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REVIEW

Synthesis and application of superparamagnetic iron oxide nanoparticles in targeted therapy and imaging of cancer

Liangqian Tong, Ming Zhao, Shu Zhu, Jing Chen (✉)

Department of Nuclear Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

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Abstract **Superparamagnetic iron oxide (SPIO) nanoparticles have become a popular strategy of cancer treatment and molecular imaging because of their versatile properties and biocompatibility. A variety of studies have shown the exciting potential of functionalized SPIO nanoparticles, such as surface-coated, targeted ligand-conjugated, and/or drug-loaded SPIO nanoparticles, as powerful tools for targeted imaging and therapy. Moreover, the applications of SPIO nanoparticles that integrate diagnosis and therapy in SPIO nanoparticles facilitate the monitoring of therapeutic efficacy during treatment. In the present review, we primarily concentrate on the recent advancements in the field of SPIO nanoparticles in terms of synthesis, targeted therapy, and cancer imaging.**

Keywords nanoparticles; superparamagnetic iron oxide; targeted therapy; molecular imaging; cancer

Introduction

Cancer is one of the leading causes of death worldwide. To date, cancer treatment remains a challenge because isolating cancer cells from healthy cells is difficult, and most effective treatments, namely, chemotherapy and radiotherapy, cause inadvertent damage to healthy cells [1]. Tumor-specific targeting has therefore become crucial. Therapeutic agents that specifically target cancer cells and are nontoxic to normal cells are required. Innovative approaches for the early detection of cancer have also become increasingly desirable.

Superparamagnetic iron oxide (SPIO) nanoparticles, an unusual category of the nanoparticle family, have quickly become a popular strategy of cancer treatment and molecular imaging because of their versatile properties and biocompatibility [2]. In the present review, we primarily concentrate on the recent advancements in the field of SPIO nanoparticles in terms of synthesis and targeted therapy, as well as cancer imaging.

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Correspondence: wellcj@sina.com

Superparamagnetic iron oxide nanoparticles

SPIO nanoparticles, unusual members of the nanoparticle family, have diverse functions because of their nanosized diameter (between 1 nm and 100 nm) and good magnetic response [3]. In recent decades, SPIO nanoparticles have been applied broadly in bioscience and clinical research, including targeted drug delivery [4,5], gene delivery [6–8], hyperthermia [9,10], and contrast agents in magnetic resonance imaging (MRI) [4,11–13].

SPIO nanoparticles consist of magnetite (Fe_3O_4) and/or maghemite (γ-Fe_2O_3) [13]. They possess superparamagnetism, that is, they can be oriented in the direction of magnetic field and can be restored to their original state of suspension after the magnetic field is removed. SPIO nanoparticles are positively charged and can easily be combined with materials that are negatively charged [14,15], such as anticancer drugs and nucleic acids, which also contribute greatly to targeted delivery and other applications.

Size and structural features

Monodispersed submicro-diameter SPIO nanoparticles are flexible. If the diameter of naked iron oxide nanoparticles is greater than the critical size for superparamagnetism, they

lose their superparamagnetism. The optimal size of coated or surface-modified SPIO nanoparticles should be under 100 nm. Otherwise, they will cause the embolization and non-specific uptake by the reticuloendothelial system [14]. Lunov *et al*. revealed that 6 to 60 times more SPIO nanoparticles (60 nm) are phagocytosed by macrophages, whereas the ultrasmall superparamagnetic iron oxide nanoparticles (20 nm) stay in the vessels [16]. Predictably, the size of the resulting nanoparticles is an important factor that affects their endocytosis by cells. Some researchers used SPIO nanoparticles of different sizes as positive contrasting agent in the liver and spleen MRI and as an enhanced contrast agent for blood pool MRI [14]. The resulting size of the coated SPIO nanoparticles could affect passive targeting as well. Nanoparticles of different sizes are phagosytized by monocytes and macrophages differently. Monocytes and monocyte-derived macrophages (MDM) are used as vehicles for passive targeting, and the results indicate that larger-sized nanoparticles cause increased uptake and concentration in monocytes and MDM [14,17].

