TOPICAL REVIEW— Magnetism, magnetic materials, and interdisciplinary research

Magnetic nanoparticle-based cancer therapy*

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Nanoparticles (NPs) with easily modified surfaces have been playing an important role in biomedicine. As cancer is one of the major causes of death, tremendous efforts have been devoted to advance the methods of cancer diagnosis and therapy. Recently, magnetic nanoparticles (MNPs) that are responsive to a magnetic field have shown great promise in cancer therapy. Compared with traditional cancer therapy, magnetic field triggered therapeutic approaches can treat cancer in an unconventional but more effective and safer way. In this review, we will discuss the recent progress in cancer therapies based on MNPs, mainly including magnetic hyperthermia, magnetic specific targeting, magnetically controlled drug delivery, magnetofection, and magnetic switches for controlling cell fate. Some recently developed strategies such as magnetic resonance imaging (MRI) monitoring cancer therapy and magnetic tissue engineering are also addressed.

Keywords: magnetic nanoparticles, cancer therapy, hyperthermia, drug delivery, targeting, magnetic resonance imaging

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1. Introduction

Nanoparticles (NPs), with sizes of 1 nm–100 nm, possess unique physical and chemical properties and play an important role in different research areas nowadays ranging from electronics[1–3] to energy[4–6] and biomedicine.[7,8] Various features of NPs like easy surface modification, attachment of bio-compatible polymers, and molecules, such as antibodies, ligands, and proteins onto their surface, make them an attractive vehicle for biomedical applications.[9,10] Among the numerous kinds of NPs, magnetic nanoparticles (MNPs) shown great promise due to their unique properties in a magnetic field with no depth-penetration limit in the human body.[11] Various kinds of MNPs have been used, including iron oxide (e.g. Fe_3O_4,[12–14] and MFe_2O_4 (M=Mn, Co, Zn)[15,16]), alloys (e.g. FePt,[17–19] PtCo,[20] and FeCo[21,22]), and multifunctional MNPs with core/shell,[23,24] dumbbell[25,26] or multicomponent hybrid structures.[27,28]

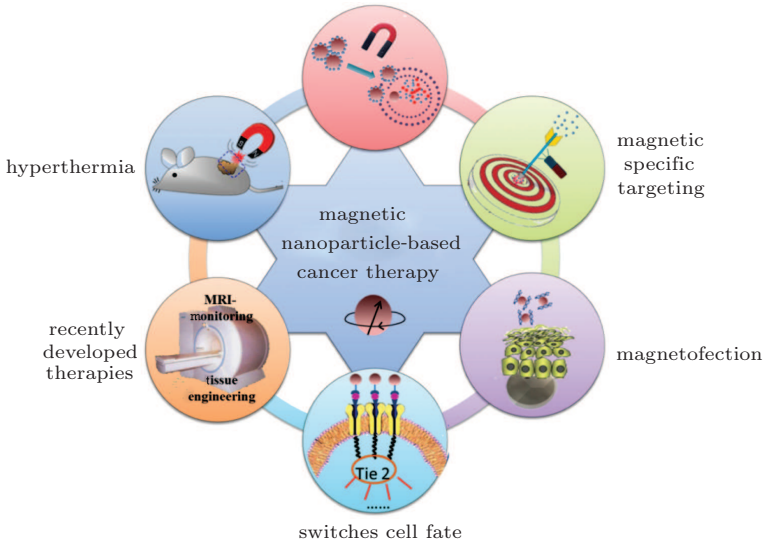

Fig. 1. Schematic illustration of MNP-based cancer therapies.

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As is well known, cancer has become one of the major causes of death due to the difficulty in accurate diagnosis and treatment. For many years now, enormous efforts have been devoted to improve the sensitivity and efficacy of cancer therapies. MNPs, with their ability to respond to a magnetic field, can target a specific site, and are potentially applied in targeted drug delivery, magnetofection, and tissue engineering. Moreover, when in a magnetic field, MNPs usually can absorb magnetic energy, raising their temperature, which may eventually induce hyperthermia, immunotherapy or controlled drug/gene release. In addition, MNPs have proved to be serviceable as contrast agents for magnetic resonance imaging (MRI) that can also be utilized as a tracing technique for drug and gene delivery. Recent progress in biomedicine has demonstrated that MNPs are becoming very important in cancer diagnosis and therapy.

In this review, we mainly focus on the MNP-based strategies for cancer therapies, like magnetic hyperthermia, magnetic specific targeting, magnetically controlled drug delivery, magnetofection, magnetic switches for controlling cell fate, and some recently developed methods (Fig. 1).

2. MNPs-based cancer therapy

2.1. Magnetic hyperthermia

Hyperthermia, treatments based on the generation of heat at a tumor site, is an attractive method for tumor therapy. According to the range of increased temperature, hyperthermia treatments can be classified into three types, i.e. thermo ablation (tumor subjected to temperatures > 46 °C), moderate hyperthermia (41 °C $< T <$ 46 °C) and diathermia ($T <$ 41 °C).[29] The traditional hyperthermia is moderate hyperthermia, which results in activation or initiation of many intra- or extra-cellular degradation mechanisms that kill cancerous tissues. More interestingly, by using such methods, cancer cells can be destroyed at these temperatures, while the normal cells survive.

The early applications of hyperthermia usually heated the whole body, which benefited from the different tolerance of temperature between cancerous and normal tissue. However, heating for a long time and on a large scale potentially harms a human body. Later on, hyperthermia was carried out by using external devices that transfer other energy, such as ultrasound, microwave or infrared radiation, into thermal energy. However, using these external devices still poses a serious threat to the normal tissues. With the probability of converting magnetic energy into thermal energy, the heating phenomenon of magnetic materials was first investigated by Gilchrist *et al.* in 1957.[30] Subsequently, Gordon *et al.*[31] applied this concept to intracellular hyperthermia by using dextran magnetite in 1979, and in 1993, the first prospective study for clinical applications in humans was reported.[32] Since then, hyperthermia using MNPs has been developing as a research hotspot, due to its unique benefits like regulation by a magnetic field, localized heating, and permeability through the blood-brain barrier.[16,33,34] In 2010, MNPs-based hyperthermia has passed preclinical stages, and received regulatory approval as a new clinical therapy, opening a new era for magnetic hyperthermia.[35]

