase carbon nitride (g-C₃N₄) coated Fe₃O₄ nanospheres (Figure 12). Due to the T₁ contrast agents of Gd and T₂ contrast agents of Fe, this platform showed the magnetic targeting ability under the guide of external magnetic field, and further supervised the therapeutic effect by dual-modal imaging precise localization.

![Figure 12. (A) The schematic diagram of synthesis process of Fe₃O₄@g-C₃N₄-UCNPs-PEG. (B,C)](image-url)
In vivo $T_2/T_1$-weighted MRI of samples, preinjected and after injection in situ. Adapted with permission from [200], copyright 2017 Advanced Healthcare Materials.

5. Fe$_3$O$_4$ NPs for Imaging-Guided Tumor Treatment

5.1 Nanozyme

In recent years, Fe$_3$O$_4$ NPs are reported to have the intrinsic biocatalytic activity akin to horseradish peroxidase (HRP) [201]. Therefore, inorganic nanoparticles have been used as nanozyme for specific biomedical applications [202, 203]. The mechanism of Fe$_3$O$_4$ as nanozyme in killing tumor cells can be understood as the Fenton reaction, which utilizes Fe$^{3+}$/Fe$^{2+}$ ions reaction with excessive H$_2$O$_2$ in tumor tissues to generate excessive reactive oxygen species (ROS) [204-206]:

$$Fe^{3+} + H_2O_2 = Fe^{2+} + HO_2^{-} + H^+$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + •OH + OH^-$$

Due to the chain reaction between Fe$_3$O$_4$ (Fe$^{2+}$, Fe$^{3+}$) and H$_2$O$_2$, the Fenton reaction consumes the H$_2$O$_2$ in the tumor tissues and produce the •OH with cytotoxicity [207]. Based on the excellent enzyme-like activities, Fe$_3$O$_4$ NPs have been developed as enzyme mimetics for many novel biomedical applications [201]. It is worth mentioning that the efficient Fenton reaction usually require specific conditions, such as lower pH (3.0 ~ 4.0), higher temperature or UV/vis light irradiation. However, the pH value of tumor microenvironment is around 5.5-6.5, which cannot achieve the optimal Fenton reaction conditions [208]. Therefore, for achieve the best therapeutic effect, the UV/vis light or thermotherapy are always used to assist the Fenton reaction to enhance the ability of producing •OH [209].

Gu et al. [210] verified the enzyme-like activities of Fe$_3$O$_4$ NPs on role position and pH values. When the Fe$_3$O$_4$ NPs were internalized into tumor cells mainly concentrated in lysosomes and the cytotoxicity is related to the concentration. Fe$_3$O$_4$ show stronger toxic potancy than γ-Fe$_2$O$_3$. Due to the acidic microenvironment of lysosomes, Fe$_3$O$_4$ NPs presented the peroxidase-like activity and the cell damage would be enhanced by the induced H$_2$O$_2$. In neutral pH conditions, both Fe$_3$O$_4$ and γ-Fe$_2$O$_3$ NPs cannot produce hydroxyl radicals (•OH), but just catalyze decomposition of H$_2$O$_2$ into H$_2$O and O$_2$ directly. These results show that the cytotoxicity of Fe$_3$O$_4$ nanozyme are decided by the external environment and distribution intracellular. Lin et al. [211] reported a strategy for enhanced
the chemodynamic therapy (CDT) effect by the synergistic of photothermal therapy, which combined the typical Fe\textsubscript{3}O\textsubscript{4} nanozyme and the semiconductor Bi\textsubscript{2}S\textsubscript{3} as shown in Figure 13A. The Fe\textsubscript{3}O\textsubscript{4}@Bi\textsubscript{2}S\textsubscript{3} nanocatalysts could kill the cancer cells through the effect of photothermal treatment under 808 nm laser, and sequential thermal effect enhanced the Fenton action of Fe\textsubscript{3}O\textsubscript{4} NPs, which could efficiently convert H\textsubscript{2}O\textsubscript{2} into highly toxic •OH, thus realizing a remarkable synergistic anticancer achievement.

Figure 13. (A) The synthesis mechanism diagram of Fe\textsubscript{3}O\textsubscript{4}@Bi\textsubscript{2}S\textsubscript{3} nanocatalysts. (B) The schematic diagram of in vivo photothermal and chemodynamic therapy. (C) The photographs of anatomical tumors in vivo of treating groups. Adapted with permission from [211], copyright 2020 ACS Applied Materials & Interfaces.

