

• Review •

# Application and development of magnetic iron-oxide nanoparticles in tumor-targeted therapy

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**[Abstract]** Much attention has been paid over the past few years to the studies in nanometer-sized magnetic particles due to their particularly large surface-to-volume ratio, quantum-size effect, magnetic character as well as their potential application in the area of bioscience and medicine. The most promising nanoparticles are magnetic iron oxide nanoparticles with appropriate surface modification, which have been widely used experimentally for numerous in vivo applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, drug delivery, hyperthermia and cell separation. To focus on one of the most important and fascinating subjects in nanobiotechnology, this review describes the current situation and development of magnetic iron oxide nanoparticles and their applications in drug delivery and hyperthermia in tumor-targeted therapy. The possible perspectives and some challenges to further development of these nanoparticles are also analyzed and discussed.

**Key words:** Nanotechnology, magnetic nanoparticles, iron oxide, drug delivery, hyperthermia, tumor-targeted therapy

Nanotechnology is defined as researches and technologies at atomic, molecular and macromolecular levels, which is involved in investigation and controllable operation of structure and equipment in a 1-100 nm scale range. Nanotechnology, which has been gradually developed since the 1970s, is a newly emerged science and technology frontier field with interdisciplinarity.<sup>1</sup> Material world in nanometer scale and its characteristics are relatively strange to human beings. Nanomedical research has a tremendous application prospect in modern biomedical research, disease diagnosis, and therapy. Magnetic materials are long-standing functional materials with very extensive applications. With the development of nanotechnology, magnetic nanoparticles became a new type of magnetic materials with great vitality and application prospect after the 1970s.<sup>2</sup> Recently, magnetic nanoparticles have been

applied more and more frequently in studies on biomedicine and biotechnology, including targeted drug delivery, tumor magnetic hyperthermia therapy, contrast enhancement of MRI, biosensor, rapid separation in environmental biology and concentration tracing of specific targets, such as bacteria, leukocyte, and protein.<sup>3</sup> Nowadays, cancer is one of the three major diseases which seriously threaten the health of human beings. Although medication has been extensively used to play a cancer suppressive and anticancer role in clinical tumor treatment, toxic and side effects of the drugs on normal organs and tissues are unignorable. Therefore, to increase the drug selectivity and decrease the drug aggregation to non-target sites are the keys to enhance the efficacy of anti-tumor drugs.<sup>4</sup> In recent years, rapid progress has been achieved in the studies on targeted therapy of malignant tumors. Therein, attentions were increasingly paid to the studies on tumor-targeted therapy with magnetic nanoparticles.<sup>5</sup> In 2005, National Institute of Health (NIH) of the U.S. initiated the "Cancer Nanotechnology Plan", which aimed to eliminate cancer pains and death by 2015 through a series of researches combining nanotechnology, cancer research, and molecular biology.<sup>1</sup> So far, magnetic nanoparticles in clinical application are mainly composed of magnetic iron oxide which is also the only clinical magnetic nano-material approved by American Food and Drug Administration (FDA). This review emphasizes introducing the current situation and development of magnetic iron oxide nanoparticles, elaborating the effects of drug delivery and magnetic hyperthermia in tumor-targeted therapy

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and discussing its potential development perspectives and challenges.

## Current situation and development of magnetic iron oxide nanoparticles

Compared with other magnetic materials, magnetic iron oxide nanoparticles, which are mainly composed of  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-Fe}_2\text{O}_3$ , have good chemical stability, magnetic responsiveness and biocompatibility. Moreover, the preparation method of magnetic iron oxide nanoparticles is relatively simple. During the past decades, magnetic iron oxide nanoparticles attracted attentions of numerous researchers at home and abroad because of its remarkable functional characteristics in disease diagnosis and therapy.<sup>6</sup> There has been increasingly higher expectation of the magnetic iron oxide nanoparticles due to their magnetic characteristic and low cytotoxicity.

