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REVIEW

An update on the applications and characteristics of magnetic iron oxide nanoparticles for drug delivery

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ABSTRACT

Introduction: In the field of drug delivery, controlling the release of therapeutic substances at localized targets has become a primary focus of medical research, especially in the field of cancer treatment. Magnetic nanoparticles are one of the most promising drug carriers thanks to their biocompatibility and (super)paramagnetic properties. These properties allow for the combination between imaging modalities and specific release of drugs at target sites using either local stimulus (*i.e.* pH, conjugation of biomarkers, …) or external stimulus (*i.e.* external magnetic field).

Areas covered: This review provides an update on recent advances with the development of targeted drug delivery systems based on magnetic nanoparticles (MNPs). This overview focuses on active targeting strategies and systems combining both imaging and therapeutic modalities (*i.e.* theranostics). If most of the examples concern the particular case of cancer therapy, the possibility of using MNPs for other medical applications is also discussed.

Expert opinion: The development of clinically relevant drug delivery systems based on magnetic nanoparticles is driven by advantages stemming from their remarkable properties (*i.e.* easy preparation, facile chemical functionalization, biocompatibility, low toxicity, and superior magnetic responsiveness). This literature review shows that drug carriers based on magnetic nanoparticles can be efficiently used for the controlled release of drug at targeted locations mediated by various stimuli. Advances in the field should lead to the implementation of such systems into clinical trials, especially systems enabling drug tracking in the body.

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Drug delivery; magnetic nanoparticles; medical imaging; cancer therapy

1. Introduction

The recent decades have witnessed the use of nanotechnologies in biomedical field with hope to highlight efficient diagnostic and therapeutic tools [1]. In this context, the development of drug nanocarriers ensuring the controlled delivery of a therapeutic substance at target sites, by ensuring its protection from metabolization or fast excretion while mitigating its potential side effects has become an important issue [2]. Nowadays, targeted drug delivery is playing an increasingly important role in biomedical research, more particularly in the field of cancer therapy [3]. By definition, drug delivery implies the release of conjugated therapeutic agent (grafted, physisorbed, or encapsulated) following the administration of the bearing-nanovehicle [4]. Briefly, there are two main routes for drug release: locally activated; or externally activated. The former can take place by simple diffusion and/or through different endocytic mechanisms requiring chemical and biochemical stimuli (*i.e.* pH, hydrolysis, enzymatic activities, *etc.*) triggering the drug release. Externally activated targeting, on the other hand, is based on external factors, such as magnet, light, and ultrasound [5].

Over the years drug delivery systems have become so sophisticated that they are able of combining therapeutic action from a drug or an external stimulus (magnetic or optical) with imaging capabilities such as magnetic resonance imaging (MRI), near-infrared optical imaging (NIR) or magnetic particle imaging (MPI) [6,7]. Such combination of imaging with therapy, called theranostics, allows for more precise delivery of a treatment, thus, increasing its efficacy [8]. To construct an efficient theranostic drug delivery carrier, one needs to combine in a single vehicle the capacity to adsorb and release a drug, imaging properties, as well as some enhancing actions and/or targeting properties. Owing to these characteristics, one may understand the privileged position occupied by magnetic nanoparticles (MNPs) which result from their biocompatibility and (super)paramagnetic properties [9,10]. These features have made magnetic particles useful as imaging contrast agents (CAs) for MRI, with different formulations (Endorem®, Resovist®, …) which have been previously used for various clinical applications [11,12]. In addition, MNPs can act as therapeutic agents by inducing a local increase in heat when submitted to alternating magnetic field. This process, called magnetic hyperthermia (MH), is particularly efficient to induce cancer cells death, because of their low tolerance to higher temperatures (*i.e.* 42–49°C), in comparison with healthy cells [13]. MNPs possess the ability to produce heat following an exposition to external AMF. Such thermal stimulus can also be used to trigger the release of a loaded drug and can

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Article highlights

- Combining therapeutic compound with magnetic nanoparticles is actively explored to provide systems able to precisely deliver compounds at targeted locations while monitoring the drug biodistribution on the body.
- The production of sophisticated carriers based on magnetic nanoparticles is facilitated by the refinement of novel technologies enabling their sustained synthesis.
- Active targeting strategies, achieved by decorating the carriers with biomarkers (antibodies, peptides, …) or with stimuli-responsive moieties, have been widely developed for various magnetic systems.
- Under the action of a localized external magnetic field, drug carriers exhibiting strong magnetic properties (nanoscale clusters, nanoassemblies, such as liposomes, micro- and nanorobots, …) can be efficiently accumulated in targeted sites.
- Various therapeutic substances have been loaded onto magnetic carriers for cancer treatment (chemotherapeutic drugs) and treatment of other diseases, such as tuberculosis, malaria, or viruses.
- Therapeutic effect of magnetic nanoparticles themselves can be induced through combination with additional therapies, such as radiation therapy or magnetic hyperthermia.

work in a synergetic way with thermotherapy to increase the efficacy of treatment [14–16].