Various kinds of mechanisms have been reported for the heat generation by MNPs under an alternating magnetic field (AMF).[36] Among them, hysteresis and relaxation behavior are the two dominant factors. Hysteresis, which originates from the internal energy of magnetic particles, is the primary mechanism for magnetic hyperthermia, especially for ferromagnetic NPs-based hyperthermia. Hysteresis loss is based on a rapid variation of magnetic moments and is basically proportional to the area of the hysteresis loop.[37] Relaxation behavior, on the other hand, which includes Brownian and Néel relaxation pathways, is another mechanism for magnetic hyperthermia, particularly in the situation of superparamagnetic NPs.[38] Brownian relaxation is caused by the entire magnetic particle rotating, while Néel relaxation is related to the magnetic moment rotating within the magnetic core (Fig. 2).[39,40] The competition of these two relaxation processes is controlled by the faster one. It is worth noting that in the case of intracellular MNPs, the intracellular components usually hinder the movement of NPs, which results in the fact that heat contribution is mostly from Néel relaxation.[29,41]

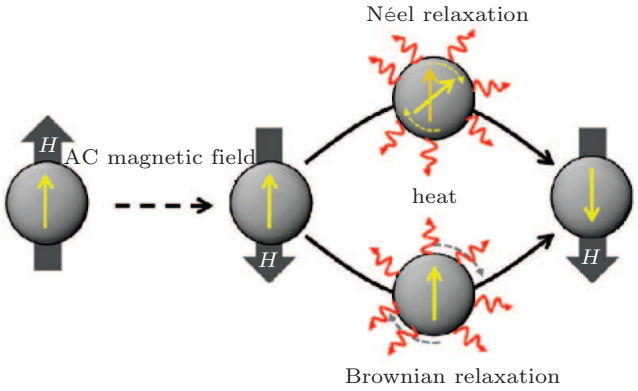

Fig. 2. Néel and Brownian relaxation processes. Reproduced with permission from Ref. [40]. Copyright 2012 American Chemical Society.

According to the heat generation mechanisms, several factors have been found to influence the heating power, including the AMF and the structures and magnetic properties of NPs. For AMF, generally, the heating power is enhanced by increasing amplitude and frequency.[37] However, the impacts of NPs are somehow more complicated. Certain properties of MNPs (e.g. magneto-crystalline anisotropy, diameter, magnetization, and homogeneity of NPs) can affect the heat generation. For example, high magnetization can increase the area of the hysteresis loop which eventually leads to a temperature increase. Poly-dispersed NPs exhibit considerable low heat generation rate compared with mono-dispersed ones; heating power is inversely proportional to the size distribution.[42] Heat

released is also strongly dependent on anisotropy (K) and diameter (D). It has been reported that in order to achieve high heat generation, K and D should be in their optimal ranges, both of which differ from one kind of MNPs to another.[43] Simply put, by tuning the structure, morphology, and size of MNPs, high heat efficiency can be achieved.[16,44,45]

By using the thermal energy generated by MNPs, cancer can be conquered by triggering cell death directly or prompting the immune system, and cancer cell death occurs by either necrosis or apoptosis.

2.1.1. Magnetic hyperthermia stimulated apoptosis

Apoptosis, which retains most cell membrane functionality and does not elicit inflammation, induces cells death in a programmed and biochemically active way. This process can be identified by cell shrinkage, the chromatin condensation and DNA fragmentation.[46] Remarkably, during apoptosis, several key morphological events occur, including the formation of membrane blebbing, cell rounding, and detachment of actin.[47] It has been reported that γ-$Mn_xFe_{2-x}O_3$ $(0 \leq x \leq 1.3)$ based magnetic hyperthermia can lead to cancer cell apoptosis.[48] Compared to cells without AMF and magnetic particles, cells with γ-$Mn_xFe_{2-x}O_3$ exposed to AMF for 30 min and further incubated for 4 h show membrane blebbing on their surface. After 8-h incubation, the actin cytoskeleton was significantly disrupted, and 12-h incubation completely destroyed the actin cytoskeleton. The mechanisms of these apoptotic phenomena are attributed to the stimulation of "initiator" cysteinyl aspartate-specific proteases (caspases) to unfold the extrinsic or intrinsic pathway, such as activation of the tumor necrosis factor (TNF) and death receptors-4 and -5 (DR4/5).[49] Among all of the caspases, caspases 3 and 7 are known as essential proteases, playing dominant roles in triggering apoptotic processes in mammalian cells.[50] Cheon *et al.*[45] reported that by using chitosan oligosaccharide-stabilized ferromagnetic iron oxide nanocubes (Chito–FIONs), both caspases 3 and 7 can be stimulated by magnetically modulated cancer hyperthermia, i.e. only Chito-FION bound cells in the area exposed to a magnetic field significantly enhanced the activation of the caspases (Figs. 3(a) and 3(b)), and this method showed much higher apoptotic population compared with commercially available Feridex (Fig. 3(c)), with excellent antitumor efficacy on an animal tumor model without severe toxicity.

Also, a 3-fold increase in TNF-α gene expression has been produced by magnetically induced heating, and this induces cell death at the tumor area.[51] Astonishingly, apoptosis induced by magnetic hyperthermia is more severe than that by hot water, indicating an additional effect on cell viability in magnetic hyperthermia. Although the mechanisms for the increased apoptosis are still being investigated, this highlights the potential of magnetic hyperthermia for more efficient cancer therapy.[52]

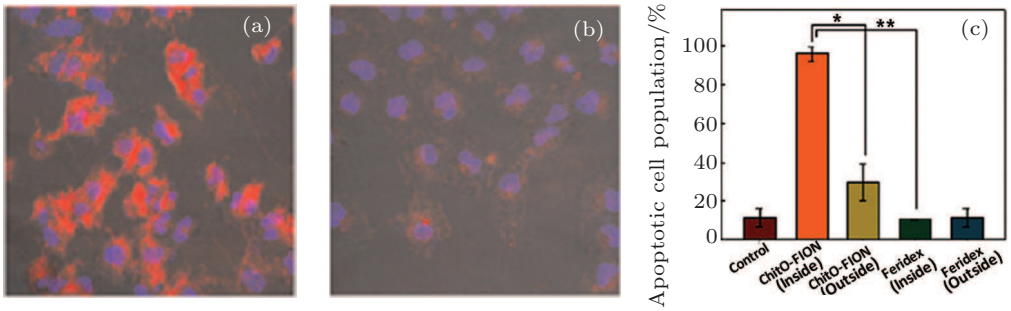

Fig. 3. (a) and (b) Cellular apoptotic activity of Chito–FIONs-treated A549 cells inside (a) and outside (b) the area exposed to a magnet and subsequent application of an AMF. Cellular apoptotic activity was detected by using a red fluorogenic substrate for caspases 3 and 7 (red), and cell nuclei were stained with DAPI. (c) Apoptosis of A549 cells induced by the localized magnetic hyperthermia with Chito–FIONs or Feridex. Statistically significant difference between two groups, $^{*}p < 0.01$, $^{**}p < 0.001$. Reproduced with permission from Ref. [45]. Copyright 2012 American Chemical Society.