Magnetic iron oxide nanoparticles have been successfully prepared in the forms of aqueous phase or organic phase. The surface modification, which is performed by coating desirable molecular materials on surfaces of nanoparticles, is indispensable in order to improve stability, prevent aggregation of nanoparticles, ensure nontoxic status in physiological conditions and enhance the targeting function. However, coated material must be prudently selected. Perfectly coated material should have advantages of good affinity to iron oxide core, good biocompatibility (that is, non-immunogenicity, no antigenicity, resistance of plasma protein opsonization), good biodegradation, high colloid stability, and so on.<sup>7</sup> In addition, coated material should also be able to recognize and bind to specific bioactive molecules, including monoclonal antibody, lectin, peptides, hormones, vitamin, nucleotide or drug. Generally, coated material can be divided into two types: artificial synthetic material and natural macromolecular material. Typically artificial synthetic materials include polyethylene, polyvinylpyrrolidone (PVP), polyethyleneglycol (PEG), polyvinylalcohol (PVA), and others, whereas natural macromolecular materials include gel, dextran microsphere, chitosan, and amylopectin. Currently, most of coated materials in clinical application are carbohydrates and carbohydrate-derived polymer materials. In addition to good hydrophilicity, biocompatibility and biodegradation, their inherent affinity to the core of iron oxide and property of simulating glycoprotein in biosystem are very important.<sup>8</sup>

Chitosan (CS) is a kind of linear polysaccharide consisting of repeated units of two amino and two deoxy- $\beta$ -dextro-glucan, and has good biocompatibility, biodegradation, and low cytotoxicity. Moreover, the chemical modification process of CS was also simplified because of its unique chemical and biophysical properties of the main chain of CS simultaneously containing active amido and hydroxyl.<sup>9</sup> Kekkonen *et al.*<sup>10</sup> synthesized magnetic iron oxide nanoparticles with glycosylation by chemical coprecipitation method, and surface characteristics and cell survival/cytotoxicity *in vitro* were also investigated.

Lactobionic acid is another kind of carbohydrate material which is commonly used in surface coating. Lactobionic acid is composed of two parts: galactose residues and gluconic acid,

and these two parts were linked together by an ether group. It was well known that lactobionic acid is an effective ligand which can be used to investigate the interaction between carbohydrate and hepatocytes. Selim *et al.*<sup>11</sup> demonstrated that surface modification with lactobionic acid could enhance the endocytosis of magnetic iron oxide nanoparticles, and the related mechanism may be receptor mediated endocytosis. It was also suggested that these nanoparticles had good biocompatibility as there was no change of cell morphology.<sup>11</sup>

In addition to CS and lactobionic acid, special attention has been paid to PEG in recent years. PEG not only had good biocompatibility, but also was easily conjugated with magnetic iron oxide nanoparticles with targeting molecule, which provided an “immunological ignorance” effect for nanoparticles to decrease, and even avoid the phagocytosis by reticuloendothelial system (especially hepatic Kupffer cells).<sup>12,13</sup> Moreover, coating magnetic iron oxide nanoparticles surface with amphiphilic multimer surfactant such as PEG could reduce or eliminate adsorption of nanoparticles on plasma protein in the maximal degree so as to increase blood circulation time of nanoparticles. In order to avoid recognition of nanoparticles by phagocytes, resist adsorption of protein, and promote phagocytosis of nanoparticles into specific tumor cells, Zhang *et al.*<sup>14</sup> tried to modify the surface of magnetic iron oxide nanoparticles with PEG. The inducement-paired plasma emission spectrometry demonstrated that the content of PEG-modified nanoparticles in mouse macrophages (RAW 264.7) was much lower than that of non-modified magnetic iron oxide nanoparticles.