In the field of drug delivery, the ability to bring the carrier to specific sites can be triggered either by passive targeting, or by active targeting [17]. Passive targeting system is a natural targeting system in which nanoparticles are delivered through normal physiological processes. In the field of oncology, tumoral passive targeting is supposed to occur via the enhanced permeation and retention (EPR) effect, even if this effect remains controversial and greatly dependent of the tumor type [18]. On the other hand, active targeting system implies the delivery of drug-loaded carriers to the targeted areas for enhancing the local drug concentration. For targeted delivery, carriers' surface is generally modified with biomarkers (*e.g.* anti/nano-bodies, peptide, aptides, …) [19] which could specifically bind to the receptors of targeted cells. Depending on the particle properties (*i.e.* the size, shape, or multiparticle assemblies), drug-loaded MNPs could be magnetically guided to reach and accumulate within the tumor by means of external magnetic field.

This literature review covers the utilization of drug-loaded MNPs for therapeutic applications with a special focus on cancer treatment. Other examples illustrating their usefulness for other biomedical applications are also provided.

2. Methods for the preparation of MNPs

To date, a huge amount of research has been achieved regarding the preparation of drug delivery systems based on MNPs. Upon all existing methods (Table 1), coprecipitation and thermal decomposition methods are the two most widely used chemical routes, affording the preparation of iron oxide nanoparticles with versatile properties (various sizes, morphologies, and surface properties) through the manipulation of reaction parameters. Coprecipitation of ferrous (Fe^{2+}) and ferric ions (Fe^{3+}) in alkaline medium is the most appropriate method for the large-scale production of iron oxide nanoparticles readily dispersible in aqueous media. On the other hand, thermal decomposition approaches, involving the decomposition of iron organic complexes at high temperature, are best suited for producing nanoparticles with better size control, narrower size distributions, and fewer defects [20].

Over the years, various strategies have been pursued to improve the ability of these methods to yield MNPs in a reproducible way and with excellent size control. However, when larger amounts of nanoparticles are required, poor batch-to-batch reproducibility remained as a major obstacle restricting the use of MNPs in emerging applications of the biomedical field. Recently, the use of continuous flow microreactors has been introduced as a novel alternative allowing for high throughput synthesis of nanomaterials. Due to their reduced dimensions, microreactors benefits from significant processing advantages, such as improved heat transfer, mixing control and precise temperature control, and foremost the possibility to operate continuously under-automated conditions. The coprecipitation method has been adapted for the preparation of iron oxide nanoparticles with sizes ranging between 2 and 9 nm. Automated systems operating at flow rates up to 60 $mL.min^{-1}$ were demonstrated to be efficient for the large-scale synthesis of iron oxide NPs, combined with reduced cost [23]. Similarly, thermal decomposition synthesis was adapted using continuous flow reactors. Vangijzegem *et al*. developed a method for obtaining very small iron oxide NPs (inorganic core below 5 nm) in polytetrafluoroethylene (PTFE) tubular reactors [24] using flow rates up to 2 $mL.min^{-1}$. Jiao *et al*. developed a similar procedure for the synthesis of 4.6 nm PEGylated nanoparticles, demonstrating that such process is efficient for the preparation of particles with narrower size distributions compared to batch preparation [25]. In other study, Besenhard *et al*. developed a reactor capable of synthesizing particles in the range of 5–7 nm [26]. Long-term operation of such systems could provide a sustainable way for producing larger quantities (*e.g.* several grams per day) of nanoparticles displaying well-defined properties for purpose of drug delivery applications.

3. Magnetic nanocarriers for cancer therapy and diagnosis

Over the decades, cancer has revealed as being one of the humanity's biggest challenge due to its worldwide incidence. This disease is considered as the second cause of death in economically developed countries, accounting for nearly 10 million deaths in 2020 [27]. The main treatments used clinically are surgery, radiotherapy, and chemotherapy. Compared to surgery and radiotherapy, chemotherapy treatment is generally associated with lack of specificity, caused by simultaneous and uncontrolled destruction of both cancerous and healthy cells as the drug is distributed throughout the body. To overcome this issue, efforts have been made to develop nanosystems able to specifically deliver therapeutic moieties to pathological cells [28]. In this context, the use of targeted magnetic nanoparticles appears as promising by providing a better tumor selectivity [29]. When considering such applications, MNPs can be used either as individual nanoparticles (*i.e.* monocore systems [30]) or as

Table 1. Comparison of various synthesis methods for the preparation of MNPs. Adapted from [9,21,22].