2.1.2. Magnetic hyperthermia induced necrosis

Apoptosis usually takes place at the early stage of hyperthermia. With the increase of temperature, cell apoptosis will decrease, with a concomitant increase in necrosis.[49,53] Necrosis is a process of cell death triggered directly by destroying the cellular structure, and complete dysfunction of metabolic pathways leads to cell death. It is well known that the integrity of the plasma membrane is a key factor for cell viability, and therefore, the most commonly used method for hyperthermal necrosis is to destroy the membrane integrity by heat. It is regarded that after being engulfed in an endosome and exposed to AMF, MNPs can disrupt the endosomal membrane by heat, and the released endosomal content may induce damage to the cell membrane.[35] It is also reported that dextran coated iron oxide can be used to induce a necrotic-like cell death, including loss of membrane structure and shrinking of the cells (Fig. 4).[54] Moreover, heat generated from NPs dropped on the membrane (not engulfed into cells) can also disturb the membrane structure directly.[44] Thereby, combined with magnetic hyperthermia induced apoptosis, hyperthermal necrosis may also be a promising means of cancer therapy.

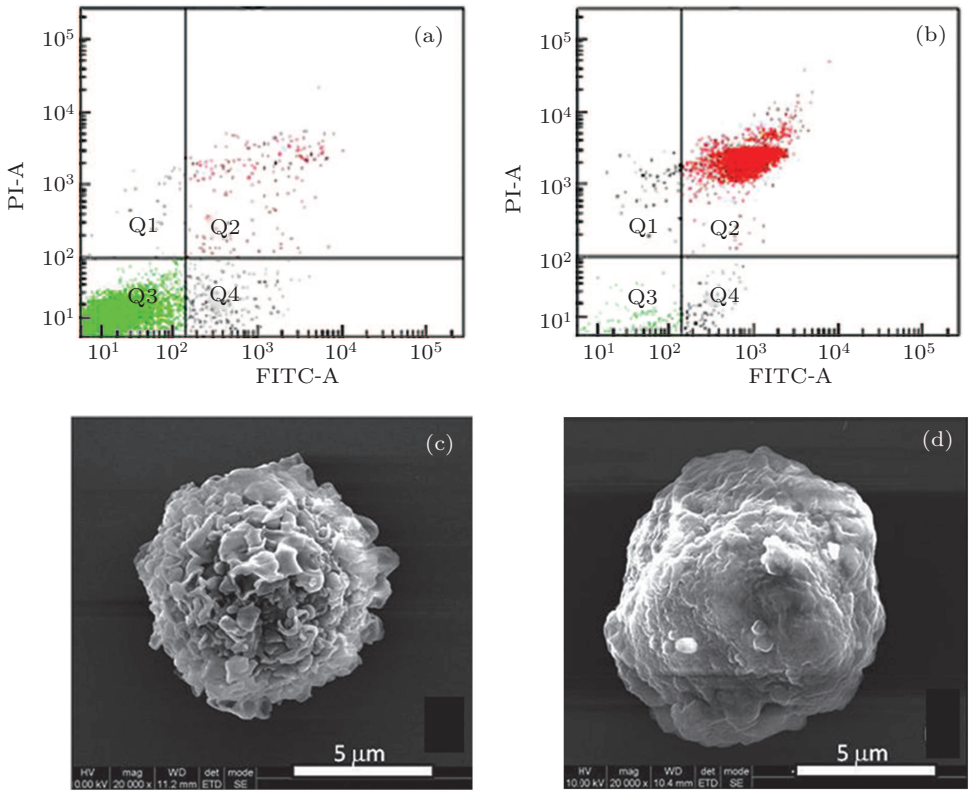

Fig. 4. Viability from FACS for (a) non-loaded cells with magnetic field off and (b) cells loaded with magnetic particles after application of AMF for 30 min, and (c) and (d) the corresponding SEM images of cells. Reproduced with permission from Ref. [54]. Copyright 2011 IOP Publishing.

2.1.3. Heat-inducible immunotherapy

Not only can hyperthermia treatment kill cancer cells directly by heating, but it is also able to activate an immune response by heat shock. This effect gives rise to the reduction of both primary tumors and metastatic lesions.[55,56] It is reported that natural killer cells, such as T cells, can be activated under a temperature of about 42 °C, due to the presence of heat shock proteins (HSP). Meanwhile, HSPs, which can be induced by heat stress, not only protect cells from heat-induced apoptosis,[57] but also can chaperone tumor antigens, and thus can induce an antitumor immunity.[58] Kobayashi *et al.*[59] observed this phenomenon in 1998, in transplanting a T-9 rat glioma tumor model into each femur of a rat. Despite the fact that only one tumor was subjected to hyperthermia with magnetite liposomes, both tumors disappeared after therapy. Moreover, the tumor can only be inhibited in immune-competent syngenic rats after hyperthermia, and cannot in nude mice,[60] suggesting that the therapy was effective through an immunity mechanism. This interesting phenomenon is attributed to the heat generation after cellular uptake of MNPs and AMF exposure. The heat released can increase the intracellular HSP, followed by formation and augmentation of HSP-peptide complexes (upper route in Fig. 5),[55] and induce necrotic cell death as discussed previously (lower route in Fig. 5).[61] Both of these routes can release HSP or their peptide complexes, activate neighboring T cells,[62] stimulate monocytes, produce proinflammatory cytokines,[63,64] and eventually stimulate the innate immune system to treat the cancer. This hyperthermia triggered immunotherapy shows great promise for tumor therapy, especially for metastatic tumors, which are still a challenge.

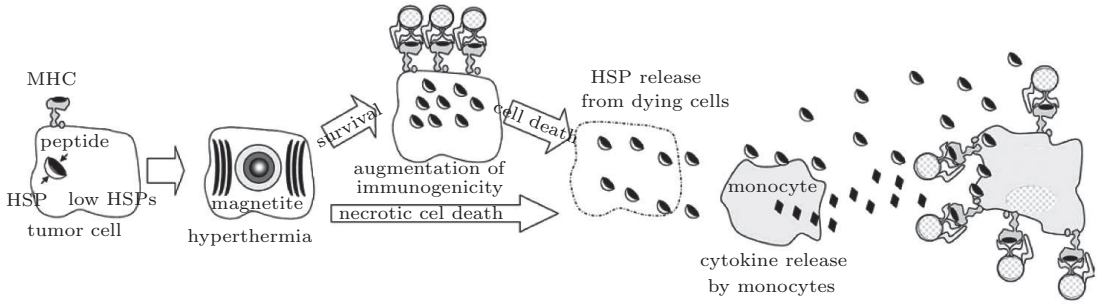

Fig. 5. Proposed scenario for the mechanism of anticancer immune response induced by hyperthermia. Reproduced with permission from Ref. [61]. Copyright 2005 Springer-Verlag.