Other than necessary surface modifications, attention should be paid to particle size in the design of magnetic iron oxide nanoparticles. Particle size is the most important characteristics of nanoparticles. Particle size not only influenced the physical property of the particle such as magnetic moment (responsiveness to applied magnetic field), but also affected biological outcomes after nanoparticles were injected into the human body (blood circulation time and bioavailability of particle *in vivo*). When particle size is less than 10 nm, nanoparticles can be rapidly cleared due to easy exosmosis or renal excretion. If the particle size is more than 200 nm, nanoparticles are easy to be mechanically filtered by spleen or phagocytized by macrophages in reticuloendothelial system, leading to decrease of blood circulation time. Nanoparticles with a particle size of 10–100 nm are ideal particles for intravenous injection. It was confirmed that this kind of nanoparticles had the longest blood circulation time. The volume of particles with 10–100 nm in size is small enough to escape the phagocytosis of reticuloendothelial system and penetrate into capillary vessels in body tissues, which ensures an effective distribution in specific tissues.<sup>14</sup>

## Application and progress in research of magnetic iron oxide nanoparticles in tumor therapy

### As drug carrier for targeted drug delivery

Recently, efforts have been made to improve the distribution of anticancer drugs in the human body and decrease the toxic

effect of anticancer drugs. Drug delivery system has emerged as a novel technology. It can not only increase the concentration of drugs in target area but also decrease the damage of normal tissues simultaneously. In numerous drug delivery systems, magnetic targeting drug delivery was considered to be the most efficient and popular system.<sup>15</sup>

In the 1970s, Widder *et al.*<sup>16</sup> proposed the concept of magnetic targeting delivery, and conducted studies on drug-loaded magnetic microparticles. As drug carrier, magnetic iron oxide nanoparticles could enter into the human body through administration with an arterial duct, intravenous or oral administration, or direct injection. Nanoparticles were distributed in specific tumor areas under an extracorporeal magnetic field with enough strength, so that loaded drugs were efficiently and directionally delivered into tumor tissues. The released drugs exerted therapeutic effects at tissue, cell or subcellular levels, and no significant influences on normal tissues were found. This is magnetic drug targeting (MDT).<sup>17</sup> By MDT administration, therapeutic dose of drug can be reduced, so that adverse effects can be reduced to a maximal degree. Widder *et al.*<sup>16</sup> have demonstrated that adriamycin-magnetic albumin microspheres (MM-ADR) were feasible and effective in experimental treatment using animal tumor models. In aspects of tumor volume and animal survival rate, the efficacy in MM-ADR group was more significantly raised than that in adriamycin alone group. Since the creative work by Widder, rapid progress has been achieved in the studies on magnetic iron oxide nanoparticles as a new sustained-release targeting drug delivery system in the aspect of tumor-targeted therapy. Magnetic iron oxide nanoparticles became the focus and hot topic of the studies on dosage forms of anticancer drug at home and abroad.<sup>18</sup> In 1994, Zhang *et al.*<sup>19</sup> injected MM-ADR into rats with hepatic tumor implantation, and found that MM-ADR aggregated in the cancerization site of rats. After 7 days, most tumor cells were inhibited, and lump of tumor disappeared. Moreover, magnetic iron oxide nanoparticles could carry more adriamycin, and the release rate of adriamycin slowed down so that the release time was extended to more than one week. Consequently, the damage of chemotherapeutic drugs on liver can be avoided. Gallo *et al.*<sup>20</sup> elucidated ultrastructural characteristics of MM-ADR using normal rats. The transmission electron microscopy showed extravascular transportation process of magnetic microspheres 2 h after injection. It was also observed that the retention time of microspheres in extravascular tissues was as long as 72 h. To some extent, it was suggested that magnetic microspheres were possible drug storage places which promoted slow and sustained release of drugs into target tissues. Magnetic iron oxide nanoparticles could enter into the main supply arteries in target tissues after injection, and were subjected to sufficient uptake and adsorption by target tissues. Because the diameter of these nanoparticles was less than 1  $\mu\text{m}$ , they could enter into microvessels in target organs prior to systemic clearance. Subsequently, these nanoparticles could be retained in arterioles and capillary vessels of target organs under extracorporeal magnetic field. The retained nanoparticles were absorbed through extravascular routes, which finally led to intracellular absorption of cells (tumor cells), exerting their

therapeutic effects consequently.