Method	Product morphology	Advantage(s)	Disadvantage(s)
Coprecipitation	Spheres, irregular shapes	Simple and effective, mild reaction conditions, easy to scale up, synthesis in H_2O	Low control, presence of mixed phases, broad size distributions
Thermal decomposition	Numerous shapes (Spheres, cubes, …)	Narrow size distributions, tunable properties, size control	Toxic organic solvents, high temperature, need for phase transfer
Microemulsion	Spheres, cubes	Improved size control, tunable properties, uniform magnetic properties	Low reaction yield, poor crystallinity, need for large amounts of organic solvents
Hydrothermal	irregular spheres, and numerous shapes	Particle size and shapes are easily controllable, synthesis in H_2O	High pressure and reaction temperature, need for special reactors or autoclaves
Electrochemical	nanorods, hexagonal nanocrystals, and	Controllable particle size	Inability to reproduce
Sonochemical	Bipyramids, rods, spheres	Short reaction time, narrow size distributions, high crystallinity	Need for ultrasound wave irradiation equipment
Flow injection	Small rods, irregular spheres, sheets, or rhombic shapes	Homogeneity with high mixing with accurate control of the procedure and good reproducibility	Under a laminar flow regime in a capillary reactor, it requires continuous or segmented mixing of reagents

magnetic nanoassemblies encapsulated in macromolecular matrices (*i.e.* multicore systems) [31]. If most of the reports describe the use of nanoclusters stabilized by an (in)organic polymeric matrix [32–38], some recent original approaches described the use of mesoporous magnetic nanostructures obtained by nanoreplication and presenting remarkable surface area particularly useful for drug loading applications [39–41].

3.1. Image guided drug delivery monitoring

The addition of image guidance to a traditional drug delivery system is expected to achieve highly efficient treatment by tracking the drug carriers in the body and monitoring their effective accumulation in the targeted tissues. *In vivo* image-based drug release monitoring is expected to provide a visualization of the spatial distribution of drug and monitoring their accumulation within targeted tissues, eventually enabling real-time adjustments of delivered doses in order to remain within the therapeutic window. The benefits of such noninvasive process are numerous and include the improvement of the treatment efficacy alongside with side effects reduction.

As an example, Huang *et al*. reported the development of MNPs modified with folic acid for the diagnosis and treatment of breast cancer [42]. Doxorubicine (DOX) was loaded onto these nanocarriers and their efficacy as drug delivery systems was evaluated in xenograft MCF-7 breast cancer tumor model. The tumor accumulation was assessed by MRI thanks to the high transverse relaxivity of the carrier.

Another example arises from Zou who reported the use of polyethylenimine (PEI)-based hybrid nanogels (NGs) loaded with MNPs and DOX [43]. In addition to possess good colloidal stability and pH-dependent release of DOX under acidic pH, these nanoassemblies enabled effective inhibition of tumor growth under the guidance of T_1-weighted MR imaging.

While most studies involve the use of MRI [44–46] or optical imaging as monitoring technique, some recent reports describe the use of a young innovative imaging technique called Magnetic Particle Imaging (MPI). MPI detects signals from MNPs which are generated by a fast-moving magnetic field-free region (FFR) [47]. It has been demonstrated that the resulting signal can be processed to reflect tracer spatial location and concentration, opening the doors for quantitative imaging with good spatial resolution (≈ 1 mm) and sensitivity (≈ 100 µM Fe) [48]. Moreover, as MNP tracers are naturally absent from the body, MPI has nearly zero background compared with contrast-enhanced MRI [49,50].

Zhu and collaborators [51] designed MNPs/poly(lactide-co-glycolide acid) (PLGA) core-shell nanocomposite loaded with DOX that serves as a dual drug delivery system and MPI quantification tracer. They showed that the nanocomposite-induced change in MPI signal correlates linearly with the release rate of DOX over time upon acidolysis, establishing quantitative monitoring of the release process in cell culture. *In vivo* studies using murine breast cancer model allowed to validate the monitoring process and assessed the induced tumor cell death (Figure 1).

3.2. Active targeted therapy

Tumor-targeted therapy involves the delivery of certain anti-tumor bioactive compounds to cancer cells through specific carriers with limited influence on healthy tissues, resulting in higher therapeutic efficacy and lower toxicity. So far, researchers have drawn attention to the size of their nanosystems, so that the drug can be enriched within tumor tissues through the EPR effect. To enhance the accumulation and/or the nanocarriers uptake, various active targeting strategies have been explored, including the use of antibodies, peptides or aptamers attached to the MNPs outershell.

Exosomes are a class of naturally occurring nanoparticles that are secreted endogenously by mammalian cells [34], which exhibit strong cargo-loading capacity and have the ability to cross the blood–brain barrier (BBB). In their study, Jia *et al* [52]. incorporated MNPs and curcumin (Cur) into exosomes modified with neuropilin-1-targeted peptide (RGERPPR, RGE) to obtain glioma-targeting exosomes. When administered to glioma cells and orthotopic glioma models, they found that such exosomes