2.2. Magnetic specific targeting

Although the potential benefits of NPs are considerable, potential toxicity has been reported.[65,66] A lot of methods have been developed to reduce the dose of NPs in order to reduce the potential toxicity, including passive targeting based on the enhanced permeability and retention (EPR) effect and positive targeting by conjugating active tumor-specific molecules, such as folic acid,[67] anti-Her2,[68] and RGD.[69] Unlike other NPs, MNPs have their unique targeting properties allowing exploitation of their response to an external magnetic field, which is denoted as magnetic targeting. After being exposed to an external magnetic field, MNPs can be magnetized, driven magnetically, and concentrated at a specific target site (Fig. 6).[70] The magnetic local targeting strategy reduces systemic distribution of the NPs, consequently reducing the dosage required and eliminating associated side effects.[71] Furthermore, the magnetic targeting can fix particles at a local site when it is desired to keep them away from the reticuloendothelial system (RES).[72] After NPs are intravenously administered and concentrated within the body, a competition forms between forces exerted on the particles by the blood vessel and magnetic forces. When the magnetic forces exceed the blood flow rates in arteries (10 cm/s) or capillaries (0.05 cm/s), the MNPs are retained at the target site and may be internalized by the endothelial cells of the targeted tissue.[73]

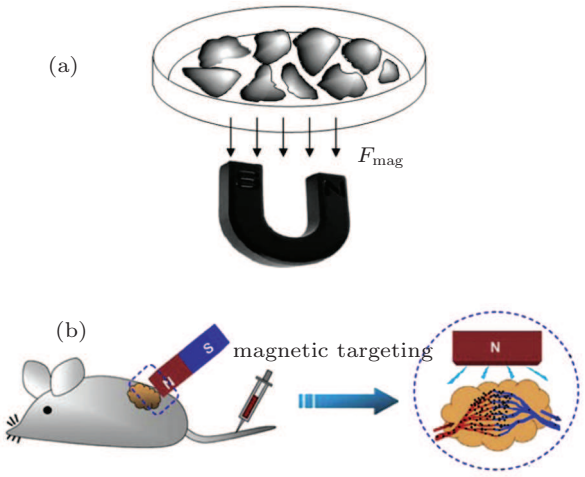

Fig. 6. A schematic illustration shows the concept of magnetically targeting *in vitro* (a) and *in vivo* (b). Reproduced with permission from Ref. [70]. Copyright 2006 Nature Publishing Group. And reproduced with permission from Ref. [78]. Copyright 2012 Elsevier.

Magnetic targeting was first developed in 1963 when Meyers *et al*.[74] accumulated magnetic iron particles in the leg vein of a dog by applying a horseshoe magnet, followed by some similar work in the decades after that.[75–77] However, further progress was halted until the blooming development of MNPs that showed great magnetic susceptibility. Recently, Liu *et al*.[78] discovered that by placing a magnet near a tumor, MNPs can be migrated toward the tumor after intravenous injection with about 8-fold higher accumulation in the tumor than would occur without magnetic targeting. This remotely and noninvasively targeting control process is unique and highly effective, and can be used as a technique of actuation for drug delivery and gene translation.

2.3. Magnetically controlled drug delivery

Various kinds of nanomaterials have been developed for drug delivery, including inorganic NPs, micelles, and polymers. However, the major problems of therapeutic drug delivery come essentially from the lack of specificity with high cytotoxicity, which results in high side effects.[79] To improve drug localization, magnetic force with an implanted or externally applied permanent magnet has been exploited as magnetically targeted drug delivery. This method dates back to 1978, when it was reported by Widder's group.[80] Since then, a great deal of work has been reported aiming to achieve a high drug concentration in the diseased area with fast response time and minimum side effects, including the delivery of drugs to a cancerous lung,[17,81] prostate,[82] brain,[83,84] melanoma,[85] breast,[86–88] or liver.[89,90] The pioneering work for the first ever Phase-I was carried out by Lübbe *et al.* in 1996,[91] followed by the second clinical trial, conducted by Koda *et al.* in 2002, and a third in 2004.[92–95] It is worth noting that hollow MNPs, with higher absorbance areas, can achieve greater drug loading efficiency than solid ones and consequently are more effective in killing cancer cells. We found that hollow iron oxide NPs can be loaded with more of a drug than solid iron oxide NPs of the same core size using the same coating strategy. Unaffected by the drug efflux phenomenon that exists in resistant cancer cells, hollow iron oxide NPs can be taken up by multidrug resistant OVCAR8-ADR cells more effectively than free drugs (Figs. 7(a) and 7(b)),[96] suggesting the NPs' potential as drug delivery vehicles, especially for multidrug resistant cells.

MNP-based drug delivery not only transports the drugs to a specific site but also permits remote control of drug release. Drugs attached to MNPs through a heat sensitive linker enable them to be released in a controlled manner by varying the AMF, which can generate heat.[97,98] Also, drugs can be attached to NPs through π–π interaction or hydrophobic interaction, in which the improvement of drug release can also be enhanced after magnetic heating, as the desorption of drugs has been proved to be an endothermic process.[99] Moreover, in some cases, drugs can be loaded into porous materials with valves (Fig. 7(c)). The magnetic heat generation can build up pressure inside the porous NPs, subsequently removing the molecular valves and triggering the drug release.[40,100] In some other situations, drugs and MNPs can be co-embedded within temperature-sensitive polymers. By local heating or mechanical force induced by magnetic particles, the polymers crack, shrink or deform, followed by the desired drug release (Fig. 7(d)).[101–104] Interestingly, compared to normal heating at 45 °C, the drug release rate of temperature-sensitive

polymer-coated iron oxide during AMF condition, is 20 times improved, due to recrystallization of NPs and shrinkage of polymers.[105] It has been demonstrated that the synergistic effect of magnetic heating, magnetic disruption, and recrystallization can initiate the drug release with high precision, and this highlights the prospect of using MNPs for controlled drug delivery and release. Recently, Zhang *et al.*[106] reported a new method of magnetically controlled drug release and showed that the drug release rate decreased dramatically due to the aggregation of the MNPs, and increase of the drug diffusion path.

Although no magnetic nanosized drug carriers are used clinically yet,[55,107] it is still anticipated, due to the promising results in preclinical investigations.