Transmission electron microscopy (TEM) has shown that naked SPIO nanoparticles are spherical crystals. The coated and surface-modified SPIO nanoparticles are basically core-shell structured [18] with high stability, good water solubility, high drug- and gene-loading capacity, and specific targeting of cells or tissues. Maeng *et al*. [19] experimented on multifunctional SPIO nanoparticles called YCC-DOX, coated with poly(ethylene oxide)-trimellitic anhydride chloride-folate (PEO-TMA-FA) and loaded with doxorubicin, in rat and rabbit liver cancer models, and discovered that they can be used for targeted therapeutic strategies and the progress monitoring of liver cancer in MRI.

Synthesis

Several physical and chemical approaches for synthesizing SPIO nanoparticles are known, such as electron-beam-induced gas phase deposition approach [20,21] and chemical precipitation-based approach, which includes coprecipitation [22–24], thermal decomposition [11,25], and reverse micelle [26,27] (Fig. 1). Physical approaches are less commonly used now because of their inability to control the nanometer size distribution of SPIO nanoparticles [21]. Coprecipitation and thermal decomposition approaches are the most commonly used approaches in synthesizing coated SPIO nanoparticles with better sizes. Babes *et al*. [22] reported in 1999 the successful synthesis of SPIO nanoparticles with hydrodynamic diameters of 30 nm to100 nm for MRI contrast agents through direct coprecipitation of iron salts and polysaccharides. Lee *et al*. [23] also claimed that 5 nm core-sized polyaspartic acid (PASP)-coated SPIO nanoparticles synthesized through coprecipitation with hydrodynamic diameters of (45 ± 10) nm were labeled with ^{64}Cu, and applicable as dual contrast agents in positron emission tomography and MRI. Approximately 21 nm diameter silica-coated SPIO nanoparticles with narrow size distributions have been synthesized through thermal decomposition [25].

Some factors that influence the size, composition, and even shape of the resulting SPIO nanoparticles mainly include temperature, the Fe(II)/Fe(III) ratio, the pH of reacting liquids, and the ratio of Fe/coating materials. Conventionally, in precipitation-based approaches, magnetite is prepared by adding a base of pH 9 to 14 to Fe(II) and Fe(III) chloride (molar ratio 1:2) mixture under oxygen-free conditions at about 60 °C [28]. Chen [23] added a base made from mixed PASP and ammonia dropwise to a mixture of 6 ml 0.6 mol/L $FeCl_3 \cdot H_2O$ and 6 ml 0.3 mol/L $FeCl_2 \cdot 4H_2O$ at 100 °C in argon atmosphere, and gained ASP-coated IO nanoparticles with (45 ± 10) nm diameter when the color of the reacting solution changes to black from yellow after stirring for 1 h at 100 °C.

Coating and surface modification

Naked SPIO nanoparticles without surface-coating moieties are erratic and can readily aggregate and precipitate in aqueous solutions and blood plasma, which seriously hinders their applications at the earlier stages either *in vitro* or *in vivo*. To endow SPIO nanoparticles with better water-solubility, biocompatibility, stability, and low cytotoxicity, researchers have fabricated nanoparticles with coating layers, such as polymers [29,30], dendrimers [18,31], polypeptides [32], and polysaccharides [33]. Fig. 2 shows that special elements can be added to the SPIO nanoparticles based on the core-shell structure for different purposes. Two methods can be used to fabricate surface-coating SPIO-conjugates: (1) *in situ* coating [34,35], wherein the synthesis and coating of SPIO occur simultaneously and (2) post-synthesis coating [19,36], wherein synthesis and coating are carried out stepwise. Amstad *et al*. [37] reported that SPIO nanoparticles with approximately 9 nm-thick stealth coatings of polyethylene glycol-gallol can remain stable for at least 20 months. After hydrophilic coating, the blood clearance half-life of the nanoparticles is prolonged [38,39].