Yang et al. [209] designed a novel diagnosis and treatment platform composed of Fe\textsubscript{3}O\textsubscript{4} NPs, metal organic frame MIL-100(Fe), UCNPs (Na\textsubscript{Y}F\textsubscript{4}:Yb,Tm@NaGdF\textsubscript{4}:Yb) and modified by PEG (aliased as FMUP). The UCNPs were designed for converter the near-infrared light to UV/vis light, which could excite the Fe\textsuperscript{3+}/Fe\textsuperscript{2+} ions in the Fe\textsubscript{3}O\textsubscript{4} NPs and enhance the effect of photo-Fenton reaction, thus activating the photocatalytic reaction by using MIL-100(Fe) as photosensitizer (Figure 14), which synergy combined photodynamic therapy (PDT) and photochemotherapy (PCT). The Fenton reaction produced cytotoxic reactive oxygen (•OH) with high cytotoxicity independent of the oxygen in the tumor microenvironment. Meanwhile, the Fe\textsubscript{3}O\textsubscript{4} NPs and MIL-100(Fe) could
form the heterojunctions that markedly inhibited the recombination of electrons and holes, thus effect enhancing the ability of PCT and PDT in producing ROS. The antitumor effect was showed by in vivo and in vitro assays, the experimental results have indicated that the FMUP with the excellent imaging ability of CT and UCL with positive synergistic treatment effect of PDT and PCT.

Figure 14. The synthesis process illustration (A) and the TEM images (B) of FMUP (Scale bars: 100 nm). (C) In vivo CT images of FMUP before (down) and after injection (up). (D) The photographs of tumor-bearing mice after 14 days of treatment. Adapted with permission from [209], copyright 2018 Chemical Engineering Journal.

5.2 Magnetic hyperthermia treatment

Magnetic nanoparticles (MNPs) provide an effective platform for biomedicine with lots of applications such as MRI, magnetic guidance delivery, magnetic separation, and thermal treatments [212, 213]. The magnetic hyperthermia treatment (MHT) is a classical therapeutic concept based the principle of cancer cells are more vulnerable than healthy cells when the environment temperatures higher than 41 °C. The thermal effect of MNPs could generate by the external alternating magnetic field (AMF) and define by the specific absorption rate (SAR), which is rate
The value of energy absorbed per unit mass of the agent when located in radio frequency. The values of SAR are influenced by the morphology and composition of the MHT agent, also be affected by the property of magnetic field such as frequency \((f)\) and amplitude \((H)\). Therefore, for achieving an effect of hyperthermia to tumors, the magnetically mediated hyperthermia nanomaterials must show the high SAR and low \((f)/(H)\) in the low content. Thereinto, the superparamagnetic \(\text{Fe}_3\text{O}_4\) NPs as the heat mediators \(\text{via}\) an oscillating magnetic field show remarkable superiority, because SAR values of superparamagnetic nanoparticles can be enhanced by increasing either \((f)\) or \((H)\) (or both increase) during the measurements \([96]\). In general, most studies on the SAR carried out on \(\text{Fe}_3\text{O}_4\) NPs were prepared by co-precipitation method or sol-gel, the resulting nanoparticles were usually in the size of 20-50 nm. Only few researches of SAR prepared by thermal decomposition methods have reported, because small size \(\text{Fe}_3\text{O}_4\) NPs usually has a low SAR value \([212]\).

Ishimura \textit{et al.} \([214]\) reported the research of superparamagnetic \(\text{Fe}_3\text{O}_4\) NPs (SPIONs) clusters for MRI and MHT. Superparamagnetism of \(\text{Fe}_3\text{O}_4\) NPs only present in the size less than 10 nm. However, this size is smaller than capillaries pores of normal tissues and will lead to the leakage of SPIONs from the normal tissues, resulting the low accumulation in tumors, and reduce the effect of the tumor diagnosis and treatment. To obtain effective accumulation and excellent therapeutic effect of magnetic hyperthermia treatment, the FA and PEG modified SPIONs nanoclusters were designed (FA-PEG-SPION NCs). The SPIONs clusters not only prevented the leakage from normal tissues capillaries, but also increased the rate of relaxivity and the specific absorption. Meanwhile, the FA and PEG could also increase the targeting of SPIONs clusters and enhance the amount accumulation in tumors. After intravenous injection the FA-PEG-SPION NCs for 24 h, the clusters would accumulate locally in cancer (not necrotic) tissues and enhance the MRI intensity. Furthermore, the FA-PEG-SPION showed excellence magnetic hyperthermia effect under the alternating current magnetic field \((f = 230\text{ kHz}, H = 8\text{ kA/m})\).