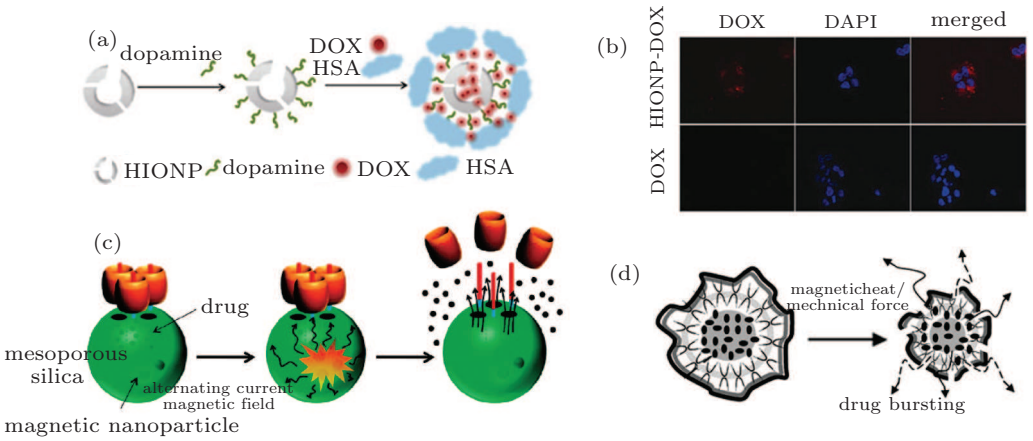

Fig. 7. (a) Schematic illustration of drug delivery system based on hollow iron oxide NPs. (b) CLSM visualization of free drug and drug loaded iron oxide NPs uptake by resistant (OVCAR8-ADR) cells. (c) and (d) Schematics of remotely controlling drug release through (c) molecular valves, and (d) shrinkage or deformation of polymers.[40,96,105] Reproduced with permission from Ref. [96]. Copyright 2012 Springer-Verlag. Reproduced with permission from Ref. [40]. Copyright 2012 American Chemical Society. Reproduced with permission from Ref. [105]. Copyright 2009 John Wiley & Sons.

2.4. Magnetofection

Numerous methods of gene transfer, including biological, physical, and chemical approaches have already been reported. However, due to the side effects of previously developed methods (e.g. unpredictable distribution, low concentration at the target site, and stimulation of immune response[108]), new strategies with high efficiency, target specificity, and considerable safety are still being explored. MNP-based transfection, often called magnetofection, is a new method, which can rapidly lead the applied vectors fully to the target cell surface within a short time.[109] It benefits from the magnetic force, which can lead both to enhanced transfection efficiency and to targeting the specific site. After attaching cells, magnetofection undergoes a similar mechanism with non-magnetic gene delivery, i.e. internalization into endosomes, escape from endosomes through a "proton sponge effect," diffusion inside cells, and transportation along microtubules.[109–112] This concept was raised by Byrne *et al.* in 2000 using an adeno-associated virus linked to magnetic microspheres.[113] Since then, magnetofection has been applied by transfecting blood vessel endothelial cells, lung epithelial, osteoblasts, aminocytes, and keratinocytes.[70]

Both DNA and RNA have been transfected by MNPs. The initial research was mainly focused on DNA, which was directly attached to MNPs or carriers via charge interactions. Compared with commercially available transfection agents or magnetic gene carriers without a magnetic field, magnetofection in the presence of a magnetic field can remarkably enhance the transfection efficiency in cell cultures,[114–116] as well as provide a better-tolerated and more easily controllable scenario *in vivo*.[117,118] Later on, some research on magnetofection of small interfering RNA (siRNA), which can knock down gene expression, was reported. It can effectively silence gene expression, i.e., knocking down eGFP expression in HeLa cells, brain cancer cells (C6 cells, SHEP cells), breast cancer cells (MCF7), prostate cancer cells (TC2 cells), and glioblastoma cells (U251 cells).[119–121] More recently, magnetic delivery of short hairpin RNA (shRNA) has also been demonstrated.[122,123]

However, so far, most of the work in magnetofection is centered on reducing the dosage required or accelerating transfection kinetics. Recently, Dobson's group found that, similar to remote control drug delivery, oscillating magnetic arrays can further enhance the overall efficiency of magnetofection.[95] This method can increase the transfection levels up to tenfold compared with magnetofection with static magnetic fields in HEK293T cells, H292 human lung epithelia cells, mouse embryonic fibroblasts cells, and human umbilical vein endothelial cells.[70,124,125] Although the underlying mechanisms are not well understood, based on the fact that the mechanical stimuli affects cellular membrane traffic –

both endo- and exo-cytosis – Dobson *et al.*[109] attribute this to the association of magnetic vectors with membranes and the transmission of mechanical forces from the lateral movement of the magnetic field to cellular membranes (shown in Fig. 8). Therefore magnetofection may be a promising way for gene delivery in the future.

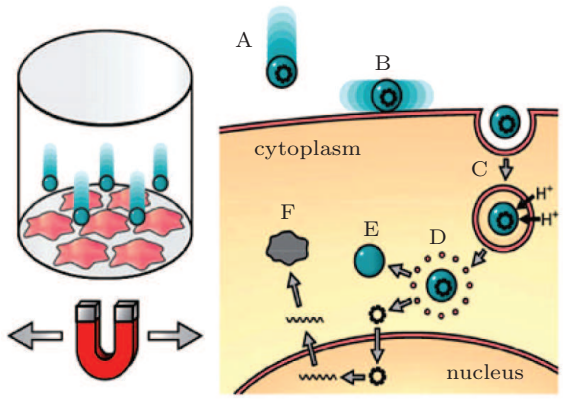

Fig. 8. Principle of oscillating nanomagnetic transfection. Plasmid DNA or siRNA is attached to MNPs and incubated with cells in culture (left). An oscillating magnet array below the surface of the cell culture plate pulls the particles into contact with the cell membrane (A) and drags the particles from side to side across the cells (B), mechanically stimulating endocytosis (C). Once the particle/DNA complex is endocytosed, proton sponge effects rupture the endosome (D) releasing the DNA (E), which then transcribes the target protein (F). Reproduced with permission from Ref. [125]. Copyright 2012 Indian Academy of Sciences.