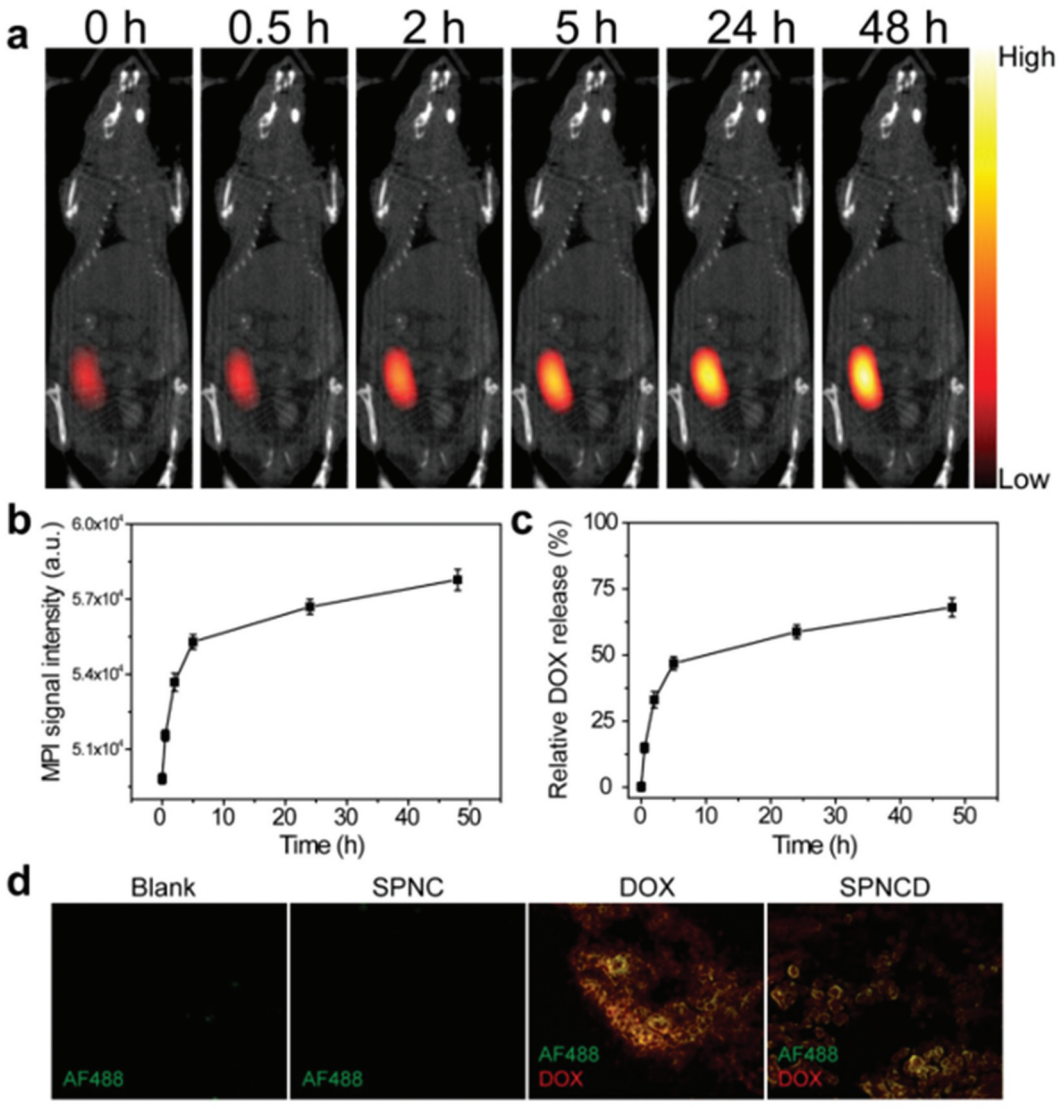

Figure 1. SPNCD for MPI-guided drug release monitoring in tumor-bearing mice. (a) MPI and X-ray computed tomography (CT) merged images (CT is employed to overlay anatomic structures of the animal over the MPI image) of an MDA-MB-231 tumor-bearing nude mouse injected intratumorally with SPNCD. MPI signals are shown in pseudo-color. MPI signal intensity gradually increased with time after injection of SPNCD into mice. (b) Quantification of MPI signal intensity from the tumor site in MDA-MB-231 tumor bearing nude mice at a series of timepoints from 0 to 48 h post-injection of the nanocomposite (N = 3 mice). Error bars are presented as S.E.M. (c) Relative DOX release percentage in the tumor site of MDA-MB-231 tumor-bearing nude mice over time, calculated based on the MPI signal in b and the calibration curve. Error bars are presented in S.E.M. (d) Tumor sections of MDA-MB-231 tumor-bearing nude mice injected intratumorally with saline (Blank), SPNC (negative control), DOX only (positive control), and SPNCD 48 h post-injection. A TUNEL assay was used to evaluate apoptosis in tumors with different treatment conditions and representative images are shown from N = 3 mice per condition. Green signal (Alexa Fluor 488, AF488) highlights the apoptotic regions in the tumor and red signal represents Doxorubicin (DOX) fluorescence, which overlap. Reprinted with permission from [33] Copyright 2019 American Chemical Society.

could cross the BBB and provided good results for targeted imaging and therapy of glioma, highlighting a potent synergistic antitumor effect between magnetic hyperthermia and cur-mediated therapy.