Liang \textit{et al.} \([215]\) designed a multifunctional system consisting of Pd modified \(\text{Fe}_3\text{O}_4\) nanoparticles (JNPs) with the property of dual-mode MRI and PA (photoacoustic) imaging, synergy of photothermal therapy, magnetic therapy and chemodynamic therapy (Figure 15). The plasmonic photothermia property of Pd nanosheets could achieve synergistic effects for enhancing the magnetic/photothermal effect of \(\text{Fe}_3\text{O}_4\) NPs, and the chemodynamic therapy could be attributed to the •OH, which generate from the Fenton reaction of \(\text{Fe}_3\text{O}_4\) NPs and catalytic properties of Pd.
nanosheet in an acidic environment of tumor H$_2$O$_2$. Meanwhile, the ability of reactive oxygen species production could also be further enhanced under alternating magnetic field and near-infrared light irradiation.

Figure 15. (A) The illustration of the design Fe$_3$O$_4$-Pd JNPs. (B) TEM images of Fe$_3$O$_4$-Pd JNPs (TEM (a), enlarged TEM (b) and HRTEM (c)). (C) The photos of tumor tissues harvested from mice at the end of treatment. Adapted with permission from [215], copyright 2019 Nanoscale Horizons.

5.3 Photothermal therapy

Photothermal therapy (PTT) is widely used for tumor therapy in recent years, relying on the nanoparticles in tumor tissues to convert optical energy into thermal energy to kill cancer cells [216]. Compared with surgical management and radiotherapy chemotherapy, PTT is more controllable,
highly efficient and lower invasive [217]. This treatment takes the advantage of near-infrared light (NIR) with excellent tissue penetration effect and minimum damage in the range of 700-1100 nm, which could activate specific materials to transformation energy generate thermal effect and cause damage to the tumor tissues. Many nanomaterials have been developed as PTT agents, such as noble metals, semiconductors, and special polymers, which usually have a strong optical absorbance in the near-infrared optical window [218]. The potential toxicity caused by photothermal agents is still an unresolved problem because these nanoparticles tend to accumulate in organs and the slow degradability of nanomaterials would introduce inflammatory cytokine production, increase oxidative stress and cell death, which would limit the clinical application of PTT [219, 220]. Therefore, that necessary to explore the agent with biosafe and biodegradable for PTT.

Fe₃O₄ NPs have been attention due to the excellent biocompatibility, nontoxicity, MRI and magnetic targeting capability[221]. Although the Fe₃O₄ have been approved as drugs for clinical. However, there are few reports about Fe₃O₄ NPs in photothermal therapy, due to the low molar extinction coefficient and poor photothermal conversion efficiency [222]. In order to improve the photothermal therapy effect of Fe₃O₄ NPs, much research has been done, one common way is to decorate the materials with high photothermal conversion (e.g., noble metals, copper chalcogenides, special polymer) onto the surface of magnetic Fe₃O₄ NPs to enhance the plasma resonance, another is to change the morphology of Fe₃O₄ NPs for obtaining better photothermal conversion effect [223]. Shi et al. [224] reported the photothermal effect of special shape magnetic Fe₃O₄ NPs both in vitro and in vivo experiments. The heating effect of Fe₃O₄ NPs with the shape of spherical, hexagonal and wire-like were found rapidly generated under the red and near-infrared range laser irradiation, which showed obviously damaged on cancer cell cellular organelles. Due to the photothermal effect of the special-shaped Fe₃O₄ NPs, the tumor cells were found to be significant apoptosis with tumor reduction.

Wu et al. [222] proposed a simple method to transfer hydrophobic Fe₃O₄@Cu₂₋ₓS NPs to hydrophilic with the help of red blood cell membrane (Figure 16), which showed excellent performance for T₂-weighted MRI and PTT. The obtained nanoplatform was consist of the densely Fe₃O₄@Cu₂₋ₓS nanocluster core and the red blood cell membrane layer shell. This system displayed a stable nanostructure, excellent magnetic targeting ability and photothermal conversion ability. With the advantages of the red blood cell membrane, the nanocluster was protected from the
elimination by macrophages, and showed excellent magnetic targeting under the external magnetic field. Therefore, all of these features promote the platform with high performance for MRI and PTT.