Cell response to SPIO nanoparticles

SPIO nanoparticles for cancer therapy and molecular imaging have been preclinically and clinically researched for a decade, and much attention has been concentrated on their cellular uptake and safety *in vivo*. For maximum therapeutic effect and optimal imaging, SPIO nanoparticles must be able to be selectively taken up by the targeted cells and tissues without cytotoxicity. Therefore, cellular uptake and cytotoxicity have been paid more attention to. Three ways can be used to increase cellular uptake: (1) external magnetic field, which increases the local concentration and duration of SPIO nanoparticles [28]; (2) functionalized SPIO nanoparticles by conjugating the surface with targeting ligands, such as folate, aptamer, and lactoferrin. The paired receptors are over-

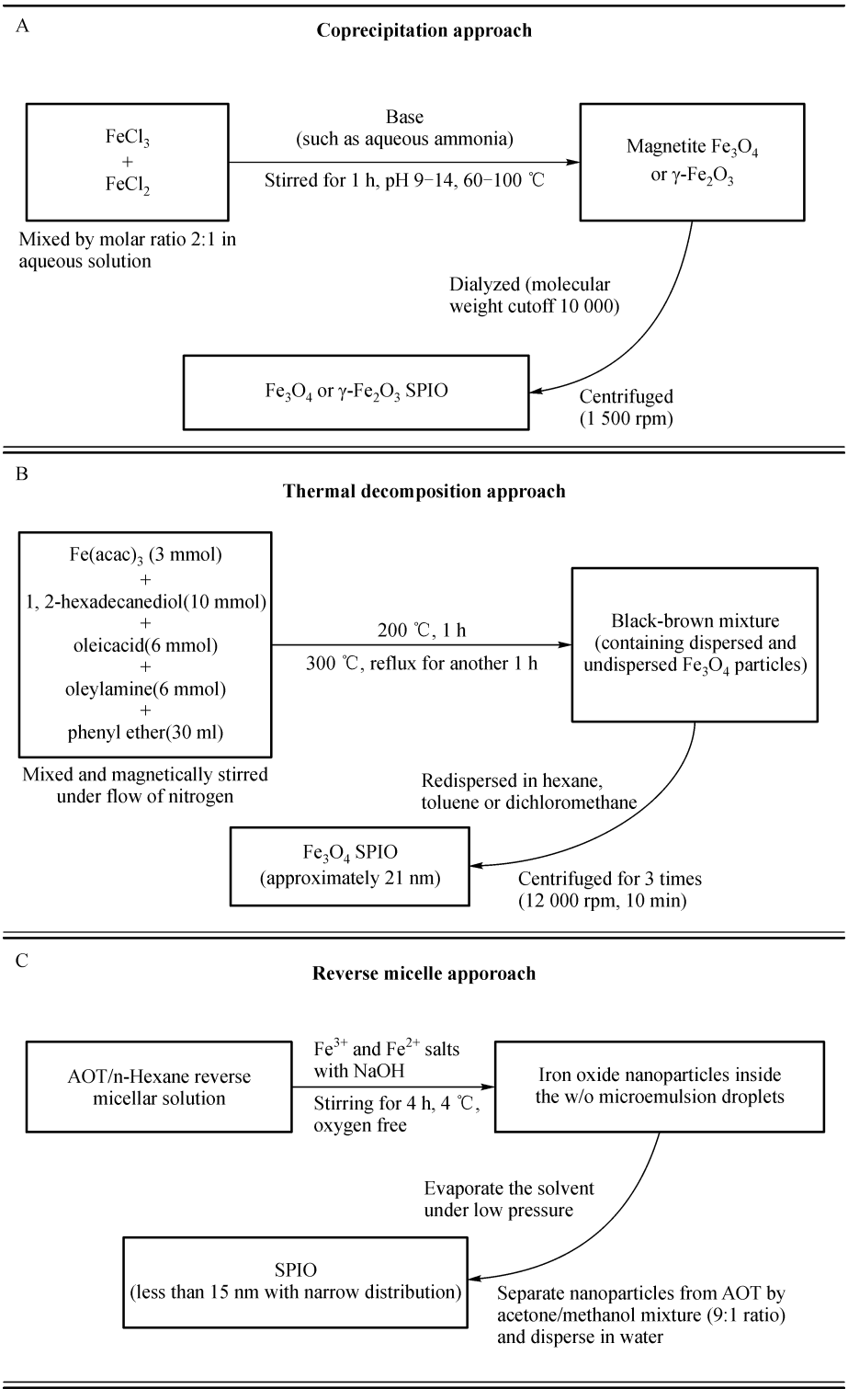

Fig. 1 The basic synthesis progress of SPIO nanoparticles. A, B, and C exhibit coprecipitation approach, thermal decomposition approach, and reverse micelle approach, respectively.