Cisplatin modified MNPs were derivatized with cell-penetrating peptides (TAT peptide; YGRKKRRQRRR) on their surface to enhance drug delivery efficacy to nasopharyngeal carcinoma cells (NPC), especially Pt-resistant NPC cells [53]. The combinatorial delivery of Pt and MNPs showed an unexpected effect on reversal of Pt resistance due to Fenton reaction with an average decrease in the half maximal inhibitory concentration of more than 80% in resistant cells when compared to Pt alone. On this basis, modification with TAT peptide significantly improved tumor intracellular uptake, devoting to better curative effects and minimized side effects. Gao *et al* [54]. proposed a similar strategy to circumvent Pt-resistance, by developing cRGD-modified liposomes co-entrapping hydrophobic iron oxide nanoparticles and artemisinin within the membrane and cisplatin in the lumen. Authors mentioned that the Fe^{2+}/Fe^{3+} resulting from degradation of MNPs motivated cisplatin and catalyzed the Fe-dependent anti-cancer drug artemisinin (ART) to generate highly toxic reactive oxygen species (ROS) through Fenton reaction, which greatly enhance anticancer effect of cisplatin while minimizing its side effects.

HER2-targeting aptides conjugated magneto-nanoclusters loaded with Docetaxel (DTX) were evaluated against HER2-overexpressing NIH3T6.7 tumors in mouse model [55]. The aptide-modified carrier showed substantial accumulation in

tumor tissue, as reflected in the relative signal increase of ≈ 45% at 3 h post-injection compared with the ≈ 15% observed for the non-targeted control.

Magnetic iron nanowires (iron nanowires with an iron oxide surface) have made a great contribution to nanomedicine because of their low toxicity and ease of manipulation with the magnetic field [56]. Their modification with anti-CD44 antibodies resulted in a 3x increase of nanowire internalization in colon cancer cells compared to control cells and did not affect the antigenicity and magnetic properties. It also increased the efficacy of killing from 35 ± 1% to more than 71 ± 2%, whereby the combination therapy was more effective than individual therapies alone.

The efficacy of a novel therapeutic strategy that combines [57] AICAR (a non-peptidic small molecule; 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) and DOX within a multifunctional platform was evaluated by Okutucu and Daglioglu. In this context, they reported the bottom-up synthesis of multifunctional nanoparticles aiming to neutralize survivin (BIRC5) to potentiate the efficacy of DOX against chemoresistance. Cellular uptake and cytotoxicity experiments demonstrated preferentially targeted delivery of nanoparticles and an efficient reduction of cancer cell viability in five different tumor-derived cell lines (A549, HCT-116, HeLa, Jurkat and MIA PaCa-2).

Recently, the bio-inspired delivery of drug using cancer cell membrane, more specifically that from the homologous tumors, has become an emerging targeting method [46]. Such delivery strategy showed great potential for precise targeting of particular tumors by simply adjusting the corresponding type of modified cell membrane. Zhu *et al* [58]. designed a theranostic nanomaterial platform based on MNPs covered with cracked cell membrane, derived from a specific tumor type, on their surface. Combination with DOX showed strong potency for tumor treatment *in vivo* with high efficacy.

3.3. MNP-based hybrid nanoparticles for imaging and therapy

Depending on the application, multimodal therapies may appear as a promising strategy for cancer treatment. Here, Xue proposed a multifunctional three-dimensional tumor targeting drug delivery system, based on the hybridization of a novel two-dimensional material graphdiyne (GDY) with magnetic metal organic frameworks (MOFs) [59]. The resulting material was loaded with DOX and served as both an anticancer drug to treat the tumor and a fluorescence probe to ascertain the location of the carrier. The results show that this drug delivery system, abbreviated FUGY, exhibits a high drug loading content (>40%) and an effective drug release at pH 5.0. In particular, fluorescent imaging demonstrates that the proposed system could deliver more anticancer drugs in tumor tissue than conventional drug delivery system

Gold-coated MNPs stabilized by polyethyleneglycol (PEG), then modified by DOX for MRI-guided chemo/photothermal-therapy were prepared by Elbialy *et al* [60]. The high efficacy of MGNP-DOX for combined chemo-photothermal therapy was observed both *in vitro* and *in vivo*, more particularly for the groups treated by the association of DOX-loaded carrier and laser irradiation.

To enhance the functionality of graphene oxide (GO), Gonzalez-Rodriguez combined it with MNPs. Such GO-MNPs conjugates retain pH-sensing capabilities to detect cancer versus healthy environments *in vitro* and exhibited fluorescence in the visible [61]. As a drug delivery platform, GO-MNPs showed successful fluorescence-tracked transport of hydrophobic DOX non-covalently conjugated to GO with substantial loading and improved efficacy allowing using eight times lower dose of DOX to achieve the same therapeutic effect of ≈ 62% cancer cell death.

An effective strategy of hyperthermia/chemotherapy regeneration for osteosarcoma treatment [36] was presented by Sabouri. MNPs were encapsulated within mesoporous silica nanoparticles, the resulting particles being bioactive thanks to the formation of a hydroxyapatite layer on their surface. Anticancer properties were obtained by mean of DOX loading. *In vitro* tests were performed using human osteosarcoma cell line (MG63). DOX loaded particles demonstrated a controlled drug release profile at pH 5.1 correlating with significant cell viability reduction.