Figure 16. Schematic illustration showing the synthesis process of red blood cell membrane coated Fe₃O₄@Cu₂S nanocluster system (A) and magnetic field triggered active targeting of MRI and PTT (B). Adapted with permission from [222], copyright 2020 Journal of Materials Chemistry B.

Recent researches have demonstrated the magnetic moment could significantly enhance with the agglomeration state [225]. Therefore, change the monodisperse magnetic Fe₃O₄ NPs into clusters provided an effective way to enhance the specific absorption rate (SAR) [226]. Yang et al. [227] reported an efficient PTT platform by self-assembly the monodisperse Fe₃O₄ nanocrystal into spherical superparticles (SPs), which could significantly increase the effect of photothermal treatment (Figure 17). For further enhance the tumor treatment effect, mPEG-PLGA copolymer were used to modify the Fe₃O₄ SPs, and immune adjuvant R837 were loaded to generate the antigens associated to tumor and induce strong antitumor immune responses synergistic treatment of tumor.
5.4 Photodynamic therapy

Photodynamic therapy (PDT) is considered as an emerging therapeutic strategy for clinical anticancer in recent years, due to the low systemic toxicity, cooperativity and negligible side effects. It includes three important parts: photosensitizers (PS), exciting light and oxygen in the tissue [200]. The principle of PDT is to trigger the reactive oxygen species (ROS) with cytotoxicity to induce tumor cells death. Meanwhile, the accurate delivery of photosensitizer to tumor tissue also play an important part of the treatment process. Magnetic targeting is a classic targeting approach, which takes the advantages of magnetic nanoparticles with the capability of being magnetized and directional movement by an external magnetic field, finally guidance the magnetic nanoparticles
concentrate in specific location. This physical interaction exhibiting potential application for tumor targeting, and as an excellent magnetic carrier, the Fe$_3$O$_4$ NPs have been concerns on the excellent MRI performance, magnetic targeted ability, high biocompatibility and chemical stability. Therefore, the effective combination of photosensitizer with Fe$_3$O$_4$ NPs could construct the photodynamic therapy nanoplatform with the magnetic targeted and MRI guided.

Lin et al. [228] synthesized a multifunctional treatment platform by covalently grafting Fe$_3$O$_4$ on the surface of MoS$_2$, then the photosensitizers indocyanine green (ICG) molecules and prodrugs Pt (IV) were loaded on the surface of MoS$_2$@Fe$_3$O$_4$ as shown in Figure 18A (Mo@Fe-ICG/Pt). The high transverse relaxivity Fe$_3$O$_4$ NPs in the Mo@Fe-ICG/Pt nanocomposites were used as an effectively $T_2$-weighted MRI contrast agent, which provided an excellent positioning effect for photothermal therapy of MoS$_2$, photodynamic therapy of ICG, and chemotherapy triggered of Pt (IV) prodrugs, thus leading to an ideal nanoplatform for tumor imaging and treatment.

Figure 18. The schematic diagram of the synthesis process (A) and TEM images (B) of Mo@Fe-ICG/Pt nanocomposites. The $T_2$-weighted MRI preinjection (C) and after injection (D) of Mo@Fe-ICG. (D) The photographs of mice after treatments by Mo@Fe-ICG/Pt. Adapted with permission.
Zhu et al. [229] designed a multifunctional tumor treatment platform composed of Fe₃O₄ core and two kinds of shells, which are MnO₂ and polypyrrole (PPy) as shown in Figure 19. The PPy was used as the agent of photothermal and photosensitizer, then combined with Fe₃O₄@MnO₂ for achieving the magnetic targeting effects. The MnO₂ shell could catalyze the H₂O₂ in the tumor tissues decompose into O₂ to improve the production of ROS under the irradiation, finally enhance the therapeutic effect of PTT/PDT. Meanwhile, the Fe₃O₄@MnO₂@PPy nanocomposite could also load DOX efficient and implement an acid response release in tumor tissues, which also realized the synergistic chemotherapy and PDT/PTT by avoiding damage to normal cells.