expressed on the surface of certain cells, which can induce positive targeting movement [40]; (3) nonselective uptake by monocyte-macrophages, which is consequentially applied in the imaging of liver, spleen, and other lymphoid tissues [10]. SPIO nanoparticles enter cells by endocytic mechanisms, as proven by TEM, fluorescence analysis, and confocal laser

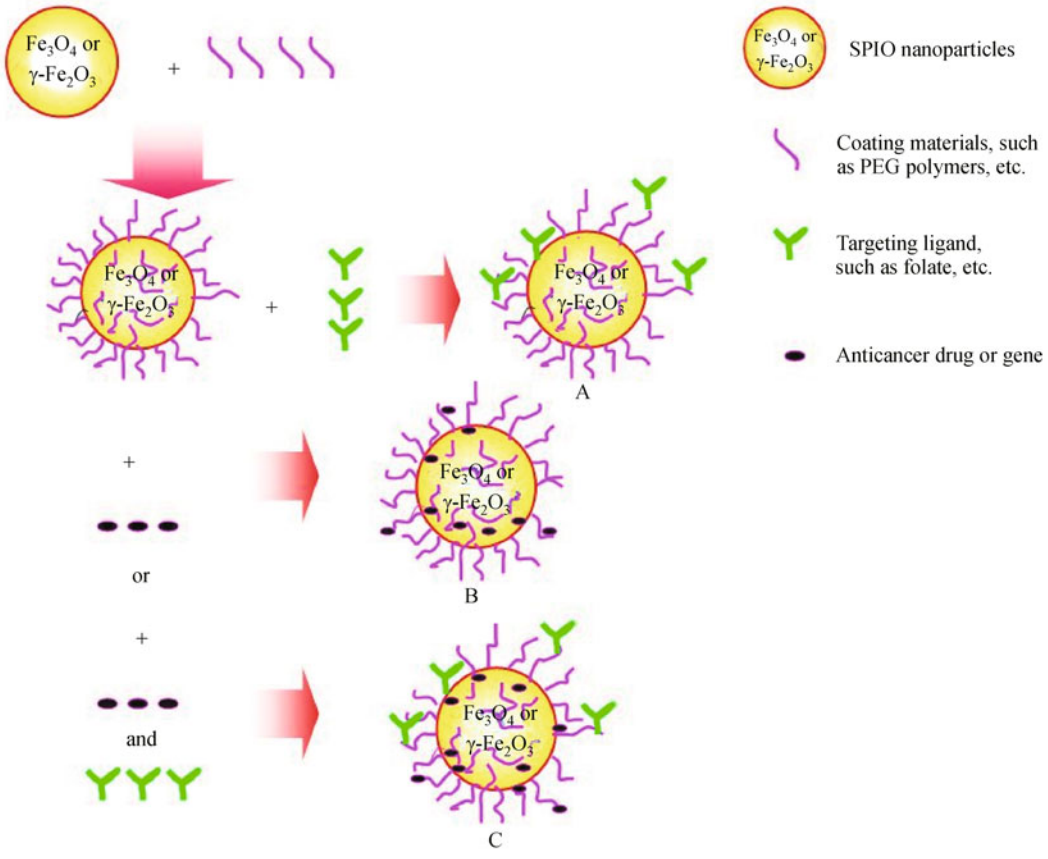

Fig. 2 Core-shell structured SPIO nanoparticles. Surface-modified nanoparticle A is coated with polymers, after which it can be used for targeted MRI contrast agents; nanoparticle B coated with polymers and loaded with drugs or genes can be used as an anticancer agent; surface-modified nanoparticle C coated with polymers and loaded with drugs or genes can be used as a diagnostic and therapeutic anticancer agent. A, B and C are three common types of diagnostic and therapeutic conjugates for cancers.

scanning microscopy, among others [4]. 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-diphenytetrazoliumromide assays, a common strategy for determining cell viability, have shown the absence of cytotoxicity to certain kinds of cells [4,19]. Jain *et al*. [41] verified the biocompatibility of magnetic nanoparticles (MNPs) in rats by examining organ function index, and claimed that no apparent long-term changes are apparent in organ functions.