2.5. Magnetic switches for controlling cell fate

While being exposed to a small magnetic field, cells may experience some changes in signaling pathways, such as F-actin arrangement, cell alignment, intra-cellular ion fluctuations, and mitochondria activation.[126–128] However, the effects on cell growth under normal culture conditions with magnetic field alone are extremely small.[129,130] By introducing magnetic materials, however, the influence of a magnetic field on cells is enhanced dramatically due to the following reasons. MNPs can produce their own magnetic field, influencing the tissue area around them through more intensive interactions or biophysical effects.[126] More importantly, MNPs can be conjugated with antibodies or some ligands, which would bind to certain receptors of interest. Then, under an external magnetic field, MNPs can move on cell surfaces or between cells generating mechanical stimulations of magnetic drag, rotation or twisting, and inducing cellular activity by influencing cell growth, differentiation or death.[131,132] Therefore, controlling cell fate by a magnetic switch is becoming more and more popular. One of the earliest biological applications of magnetic materials is to investigate the properties of cytoplasm under an applied stress, and the first application was raised by Heilbrunn and Siefriz in 1920s.[133] From then on, magnetically induced cellular response has been applied to a wide range of cell types, such as endothelial cells,[134,135] macrophages,[136] glioma cells,[137] and stem cells,[138,139] for both magnetically controlling cell fate by activation of ion channels and regulation downstream.

2.5.1. Magnetically activated ion channels

The pioneering work of magnetically controlling cell fate was focused on magnetic activation of the hydro-mechanical properties of the cells or ion channels.[140–142] In 1949, Crick *et al.* quantified the rheological properties of cells by magnetic force, a method further exploited by Ingber and his co-workers.[134,143] Because ion channels are essential to a cell's fate, two activation methods have been explored to control ion channels. The first one is to regulate the mechano-sensitive ion channels, as these channels can respond to membrane or cytoskeletal deformation (Fig. 9(a)).[133] Dobson *et al.*[144] have developed an MNPs-electromagnet model which can induce stretch-activated Ca^{2+} flux in fibroblasts by placing collagen-coated ferric oxide beads on the plasma membrane of substrate-attached fibroblasts in 1995, and then their group developed the magnetic micro- and NP mediated activation of mechano-sensitive ion channels in 2005.[145] Pommerenke *et al.*[146] also reported stimulation of integrin receptors by using a magnetic drag force device that induces an intracellular free calcium response.

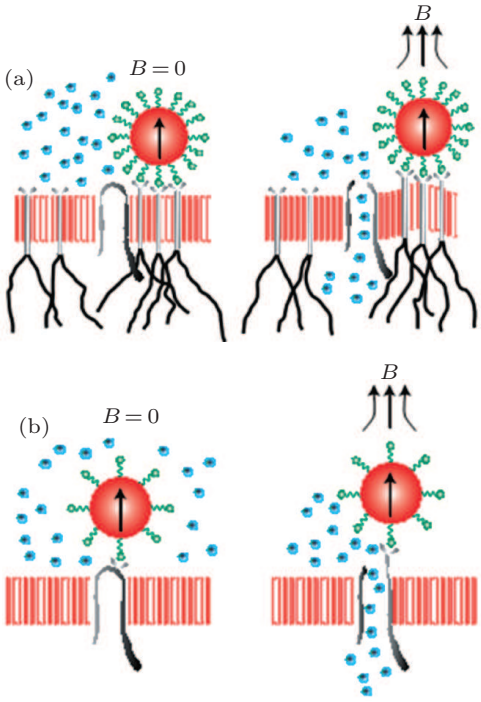

Fig. 9. Schematic representation of different types of nanomagnetic actuation of ions. (a) Mechano-sensitive ion-channel activation: magnetic particles are bound to the integrin receptors (left). Upon the application of a high-gradient magnetic field (right), the particles are pulled toward the field, deforming the cell membrane and activating adjacent mechano-sensitive ion channels. (b) Targeted ion-channel activation: MNPs are attached to an ion channel via an antibody (left). Upon activation of a high-gradient magnetic field source (right), the ion channel is forced open. Reproduced with permission from Ref. [133]. Copyright 2012 Nature Publishing Group.

However, the magnetic materials used in these experiments were almost in a micrometer-scale, so they can hardly manipulate a single receptor without disturbing other parts of cells. The inaccuracy in controlling the spots being affected makes this technique need a stronger magnetic field. With the development of nanotechnology, nanoscale magnetic materials enable switching ion channels by targeting specific ion channels and cell membrane receptors, in order to accurately initiate controlled cell responses.[133] Ingber *et al.*[147] created magnetic switching of calcium signaling in cells by applying magnetic fields to magnetize the bound superparamagnetic nanobeads and physically induce cohesion and aggregation of nanobead-receptor complexes on the cell membrane (Fig. 9(b)). Dobson *et al.*[142] also activated K^+ ion channel by using 130-nm MNPs through a member of the 'background leak' family of tandem pore potassium channels, TREK-1. With this magnetic triggering method, ion channels can be well controlled.

2.5.2. Magnetic stimulation for direct control of cell destiny

Despite the actuation of cell signal transduction by switching ion channels with a magnetic field, the direct stimulation of candidate mechano-transducers using magnetic materials can also change other cellular properties, such as cell shape, cytoplasmic viscosity, cytoskeletal organization, and even cell fate.[134,143] To accomplish this, both a static magnetic field and an alternating magnetic field have been applied.

Definitely, MNPs, especially ferromagnetic NPs, could induce strong attractive forces between the dipoles of neighboring NPs, and aggregate under a static magnetic field. This aggregation will induce the clustering of a specifically targeted protein, and finally, influence the cell fate. Although the clustering of receptors can naturally be induced by multivalent biochemical ligands,[148] MNPs, with comparable size and modified with meaningful biological molecules such as DNA or proteins, show great importance for magnetically regulation of cell fate with high flexibility. Cheon *et al.*[149] magnetically manipulated Tie2 receptors on 293-hTie2 cells by employing TiMo214 monoclonal antibody-conjugated Zn^{2+}-doped ferrite MNPs. After exerting an external magnetic field with horizontal magnetic field lines, magnetization of the NPs accelerate the aggregation, which promotes the clustering of Tie2 receptors, induces intracellular signaling processes, and finally leads to angiogenesis (Fig. 10). Clustering of death receptors, such as the TNF-related apoptosis inducing ligand (TRAIL) through docking of biochemical ligands is another way to activate extrinsic apoptosis signaling pathways for inducing apoptosis.[150] Based on this phenomenon, Cheon *et al.*[151] further developed a technique recently by using a targeting antibody conjugated with zinc-doped iron oxide NPs. The antibody can target death receptor 4 (DR4) in DLD-1 colon cancer cells. When a magnetic field is applied, aggregated MNPs induce clustering of the DR4s in a manner similar to TRAIL, and consequently promote apoptosis signaling pathways without damaging the cell membranes.