Figure 19. Schematic illustration for the fabrication and magnetic targeting, improved PDT by the catalytic decomposition of H₂O₂ and synergistic chemotherapy and PDT/PTT treatments of the Fe₃O₄@MnO₂@PPy-DOX to cancer cells. Adapted with permission from [229], copyright 2018 Journal of Materials Chemistry B.

5.5 Chemotherapy drug delivery

The most common non-surgery methods for cancer treatment are chemotherapy and radiation therapy. Nowadays, there are about 80 different kinds of antitumor drugs in the clinic, and around 400 drugs are being tested in clinical trials [230, 231]. Although they are effective in some cancer
types, most of them lack specificity towards tumor cells [232]. The normal tissues and organs will be harmed at therapeutic dosage [233]. Tumor-targeting therapy is delivering some chemotherapy drugs or other antitumor bioactive compounds to cancer cells by specific carriers, with limited influence on normal healthy tissues, resulting in higher therapeutic efficiency and lower toxicity [234, 235]. Meanwhile, the target delivery can be classified into three types based on the destination of the drugs, the primary target is reaching determined tissues or organs. The secondary target is reaching specific cells. The third-level target is interacting with some specific targets inside cells [236, 237]. So far, tumor nano-delivery systems have attracted main attention on the size so that the drug could enrich in tumor tissues owing to the EPR effect of tumor tissues [238, 239]. Meanwhile, the nano-delivery system could conjugate with some certain ligands to permits the active targeting into tumor tissues. Achieving highly specific targeting to cancer cells is the ultimate goal in cancer therapy and diagnosis [240, 241].

Figure 20. The schematic diagram of Fe₃O₄@PLGA nanocomposite for MPI-based drug release monitoring. (B) SEM image of DOX loaded Fe₃O₄@PLGA nanocomposites, and TEM image of a Fe₃O₄@PLGA nanocomposites (Inset). (C) MPI and CT merged images injected intratumorally with Fe₃O₄@PLGA. Adapted with permission from [243], copyright 2019 Nano letters.
In 1960s, Freeman et al has introduced the magnetically Fe₃O₄ NPs guided anti-cancer drugs delivery carrier is a unique method to concentrate drug accumulation of therapeutic nanoparticles to tumor tissues to improve therapeutic efficacy and used for the MRI contrast agent in 1985s [75, 242]. Meanwhile, the monitoring of drug release in vivo provides the accurate and reliable information for guiding the chemotherapy. The in vivo drug delivery based on the imaging guiding shows unique advantageous due to the noninvasiveness and the drug distribution visualization [243]. Magnetic particle imaging (MPI) due to the deeper tissue penetration and quantifiable signal intensity are widely used in detecting drug release in vivo, which mainly use the superparamagnetic Fe₃O₄ NPs as the contrast agent and sole signal source [244, 245]. Smith et al. [243] designed a core-shell nanocomposite consisting of the superparamagnetic Fe₃O₄ nanocluster and PLGA loaded with the chemotherapy drug DOX, which could be applied for drug delivery and the tracer of MPI quantification (Figure 20). The core-shell nanocomposite can occur decomposition in the tumor microenvironment (pH = 6.5), which could induce the gradual decomposition of the Fe₃O₄ nanocluster, sustained release of DOX and the change of MPI signal. The results indicated that induced MPI signal changes and the release rate of DOX over time displayed a linear correlation (R² = 0.99). Using this phenomenon, the chemotherapy drug release process in tumor tissue can be successfully monitored to assess the induced tumor cell damage.

Figure 21. (A) The schematic diagram of cracked cancer cell membrane (CCCM) embellished MNPs and the mechanism for prioritizing cancer cells recognition; (B) TEM images of MNP and MNP@DOX@CCCM. Adapted with permission from [250], copyright 2016 Nano Letters.
In recent years, the delivery of drug using cancer cell membrane, especially that from the homologous tumors has become an emerging tumor-targeting modification method [246]. In this context, a series of cancer cell membrane modified Fe$_3$O$_4$ NPs for the cancer diagnosis and treatment are reported. By modifying the source cancer cell membrane in vitro, the Fe$_3$O$_4$ NPs are identified with the self-recognition internalization, and “homing” targeting ability to the homologous tumor in vivo to precisely deliver the drugs to the appropriate tumor sites [247, 248]. As a result, Fe$_3$O$_4$-based delivery system shows strong curative effect for MRI and tumor treatment in vivo. This delivery strategy by modifying cell membrane on the traditional delivery system have shown great potentials for precise targeting to particular tumors by merely adjusting the corresponding type of modified cell membrane [249, 250]. Zhang et al. [250] devised a magnetic Fe$_3$O$_4$ NPs (MNP) based diagnosis and treatment nanomaterials platform, and modified the cracked cancer cell membranes (CCCM) on the surface coating the Fe$_3$O$_4$ NPs with the particular kind of cell membrane derived from a specific delivery system to correspond type of tumor (Figure 21). Meanwhile, the chemotherapy drug DOX can also be delivered with the CCCM modified MNP system, which showed self-recognition to the same cancer cell lines by the “homing” to the homologous tumor in vivo. These results provide a new approach to develop highly tumor-recognition self-targeting nanosystems for cancer therapy and diagnosis.