3.4. Magnetic drug targeting

Taking advantage of the superparamagnetic properties of iron oxide nanoparticles, site-specific drug delivery can be achieved by guiding the MNPs under the action of a localized external magnetic field. Such approach has shown a certain efficacy for the accumulation of nanoparticles in tumoral sites. The magnetic response of MNPs depends on their physicochemical properties, more specifically, their saturation magnetization which must be as high as possible in order to favor their accumulation toward the targeted sites. MNPs organization within stabilized nano/micro-assemblies appears as a promising strategy to reach efficient response toward external magnetic stimulus.

By using the concept of superparamagnetic magnetite colloidal nanocrystal clusters (SMCNC), Qi et al. have conceived reticulocytes (RTC) exosomes-based MNP clusters strategy for tumor-targeting drug delivery [34]. In this strategy, multiple MNPs anchor onto each exosome to form a cluster, which increases the magnetization in a controllable manner while retaining its superparamagnetic characteristics. Such a strategy separated exosomes from blood efficiently and provided exosomes with a robust targeting ability. The magnetic-targeting ability was assessed by using a microfluidic system with controlled flow rates and simulating their retention under a moderate magnetic field (MF) in blood circulation. *In vivo*, the tumor-targeting ability of Cy5.5-labeled MNPs-exosomes was investigated (Kunming mice bearing a subcutaneous H22 cancer as a model) and revealed a significant increase of the fluorescence signal at the tumoral area when an external magnetic field (1 T) was applied. In another work, Kim proposed MNPs–incorporated exosome-mimetic nanovesicles from MNPs-treated human mesenchymal stem cell (hMSCs) and evaluated their therapeutic efficacy in a clinically relevant model for spinal cord injury [62]. Magnetic guidance significantly increased the amount of magnetic carrier accumulating in the injured spinal

cord, enhancing blood vessel formation and attenuating inflammation in the injured area, improving thus spinal cord function.

Choi *et al*. incorporated DOX within nanosystems combining MNPs (from bacterial origin) and liposomes stabilized by MePEG-palmitate for stimuli-sensitive drug targeting [63]. DOX-incorporated magnetic lipocomplexes showed increased anticancer activity against CT26 mouse colorectal carcinoma cells. Stimulation with magnetic field resulted in higher cellular uptake ratio and suppression of cell growth. To assess magnetic field sensitivity, lipo-MNPs were injected via tail vein of CT26 cell-bearing mice. DOX-incorporated lipo-MNPs showed higher cellular uptake *ratio* in the presence of magnetic field and then specifically inhibited tumor cell growth.

Microscale and nanoscale robots [64], also referred as future cargo systems for targeted drug delivery, can effectively convert magnetic energy into locomotion [65]. They can be used to support drug delivery vehicles, help cross biological barriers or improve diagnosis, particularly in the case of cancer [66]. It has been a recent topic of interest for the scientific community, which focused on both theoretical (*e.g*. by proposing simulation analysis of the optimized navigation of μMNPs in blood vessels, or by proposing physical models describing the dynamic of particles in liquid in term of concentration in each point of space) [64,67] and implementation aspects. Recent progresses of untethered mobile micromotors highlighted huge potential for targeted drug delivery *in vivo*. In 2019, Sun presented a pine pollen-based micromotor (PPBM) fabricated by the encapsulation of MNPs and DOX into the two hollow air sacs of pine pollen, *via* vacuum loading [68]. Under an external magnetic field, loaded MNPs enabled PPBMs to propel precisely in complex biological fluids. Owing to the magnetic nanoparticle aggregation phenomenon under a powerful magnetic field, controlled release of the cargo was achieved using a fluid field generated by the rotating magnetic agglomerate.

Kim and coworkers proposed hydrogel sheet-type magnetically retrieval intraocular microrobot for the treatment of retinoblastoma [69]. The developed system consisted of a therapeutic layer of gelatin/PVA composed of PLGA–DOX drug particles and an MNPs layer composed of PEGDA containing MNPs. The therapeutic layer dissolved to deliver drug particles upon AMF stimulation, the MNPs layer being retrieved using an external magnetic field. The potential for the vitreous migration of the microrobot and the therapeutic effect against retinoblastoma Y79 cancer cells was assessed using *ex vivo* bovine vitreous and *in vitro* cell tests.

Other magnetic microswimmers powered by collagenase and having the ability to deliver heat when exposed to an alternating magnetic field (AMF) were developed [70]. These objects were starting from polystyrene (PS) particles, modified with three layers of oppositely charged poly(diallyldimethylammonium chloride) (PPDA) and poly(styrene sulfonate) (PSS) prior to the deposition of the MNPs (*i.e*. manganese ferrites). An intermediate poly(L-lysine) (PLL) layer was immobilized onto the resulting objects before collagen was deposited. It was highlighted that the swimmers in collagen gel in the presence of a steep calcium gradient exhibit fast and directed mobility. Finally, the successful penetration of the swimmers into 3D cell was shown and impaired cell viability following AMF-induced heat was evidenced.