In addition to MM-ADR, many other types of drug-loaded magnetic iron oxide nanoparticles have been developed in recent years. Although it was confirmed by *in vitro* studies that camptothecin (CPT) had powerful anti-tumor effects, CPT was still not applied in clinical practice due to its poor hydrophilicity, low effect *in vivo*, severe side effect, and so on. By chemical method, Zhu *et al.*<sup>9</sup> combined CPT with polysaccharides-modified magnetic iron oxide nanoparticles, and found that these nanoparticles nonspecifically bound to protein with a low degree. Moreover, the loaded drugs could be continuously and stably released. In addition, cytotoxicity experiments *in vitro* based on hepatocarcinoma cells of 7721 cases have been performed in order to detect pharmacological activity of CPT released from polysaccharides-modified magnetic iron oxide nanoparticles and evaluate the cytotoxicity of nanoparticles. Morphological comparisons were performed among cancer cells which were cultured for 24 h using complete medium (group A), drug medium without CPT (group B), and medium containing the suspension of polysaccharides-modified magnetic iron oxide nanoparticles combined with CPT (group C), respectively. Cancer cells in group A grew with a good spreading, which implied powerful cell survival in group A. The number of cancer cells in group B significantly decreased, but no morphological changes were found. The cells in group B still spread, and retained cell activity. In group C, cancer cells swelled and spread less. Morphological changes and count decrease of cancer cells suggested that the activity of CPT loaded in magnetic iron oxide nanoparticles was enhanced, and CPT could also be effectively released into cancer cells to better inhibit the activity of cancer cells. It has been proved that 5-FU was one of effective anti-tumor chemotherapeutic drugs. Zhu *et al.*<sup>21</sup> loaded 5-FU in CS-modified magnetic iron oxide nanoparticles (MNPs). The prepared CS-5-FU MNPs had advantages of small particle size, narrow size distribution and relatively better magnetic responsivity. Moreover, *in vitro* studies on CS-5-FU MNPs demonstrated that 5-FU could be slowly released from CS-MNPs in various buffer solutions. It was also suggested that CS-5-FU MNPs had a low cytotoxicity and could significantly promote the apoptosis of tumor cells.

### Application in tumor-targeted hyperthermia therapy

Gilchrist *et al.*<sup>22</sup> put forward the concept of magnetic targeting hyperthermia therapy in the late 1950s. However, there was a great disparity between research results and clinical applications due to restrictions of materials, temperature measurement, and magnetic field. With rapid development of nanotechnology in the early 1990s, Jordan *et al.*<sup>23</sup> found that magnetic iron oxide nanoparticles had a very strong thermal effect. Under the intensity and frequency range of magnetic field applied in clinical practice, the thermal effect of these nanoparticles was much stronger than that of magnetic particles in the micrometer scale, which had a great significance in clinical application. Subsequently, systemic experiments *in vitro* were performed by Chan and Jordan. The results suggested that the inactivation effect of cancer cells induced by hyperthermia therapy using magnetic iron oxide nanoparticles under alternating magnetic field was as good as that mediated by the best method of uniform

heating-water bath heating.

Under the alternating magnetic field, magnetic iron oxide nanoparticles can absorb large amounts of magnetic energy by hysteresis loss to generate thermal energy. Cancer cells can be killed when the temperature exceeded 43°C for 30 min, but normal cells can survive at relatively higher temperature.<sup>24</sup> The heat generated by nanoparticles under the alternating magnetic field was associated with the following factors:<sup>25</sup> (1) magnetic properties and particle size of nanoparticles; (2) amplitude and frequency of in vitro magnetic field; and (3) cooling rate of blood in tumor vessels. Due to unique surface and small size effects (single domain effect) of magnetic iron oxide nanoparticles with a size of about 10 nm, the energy absorption rate of these nanoparticles under the alternating magnetic field was much higher than that of other materials, and the heating effect of these nanoparticles was more significant. Although the thermal effect of magnetic iron oxide nanoparticles can be increased with enhancement of amplitude and frequency of magnetic field in vitro, Zeisberger *et al.*<sup>26</sup> found that the reasonable magnetic field parameters with which magnetic iron oxide nanoparticles exerted hyperthermia effect were 400 kHz for frequency and 10 kA/m for amplitude.