5.6 Gene delivery

Small interfering RNA (siRNA) could downregulate specific protein expression by suppressing a targeted gene selectively at the mRNA transcription level through a mechanism called RNA interference (RNAi) [251, 252], which have been widely researched for treating a variety of genetic diseases, such as cardiovascular diseases and cancers [253, 254]. Despite of these advantages, the low transport efficiency of siRNA with severe side effects still limits the clinical use [255, 256]. Fe$_3$O$_4$ NPs are well-established depend on possess magnetic properties, actively investigated as new generation for MRI, excellent biocompatibility and versatile surface functionalization capability, due to the unique characteristics, Fe$_3$O$_4$ NPs are extensively explored for various biomedical applications especially in delivery of therapeutics, antibodies, peptides, nucleic acids, and other assorted biological agents [257, 258]. Chen et al. [259] reported a multifunctional nanoclusters as an agent for dual-modal $T_1$-$T_2$ MRI imaging and siRNA delivery (Figure 22). The
hydrophilic nanoclusters were self-assembled from the hydrophobic gadolinium (Gd) embedded Fe₃O₄ nanocrystals (GdIO), after self-assembly with polyethylenimine (stPEI), the obtained GdIO-stPEI nanoclusters showed good stability, dispersity and dual-modal $T₁$-$T₂$-weight MRI properties. Meanwhile, the composite nanoclusters with the ability to deliver the siRNA, while maintaining other properties such as magnetism, biocompatibility, and imaging performance, thus providing a safe and efficient method for imaging guidance gene delivery.

![Figure 22](image)  
Figure 22. Schematic illustration of the preparation of GdIO-stPEI/siRNA complexes and their function. Adapted with permission from [259], copyright 2013 Nanoscale.

6. Conclusion and Perspective

In summary, we reviewed the recent research of Fe₃O₄ NPs from the synthesis, surface modification, and focus description the novel biomedical applications in tumor imaging and therapy. With the development of nanotechnology more high quality Fe₃O₄ NPs synthesis methods are reported, and the surface modification of Fe₃O₄ NPs also became more functional. New-generation Fe₃O₄-based contrast agents could realize the multimode and responsive imaging, provided more accurate and effective information for tumor diagnosis and guided follow treatment.

At present, the clinical application of multifunctional Fe₃O₄ is mainly in MRI contrast agent, not really implemented the integration of diagnosis and treatment [260]. First, the complex preparation
process, high cost and long tumor treatment trial period limit the clinical use of Fe₃O₄-based drugs. Second, although the magnetic targeting could increase the accumulation of Fe₃O₄-based drugs in tumor tissues, the available percentage still less than 2 % of the intravenously administrated dose. Third, there may be no EPR effect of the nanomaterials in the solid tumor of human beings comparing with the nude mouse tumor models. Last but not the least important, the potential toxicity of nanoparticles has not been properly addressed. Totally, these disadvantages limit the Fe₃O₄-based drugs for the further clinical applications. We believe these advanced reports will guide the academic researchers and industries to accurate the present translational stage for the further bioapplications utilized with these multifunctionalized magnetic iron oxide nanomaterials.