Except for inducing clustering of receptors, MNPs can produce an additional magnetic field. It has been reported that a static magnetic field can induce strong and replicable alterations of cell shape and plasma membrane in various cell types for controlling cell shape, motility, division or adhesion.[152,153] Therefore, a new approach for targeted cell therapy accomplished by controlling the cell growth has been developed recently. By conjugating cells with ferromagnetic NPs and exposing them to a magnetic field, the NPs are magnetized and form a local magnetic field. Consequently, the growth of the NP-containing cells will be significantly increased, as well as increasing the anti-apoptotic effect.[126]

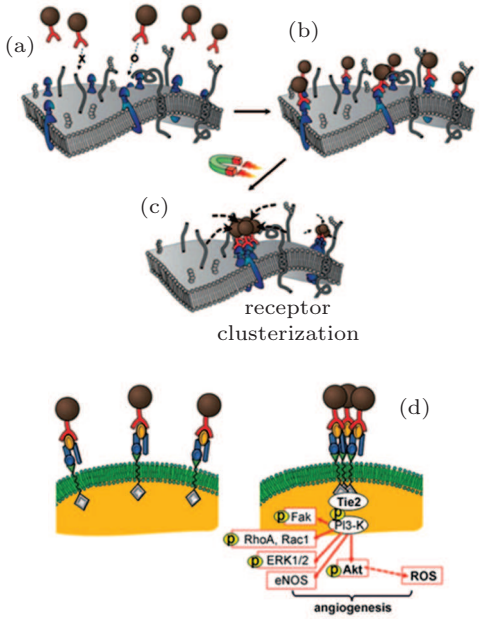

Fig. 10. Targeting and magnetic manipulation of Ab-Zn-MNPs. (a) and (b) Ab-Zn-MNPs selectively bind to the specific cell-surface Tie2 receptors. (c) In the presence of an external magnetic field, the Ab-Zn-MNPs are magnetized to form nanoparticle aggregates, and induce the clustering of receptors to trigger intracellular signaling. (d) Tie2 receptor-bound NPs before and after application of the magnetic field. Reproduced with permission from Ref. [149]. Copyright 2010 John Wiley & Sons.

Apart from the static magnetic field, an alternating magnetic field can also activate the MNPs. Compared with the common magnetic hyperthermia effect, MNPs can respond to an alternating magnetic field with a lower oscillating frequency. Under a spinning magnetic field, MNPs, especially particles with separate magnetic and non-magnetic parts, can move or rotate accordingly, and the resulting mechanical forces could become an effective tool for cancer treatment. Gao *et al.*[154] took advantage of this interesting property, and designed a nano-composite with magnetic and optical blocks spatially separated for a special therapy, called magnetolytic therapy. Due to the asymmetry in spatial distribution of magnetic components, the particle can rotate under a spinning

magnetic field after attaching to a cell, and therefore, a majority of the tumor cells are killed owing to the compromised integrity of the cell membrane and the promoted apoptosis. This magnetic control of cellular fate shows great advantages over a conventional biochemical ligand system, especially in the remote control approach, and shows tremendous promise for future applications.

2.6. Recently developed therapies

2.6.1. MRI-monitoring cancer therapy

Although various therapeutic methods have been reported in the past few decades, approaches for monitoring the bio-distribution and therapeutic efficiency of the agents still need to be established. Fortunately, magnetic resonance imaging (MRI), which is based on the response of hydrogen spin to a magnetic field, has emerged as an important diagnostic tool due to the high special resolution, and it can monitor therapeutic effects effectively.[155] MNPs have been widely used as MRI contrast agents, and among them, superparamagnetic NPs are commonly applied as T_2 contrast agents, while paramagnetic NPs are usually used for T_1 contrast agents. Consequently, MNPs can also be applied in MRI-traced cancer therapy. The most useful application of this strategy is MRI-tracked drug delivery, in which the position, the dose, and the metabolization of drugs can be traced.[156–158] Remarkably, T_1 contrast agents are preferred due to positive signal enhancement, and therefore, paramagnetic manganese oxide or manganese phosphate NPs, with five unpaired electrons, are of great research interest recently. We developed a hollow manganese phosphate NP-based drug delivery system, which is sensitive to pH value and enables tracing drug delivery by MRI. With great cellular uptake by a folic acid-mediated method, folic acid positive cells show high drug release as well as a bright T_1 MRI signal, compared with low drug delivery and dark MRI signal from folic acid negative cells (Fig. 11(a)).[159] Further, we discovered that the MRI signal can be improved by increasing the number of siRNA-loaded MnO transfected cells (Fig. 11(b)),[160] proving this approach can also be used for supervising gene therapy. This promising technique can also monitor changes of tumor size and its microenvironment, as well as atherosclerosis,[161,162] opening a new era for the efficient management of therapy, in which the mechanism and effect of therapy is "visible."

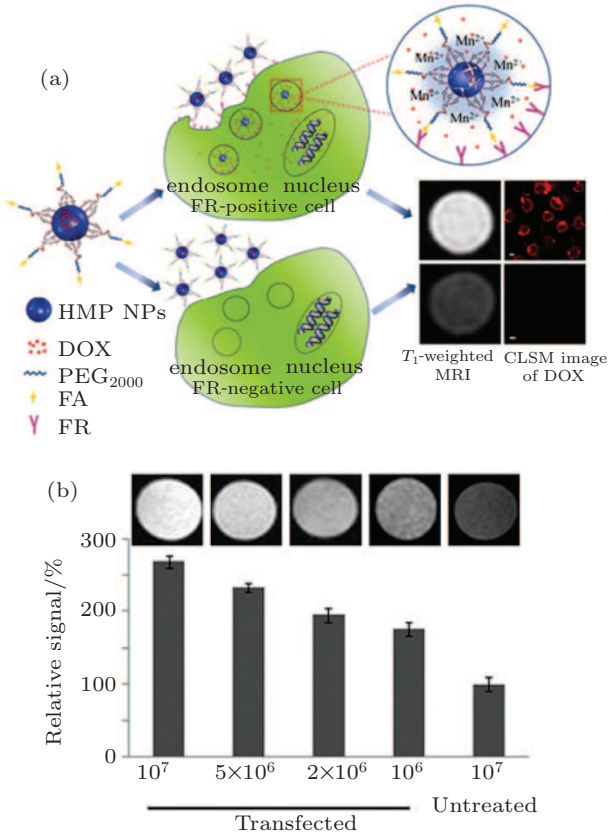

Fig. 11. (a) Schematic illustration of the concept for MRI-monitored drug delivery by DOX-loaded hollow manganese phosphate NPs. (b) Relative MRI T_1 signal intensity at different cell amounts after transfected by siRNA-loaded MnO. Reproduced with permission from Ref. [159]. Copyright 2012 Springer-Verlag. And reproduced with permission from Ref. [160]. Copyright 2011 Royal Society of Chemistry.