Targeted therapy

Targeted therapy in both malignant diseases and other diseases is the pursuit of the ideal goal of researchers and clinical doctors. Thus far, great progress has been achieved in the field of magnetic nanoparticles (especially SPIO nanoparticles). SPIO nanoparticles, as an amazing targeted therapeutic strategy for malignant tumors, are mainly focused on hyperthermia, targeted gene delivery, and targeted drug delivery [1,2].

Hyperthermia

The optimal temperature for human cells to survive is about 37 °C. Higher temperatures may lead to loss of cell function, and even immediate cell death. Magnetic hyperthermia, which uses the heat generated by magnetic nanoparticles when placed in an alternative magnetic field (AMF), is an applicable therapy for malignant tumors [42]. Furthermore, surface-modified SPIO nanoparticles possess targeting capabilities. A series of experiments were performed to investigate the clinical value of SPIO nanoparticles applied for hyperthermia in malignant tumors. Sato *et al*. [43] studied N-propionyl-cysteaminylphenol-conjugated magnetite nanoparticles (NPrCAP/M) as hyperthermia particles for targeting melanomas. When the melanoma cells exposed to NPrCAP/M or magnetite were heated to 43 °C with an AMF, the cells treated with NPrCAP/M degraded 1.7- to 5.4-fold more than the cells treated with magnetite. Therefore, NPrCAP/M could successfully be used in targeted hyperthermia for melanomas. In recent studies, magnetic hyperthermia combined with

chemotherapy or gene therapy has attracted intense interests. Pradhan *et al*. [10] proposed doxorubicin-loaded magnetic liposomes (MagFolDox) as a promising magnetic hyperthermia-triggered drug release system. MagFolDox heated with AMF to 43 °C and incubated in both phosphate-buffered saline and fetal bovine serum (FBS) for 1 h showed 70% and 50% doxorubicin release, respectively, but less than 5% doxorubicin release at 37 °C. In addition, folate receptor-overexpressing tumor cell lines (KB and HeLa cells) were exposed to folate-targeted MagFolDox, which showed 52% doxorubicin release in 50% FBS at 43 °C and increased cellular uptake of doxorubicin. Heat-inducible gene expression has been studied by Tang *et al*. [44]. They used Mn-Zn ferrite magnetic nanoparticles under an AMF as thermal energy sources. Their results show 10-fold to 500-fold expression of the heterogeneous gene compared with those of the control groups.

Targeted gene delivery

Malignant tumors are a gene-induced disease. Therefore, gene therapy is still the hot spot for malignant tumor research, and much effort has been exerted in this field with inspiring progress. Gene transfection is the most common method in gene therapy, which introduces exogenous genes into the desired cells to address the genetic defect, adds an additional gene function to the cells, or even knocks down the overexpressing gene. Scherer *et al*. [45] explored SPIO nanoparticles as magnetofection gene vectors to increase gene transfection both *in vitro* and *in vivo*. The SPIO nanoparticles increased the efficacy of gene delivery by up to several hundred-fold, with significant reduction in the transduction time. Polyamidoamine (PAMAM) dendrimer, Tat peptide-conjugated, and human epidermal growth factor receptor plasmid siRNA (psiRNA-EGFR)-loaded SPIO nanoparticles (Tat-BMPs-PAMAM/psiRNA-EGFR) have been synthesized, and their anticancer efficacies have been examined. Tat-BMPs-PAMAM/psiRNA-EGFR-treated U251 cells exhibit low EGFR expression, and the subcutaneous xenografts in nude mice grow much more slowly [46]. Kamei *et al*. [47] employed Gold/iron-oxide MAgnetic Nanoparticles (GoldMAN) to direct cellular uptake in adenovirus-mediated gene delivery. The Ad/GoldMAN showed a more than 1 000-fold increase in gene expression than Ad alone. As a result, the authors claimed that these nanoparticles could be useful tools for increasing Ad tropism and even Ad resistance, and for enhancing transduction efficiency.