Abbreviations
multifunctional magnetic nanoparticles (MNPs)
magnetic iron oxide (Fe₃O₄)
nanoparticles (NPs)
enhanced permeation and retention (EPR)
superparamagnetic iron oxide nanoparticles (SPIONs)
magnetic resonance imaging (MRI)
tumor microenvironment (TME)
gadolinium (Gd)
metal-organic frameworks (MOF)
polyvinylpyrrolidone (PVP)
polyethylene glycol (PEG)
polyethylenimine (PEI)
Oleic acid (OA)
1-octadecene (ODE)
oleylamine (OAm)
poly (lactic-coglycolicacid) (PLGA)
polystyrene (PS)
polyacrylic acid (PAA)
tetrahydrofuran (THF)
3-mercapto propyl trimethoxysilane (MPTMS)
poly(L-lysine) (PLL)
poly(propyleneimine) (PPI)
poly(glycerol succinic acid) (PGLSAOH)
poly (vinyl alcohol) (PVA)
microcapsules (MCs)
premix membrane emulsification (PME)
platelet (PLT)
near infrared (NIR)
photothermal therapy (PTT)
systematic evolution of ligands by exponential enrichment (SELEX)
doxorubicin (DOX)
alginate nanogels (AG NGs)
polyethyleneimine (PEI)
phosphine oxide (PO)
upconversion nanoparticles (UCNPs)
horseradish peroxidase (HRP)
reactive oxygen species (ROS)
photochemotherapy (PCT)
near-infrared (NIR)
photodynamic therapy (PDT)
computed tomography (CT)
upconversion luminescence imaging (UCL)
photosensitizers (PS)
singlet oxygen (¹O₂)
indocyanine green (ICG)
magnetic hyperthermia treatment (MHT)
specific absorption rate (SAR)
superparticles (SPs)
oil-in-water (O/W)
dendritic cells (DCs)
draining lymph nodes (DLNs)
cracked cancer cell membranes (CCCM)
small interfering RNA (siRNA)
RNA interference (RNAi)
gadolinium embedded iron oxide (GdIO)
stearic acid modified low molecular weight polyethylenimine (stPEI)

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Conflict of Interest
The authors have declared that no conflicts of interest exist.

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**Author biography**

Shengzhe Zhao received his B.S in Materials Physics from Nanjing University of Information Science & Technology in 2015, M.S. in Applied Chemistry from Jilin Agricultural University in 2018, then appointed as a research assistant at University of Chinese Academy of Sciences in 2018. Currently, he is a visiting scholar in School of Chemistry and Chemical Engineering in Shanghai Jiao Tong University. His research interest is theranostic multifunctional nanomaterials.
Xujiang Yu obtained his B.S. in Chemistry in 2011 and M.S. in Inorganic Chemistry in 2013 from Sun Yat-Sen University under the supervision of Prof. Zongwan Mao. Then he moved to Shanghai Jiao Tong University and received his PhD in Materials Science and Engineering in 2018, supervised by Prof. Wanwan Li. He is currently a postdoctoral fellow in School of Chemistry and Chemical Engineering in Shanghai Jiao Tong University. His research focuses on the engineering of multifunctional nanomaterials and nano-assemblies for biomedical applications, especially in cancer imaging and therapy.

Yuna Qian received her Ph.D degree in 2017 from Chongqing University. Now she is an assistant professor of Wenzhou Institute, University of Chinese Academy of Sciences. Her current research interests are mainly in nanomaterials for tumour microenvironment and inflammatory cells.
Wei Chen is a full professor of Ophthalmology and the Head of Cornea Service in the affiliated eye hospital of Wenzhou Medical University in Zhejiang Province and Director of Eye Clinic in Boao Super Hospital in Hainan province. He graduated from Wenzhou Medical University with Medical Degree in 1998 and Shanghai Fudan university with PhD in 2002. He was trained as postdoctoral research fellow in Schepens Eye Research Institute, an affiliate of Harvard Medical College and Ocular Surface Center in Baylor. He was advisory member of China cornea society and founding member of Asia Dry Eye Society. His clinical and research interests include molecular mechanism and animal model of dry eye, corneal tissue engineering, corneal transplantation.

Jianliang Shen received his B.S in Applied Chemistry from Hunan University of Science and Technology in 2007, M.S. in Inorganic Chemistry from Xiangtan University in 2010, and Ph.D. degree in Inorganic Chemistry under supervision of Prof. Zong-Wan Mao at Sun Yat-Sen University in 2014, then started as a postdoctoral fellow under supervision of Prof. Haifa Shen in Houston Methodist Research Institute. He was appointed as a full professor at Wenzhou Medical University and University of Chinese Academy of Sciences in 2017. His research interests in novel hybrid nano/micro-particles for biomedical applications.