3.5. Stimuli-responsive nanocarriers

By using stimuli-responsive systems it is believed that it can be possible to increase the percentage of drug release in specific location of the body [35,71,72]. One common strategy consists of taking advantage of the difference of pH existing between healthy and tumoral tissues, the last one being slightly acidic. Such slight pH difference was advantageously used by several authors [73] to trigger the delivery of drugs grafted onto nanocarriers by means of pH-sensitive bonds (*e.g*. hydrazone) or pH-sensitive coatings (*e.g*. polymers).

For example, Ganivada et al. reported the development of magnetic lactone-based smart biodegradable nanocarrier (PVLPEG-PVLDOXI-PCL-PHOS-Fe_3O_4) conjugated with doxorubicin using ring-opening polymerization [74]. In this study, DOX was attached to the polymeric backbone by mean of a pH-sensitive acylhydrazine linker. Reservoir capabilities of the newly designed biodegradable nanocarrier are tested by both dynamic light scattering (DLS) and transmission electron microscopy (TEM). Drug release profile from nanocarrier is monitored by fluorimetry. The release profile monitored by fluorimetry highlighted the importance of having the acylhydrazine linker that helps to release the drug in mild acidic conditions. In a similar way, Gawali *et al*. prepared pH-labile ascorbic acid-coated MNPs for the tumoral delivery of DOX [75]. In this study, DOX was covalently attached by means of pH-sensitive carbamate and hydrazone bonds. The drug-loaded nanocarriers exhibited sustained pH-triggered release of DOX in acidic medium, substantial cellular internalization, and significant toxicity toward the proliferation of mouse skin fibrosarcoma (WEHI-164), human breast cancer (MCF-7), and human lung cancer (A549) cells. In another study, the authors described a smart DOX-loaded magnetic drug carrier that consists of MNPs coated with porous silica, then modified by poly(2-dimethylaminoethyl methacrylate) [76]. While cytotoxicity experiments assessed the biocompatibility of the presented system, drug release experiments revealed an excellent pH-triggered drug release. An interesting releasing approach using magnetic mesoporous silica nanoparticles was proposed by Liu and collaborators [77]. They proposed tumor cell-specific drug delivery system by capping mesoporous silica-coated MNPs with programmable DNA hairpin sensor « gates ». The nanocarrier was activated by endogenous miRNA-21 overexpressed in tumor cells (HepG2, human liver tumor cells), which serves as an exclusive key to unlock the system through hybridization with programmable DNA hairpin, leading to a rapid drug release (Figure 2). Among tumor biomarkers, microRNA (miRNA) is a short non-coding RNA molecule regulating gene expression in many cellular processes [78], including tumor initiation, development, and metastasis

Inhibition of *O* [6]-methylguanine-DNA methyltransferase (MGMT) using *O* [6]-benzylguanine (BG) has shown promise for patients suffering from temozolomide-resistant glioblastoma multiform (GBM). However, its acute cytotoxicity has hindered its clinical use. To improve BG biodistribution and

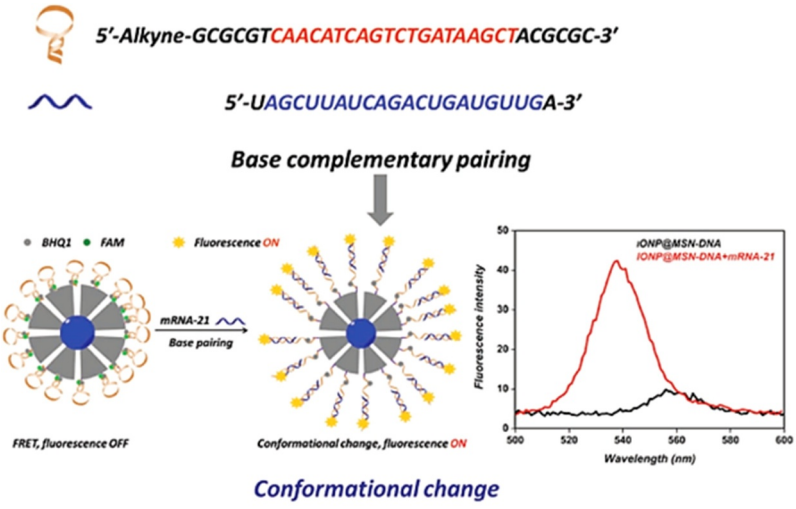

Figure 2. Sequences of the designed DNA hairpin structure, miRNA-21 and miRNA-21-induced conformational change in the DNA hairpin structure of IONP@MSN-DNA. Adapted with permission from [77] Copyright 2019 Wiley Online Library.

efficacy, Stephen *et al* [79]. developed MNPs for targeted convection-enhanced delivery of BG to GBM. These nanoparticles consist of a magnetic core coated with a redox-responsive, cross-linked, biocompatible chitosan-PEG copolymer surface coating, covalently modified with BG and tumor targeting peptide chlorotoxin (CTX) (Figure 3). Controlled, localized BG release was achieved under reductive intracellular conditions. Treated cancerous cells showed a significant reduction in MGMT activity and the potentiation of TMZ toxicity. *In vivo* (mice bearing orthotopic human primary GBM xenografts), concurrent treatment with BG-bearing nanoplatform and TMZ showed a 3-fold increase in median overall survival in comparison to untreated animals and to those treated with nanosystems without BG.