Targeted drug delivery

Traditional chemotherapy has long been used to improve the prognosis of cancer patients, especially those with distant metastasis. Despite its many benefits to patients, some disadvantages are still associated with the traditional method, such as nonspecificity for malignant cells and intense side effect. SPIO nanoparticles have been exploited as anticancer drug delivery vectors because of their extensive drug-loading ability, favorable biocompatibility, and positive targeting when added to an external magnetic field (EMF) or to a surface modified with selective ligands [48]. The principle of magnetic anticancer drug targeting is shown in Fig. 3. Yu *et al*. [49] developed glycerol monooleate-coated magnetic nanoparticles (GMO-MNPs) as anticancer drug carriers, and confirmed that they possess high entrapment efficiency (approximately 95%) for different anticancer drugs (paclitaxel rapamycin alone or in combination) together with sustained release for more than two weeks *in vitro*. In addition, human epidermal growth factor receptor-2 antibody-conjugated GMO-MNPs were tested for targeted therapy, and enhanced uptake in a human breast carcinoma cell line (MCF-7) was observed. Doxorubicin-loaded SPIO nanoparticles have also been extensively researched for targeted cancer therapy [50]. YCC-DOX, composed of poly (ethylene oxide)-trimellitic anhydride chloride-folate (PEO-TMA-FA), doxorubicin (DOX), superparamagnetic iron oxide (Fe_3O_4), and folate, used as a targeted therapy for liver cancer, has been evaluated in rat and rabbit models [51]. The authors claimed that YCC-DOX could be a promising anticancer drug for liver cancer.

Multidrug resistance (MDR), which results from the overexpression of ATP binding cassette (ABC) transporters on the tumor surface that actively pump a variety of hydrophobic chemotherapeutic drugs out of cancer cells, are present in a broad class of cancers [52]. Kievit *et al*. [51] discovered that SPIO nanoparticles could help overcome MDR. They examined free DOX and SPIO-conjugated DOX in wild C6 cells and DOX-resistant C6 cells (C6-ADR). Increased uptake and resistance of DOX was observed in both the wild C6 cells and DOX-resistant C6 cells compared to those in the free drug groups. Therefore, they suggested that DOX-conjugated SPIO nanoparticles have the potential of improving the efficacy of chemotherapy by overcoming MDR.

Targeted imaging

MRI has been intensively used as a noninvasive diagnostic tool since 1973. SPIO nanoparticles have been employed as MRI contrast agents to obtain better differentiation because of their magnetic properties and low toxicity [53,54]. Poly (TMSMA-r-PEGMA)-coated SPIO nanoparticles have been fabricated as cancer MRI contrast agents and cell resistance has been proven by exposure to 10% serum containing cell culture medium and macrophages. Moreover, less than 1 h after intravenous administration to tumor xenograft mice, the tumors could be detected in T2-weighted MR images through the accumulation of the nanomagnets. The experimental evidence shows that although no targeted ligands are present on the surface, the poly(TMSMA-r-PEGMA)-coated SPIO