2.6.2. Magnetic force-based tissue engineering

Since cells labeled with MNPs can be manipulated by magnets, Ito *et al.*[163] developed a new tissue engineering method based on magnetic force. Compared to conventional tissue engineering, which relies on substrate chemistry or physical modifications, magnetic force-based tissue engineering achieves the organization of cells on substrate by controlling the magnetic field, and can create a 3D multicellular configuration with a flexible pattern.[164] This method has proved to be very promising for applications in manufacturing thick tissue sheets,[165–167] cellular clusters of controlled size,[168,169] and specific shapes or constructs,[170,171] by varying the magnetic field and number of cells (Fig. 12).

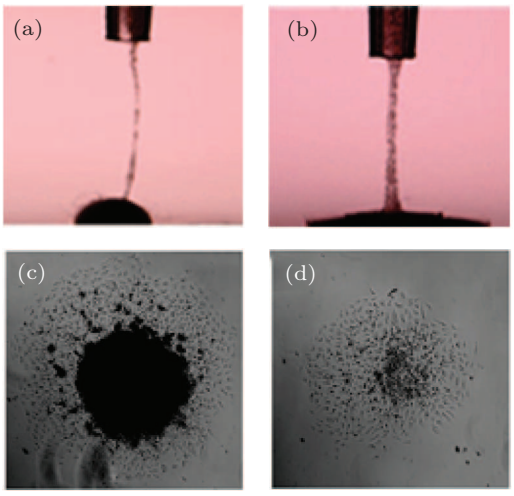

Fig. 12. (a) Magnetically labeled cells are attracted by the magnetized tip and pile up to form a 3D multicellular assembly of controlled dimensions. (b) The same cell suspension spreads over the substrate when no magnetic force is applied. (c) and (d) Photographs of cells after 24-h magnetic deposition, with high (c) and low (d) cell count. Reproduced with permission from Ref. [168]. Copyright 2009 American Chemical Society.

Furthermore, an ideal tissue engineering strategy should possess a high cell density for cell fusion and a highly unidirectional orientation for facilitating large forces.[169] Magnetic force can be regulated by the magnetic field to control the optical cell density, as well as the orientation. This technology is applied in various kinds of tissue engineering now, including stem cell research,[172–174] skeletal muscle tissues,[169,174–176] stented pericardia,[177] bones,[178] and orthopedic research.[179] Nowadays, magnetic force-based tissue engineering attracts much attention and is becoming a promising strategy in tissue engineering.

3. Conclusions and perspectives

Cancer represents one of the biggest problems for modern societies. It is estimated that by 2020, cancer deaths worldwide could reach 10 million. An important goal of cancer research is to improve anti-cancer treatment options to alleviate cancer-related morbidity and mortality. Therefore, MNPs-based cancer therapy, for its irreplaceable advantages, is becoming one of the leading topics. A variety of methods of cancer therapies based on MNPs have been reported, and they show promise in inhibiting and curing tumors. In this review, the applications of MNPs for magnetically modulated hyperthermia therapy, drug/gene delivery, and controlling cell fate are discussed. These applications may open new routes for treating cancer.

The toxicity of MNPs is under intense investigation before they are applied clinically. Although iron is an innate metal that is essential for life, most of us attribute the potential toxic effects of MNPs to excess iron released, because of its ability to accept and donate electrons by switching between ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions.[65] This reduction–oxidation reaction may cause an imbalance in body homeostasis and lead to aberrant cellular responses, such as DNA damage, oxidative stress, and inflammatory processes.[180] Efforts need to be made to decorate the MNPs in a fabricated manner that leads to a minimum of free iron being released. The size of MNPs seems to be another consideration for their toxicity. MNPs with different sizes have different metabolization mechanisms, i.e. small MNPs ($d < 30$ nm) are metabolized through the kidneys, while large MNPs ($d > 200$ nm) are metabolized through the liver. Medium-sized MNPs can linger in the body for a long time, and therefore, detailed pharmacokinetic phenomena such as drug and MNP elimination and accumulation within the body are required to determine their biocompatibility, both *in vitro* and *in vivo*.

Another hurdle in the treatment of cancer is the emergence of resistant malignant cells. The drug concentration may affect the birth and death rates of both the sensitive and resistant cell populations in continuous time. However, free drugs can be pumped out after cellular uptake in resistant cells, resulting in a low drug concentration within cells. Fortunately, we have primarily proved that the application of MNPs-based drug delivery models can dramatically improve the therapeutic effect in multidrug resistant cells, but still, relevant reports are few, and it needs to be further studied, especially by *in vivo* and clinical studies.

The inherent mechanisms of magnetic therapy are not well understood. For example, the reasons that why magnetic hyperthermia cures cancer more effectively than traditional hyperthermia are still being explored. In addition, the real downstream, which can be activated by magnetic force for controlling cell fate, are also unknown. Only when the intrinsic reasons are clearly known can we design and develop therapies accordingly. Therefore, assisted by mathematical modeling, the scientific community still needs to study the detailed therapeutic mechanisms involved in MNP-base treatments.

To make full use of magnetically responsive NPs, the magnetic properties of NPs should be improved to increase the efficiency of magnetic hyperthermia and maximize the sensitivity of magnetic response. Therefore, there is also a need to find novel materials to be screened for maximum absorption of magnetic field lines. Heavy metal-doped MNPs seem to be a new target for this purpose, but still, little work has been done to systematically explore the influence of doping in thermo-magnetic approaches. Moreover, heavy metals seem to be more toxic than iron, and their biocompatibility is another criterion for further applications.

Developing novel magnetic-responsive cancer therapies may be another way for their further applications. Inspired by the striking changes in tissue during exposure in outer space, Wang *et al.*[181] proposed a novel method for tumor treatment recently. As MNPs will be in a weightless environment, after being accumulated into target cells, the MNPs will lead the cells to an environment of "microgravity." This special cell state will affect cell growth, and eventually, inhibit tumor growth. Developing accurate multi-modal MNPs with little or no toxic effects is also urgently needed. The ways cancer is cured in different therapies are different, and there may be some synergistic effects. The collaborations of different therapeutic strategies may further reduce the dose needed. Lastly, the protocols for synthesis of MNPs and their functionalization with nucleic acids, drugs, and targeting moieties still need to be standardized along with optimization of magnetic field parameters. The field will open for broader and in depth investigations that may bring MNPs into clinical trials in the near future, with interdisciplinary collaborations from physics, chemistry, biology, pharmacy, and clinical medicine.

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