Via perineal approach, Kim *et al.*<sup>13</sup> injected magnetic iron oxide nanoparticle suspensions into the prostate of patients with prostate cancer, meanwhile, an alternating magnetic field with high frequency was applied to the patients. Because the clearance rate of nanoparticles in tumor tissues was very low, magnetic iron oxide could continuously exert hyperthermia effect with injection of unidirectional magnetic fluid solution. Patients were subjected to hyperthermia for 60 min once a week, for 6 weeks as one course of treatment. The nano-iron content in tissue specimens was detected by computed tomography. During the treatment, 90% of median temperature in hyperthermia therapy for prostate cancer exceeded 43°C, the highest median temperature being 55°C, suggesting that magnetic nanoparticle mediated hyperthermia therapy was feasible. Yang *et al.*<sup>27</sup> discovered a tumor-targeted technology of magnetic iron oxide nanoparticles-liposome complex. Experiments in vitro and in vivo demonstrated that nanoparticles encapsulated in tumor-targeted complex could be directionally delivered into target tissues and better exert hyperthermia effect.

Recently, extensive studies have been carried out on magnetic iron oxide nanoparticles in the aspect of tumor magnetic hyperthermia therapy combined with magnetic drug targeting.<sup>28</sup> Other than magnetic induced hyperthermia effect of nanoparticles themselves, hyperthermia could also enhance the cytotoxicity of anticancer drugs and improve the body immunity. In addition, studies suggested that externally applied magnetic field could inhibit the growth of cancer tissues. The related mechanisms were as follows: affecting biomagnetic field of cancer tissues, interfering with blood and oxygen supply of cancer tissues, affecting material exchange by altering the function of cancer cell membrane, inhibiting the proliferation of tumor cells, and so on.<sup>22</sup>

## Perspectives and challenges of magnetic iron oxide nanoparticles in tumor therapy

Currently, studies on magnetic iron oxide nanoparticles in tumor-targeted therapy have become hot subjects. Magnetic iron oxide nanoparticles have potential application prospect, and their superiority has come into being. With in-depth researches, magnetic iron oxide nanoparticles will bring revolutionary changes for tumor therapy, and their application in medicine will certainly bring a new round of revolution in medical technology. However, studies on magnetic iron oxide nanoparticles are still in the experimental stage, and numerous problems await urgent solutions<sup>5,29</sup> such as (1) how to increase the activity of functional groups on nanoparticle surface so as to enhance active targeting ability of nanoparticles and inhibit the phagocytosis of reticuloendothelial system; (2) how to increase the drug loading of particles, avoid drug leakage in the drug delivery process and regulate drug release amount and rate in the locations of lesions; (3) how to solve the problem that magnetic iron oxide nanoparticles are easy to aggregate; and (4) how to set the safe dose of accumulation of magnetic iron oxide nanoparticles in vivo and reduce the side effects induced by the accumulation. In addition, scientific and reasonable applications of magnetic iron oxide nanoparticles also face new challenges. Influences of new characteristics of nanoparticles on producer, consumer, public places, and environment, especially consequences of interactions between magnetic iron oxide nanoparticles and human body or environment, still remain unclear. These uncertainties suggest that nanoparticles should be considered as a double-edged sword. Before magnetic iron oxide nanoparticles are processed into industrial products, their biological effects, mechanism of action, and elimination of toxicity should be thoroughly investigated so as to provide a theoretical basis for clinical application of magnetic iron oxide nanoparticles.

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