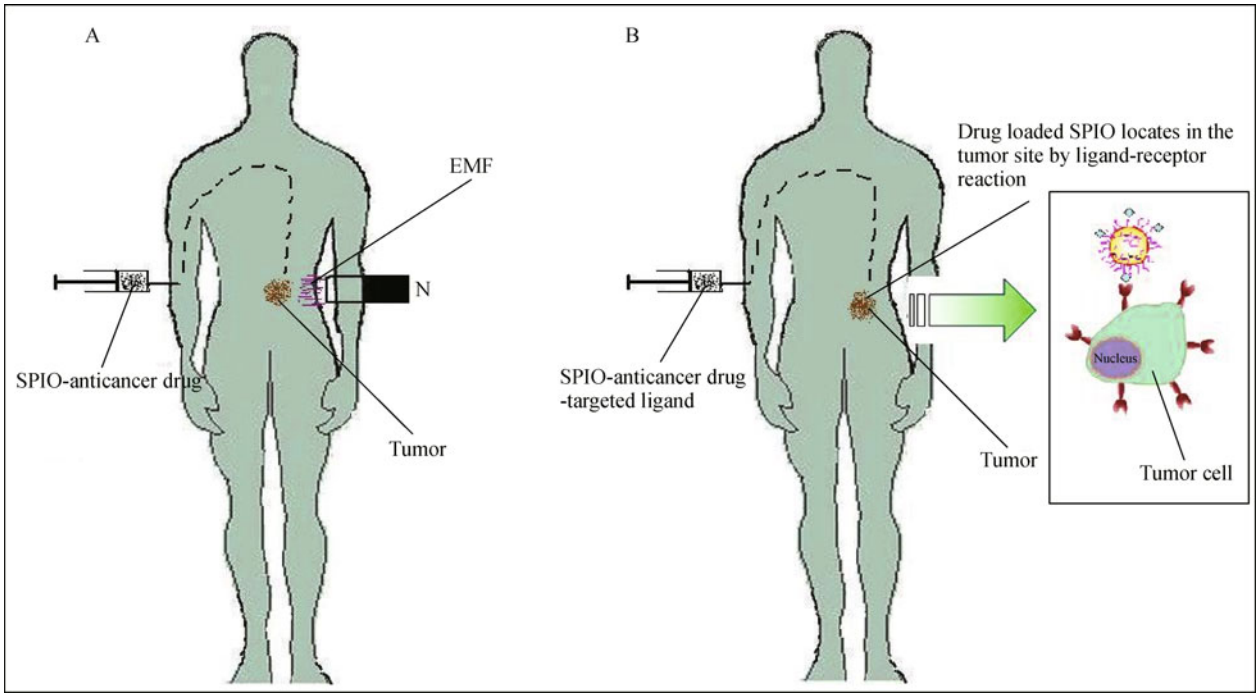

Fig. 3 Principle of magnetic anticancer drug targeting. A represents the drug-loaded SPIO concentrates in the tumor site by EMF. B represents the SPIO concentrates in the tumor site by ligand-receptor reaction.

nanoparticles are potentially useful as MRI contrast agents for cancer diagnosis *in vivo* [54]. Gadolinium-labeled SPIO nanoparticles with arginine-glycine-aspartic acid (RGD) peptide as a targeting surface ligand could be used as targeted dual-contrast T1- and T2-weighted MRI of tumors [11]. In the present study, T1 relaxivity r_1 of 4.2 $mm^{-1} \cdot s^{-1}$ and T2 relaxivity r_2 of 17.4 $mm^{-1} \cdot s^{-1}$ were manifested in the relaxivity measurements when the Gd/Fe molar ratio was 0.3∶1. These findings suggest that they can be potentially used as T1 positive and T2 negative contrast agents to improve diagnostic accuracy (Fig. 4). Nowadays, some MRI contrast agents consisting of SPIO nanoparticles, such as Feridex I.V. [37,54] and Ferumoxtran-10 (dextran T-10-coated SPIO nanoparticles) [55,56], are commercially available.

Great efforts have been exerted on studying the application of SPIO nanoparticles as contrast agents for molecular and cellular MRI. Yang *et al*. [57] bound urokinase plasminogen activator, whose receptor uPAR is highly expressed in pancreatic cancer and tumor stromal cells, to functionalize the SPIO nanoparticles for imaging pancreatic cancer with MRI. They found that uPAR-targeted SPIO nanoparticles selectively accumulated within the pancreatic tumors of orthotopically xenografted nude mice, and furthermore, they concluded that the novel nanoparticles can be used as molecular imaging agents for detecting both primary and metastatic pancreatic cancer with great potential. Galanzha *et al*. [58] employed functionalized magnetic nanoparticles as cell catchers for multiplex photoacoustic detection of circulating tumor cells. In their study, SPIO nanoparticles were functionalized with ligands that target breast cancer cells, and could concentrate the circulating tumor cells in vessels from large volumes of blood in breast tumor-bearing mice.

Concluding remarks

Cancer is a multistep process. Different selective biomarkers are overexpressed on the tumor cell surface, allowing pre-targeting diagnosis and therapy. SPIO nanoparticles have emerged as promising tools for bioscience applications because of their nanosized diameter, superparamagnetism, powerful drug and gene loading, and ability to integrate diagnosis and therapy, among others. A variety of studies have exhibited that functionalized SPIO nanoparticles, such as surface-coated, targeted, ligand-conjugated, and/or drug-loaded SPIO nanoparticles, are feasible for targeted imaging and targeted therapy. Moreover, applications that integrate diagnosis and therapy in SPIO nanoparticles facilitate the monitoring of therapeutic efficacy during treatment. However, some limitations and obstacles still need to be overcome. For example, powerful combinations of anticancer drugs with SPIO nanoparticles or flexible ligands, which yield more positive targeting of SPIO nanoparticles, are lacking. Better *in vivo* size control of SPIO nanoparticles to

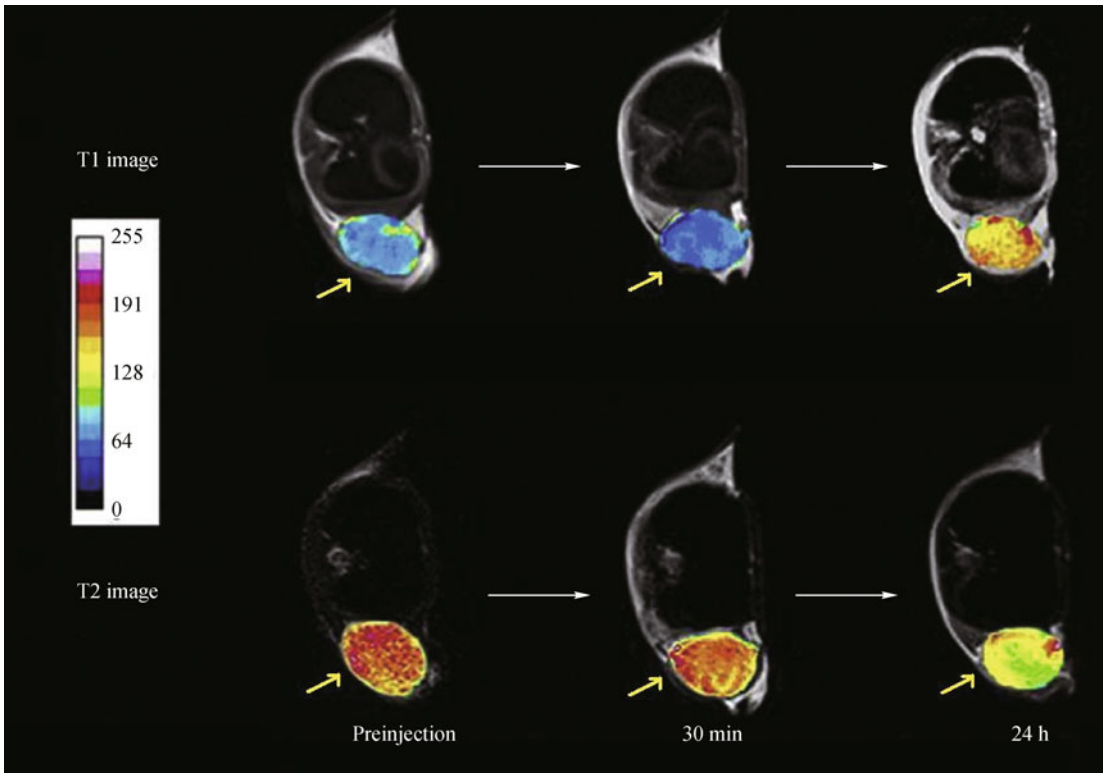

Fig. 4 T1-weighted and T2-weighted magnetic resonance images of tumor injected with Fe_3O_4-SiO_2(Gd-DTPA)-RGD NPs (yellow arrow indicates the location of tumor). T1 and T2 MR images of the tumor show no obvious changes at 30 min post-injection, but they all show significant enhancement at 24 h post-injection.

prevent quick blood clearance and nonspecific uptake by macrophages also needs to be discovered. All these limitations restrict the broader application of the nanoparticles. Although more efforts are needed to improve their tumor-targeting properties and simplify the synthesis, SPIO nanoparticles still show exciting potential as powerful tools for targeted imaging and cancer therapy.

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