Given that MNPs possess the ability to produce heat following an exposition to external AMF, thermal activation constitutes another way to increase the drug release rate. When hyperthermia is not desired and control of the dosage is required, it is necessary to design a platform in which local heating at the nanoscale releases the therapeutic cargo without bulk heating of the surrounding medium. In their study, Zink & Lin reported a design using a stimuli-responsive nanoparticle platform to control the dosage of the cargo released by an alternating magnetic field (AMF) actuation. To reach their goal, they proposed a superparamagnetic doped iron oxide core@shell nanoparticle ($MnFe_2O_4$@$CoFe_2O_4$) embedded within a mesoporous silica shell [80]. A thermo-responsive molecular gatekeeper containing aliphatic azo group (*i.e.*

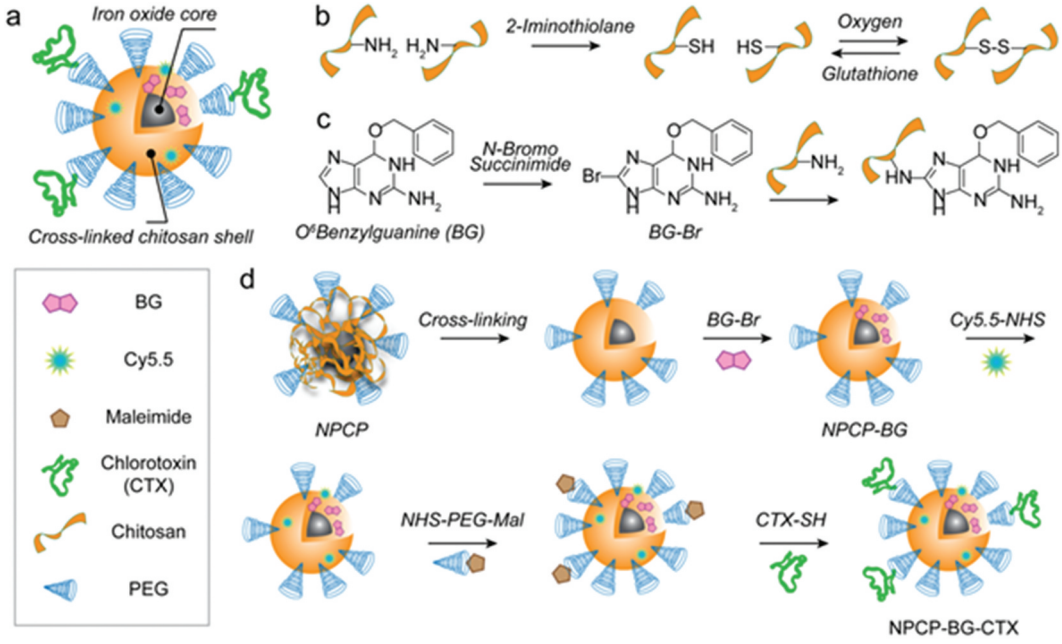

Figure 3. Synthesis of NPCP-BG-CTX. (a) Illustration of fully functionalized NPCP-BG-CTX. (b) Cross-linking of NPCP coating through intracellular reducible disulfide linkages. (c) Activation of BG by bromination and subsequent reaction with amines on the chitosan backbone. (d) Modification of NPCP with BG and CTX to produce NPCP-BG-CTX. Reprinted with permission from [79] Copyright 2014 American Chemical Society.

thermal-labile 4,4′-azobis(4-cyanovaleric acid moiety) was covalently grafted on the core@shell nanoparticles to regulate the cargo release. By applying multiple sequential exposure of AMF, they were able to release the cargo stepwise and increase the total amount of released cargo. Another original example arose from Mai *et al*. who exploited the fast rate of a photo-induced copper-mediated polymerization of diethyleneglycol methyl ether methacrylate, (DEGMEMA), and oligoethyleneglycol methyl ether methacrylate (OEGMEMA) onto the surface of magnetic nanocubes [81]. The combination of these nanosystems with DOX allowed to attain high therapeutic impact alongside with low amount of adverse side effects as observed *in vivo* for which efficacy studies showed complete tumor suppression and the high survival rate when they were exposed to magnetic hyperthermia. A strategy based on the use of molecularly imprinted polymers (MIP) for the production of stimulus-responsive drug delivery was reported by Kubo *et al* [82]. In that study, magnetic seeds (MTS) with size between 10 and 20 nm were modified with a thermo-responsive MIP by grafting polymerization and loaded with methotrexate (MTX). The MIP-coated MNPs showed selective adsorption ability toward MTX, and 80% of MTX adsorbed on the MIP-coated MTS was stimulus released at 60°C by cleaving hydrogen bonding in the recognition sites. Kim proposed the use of microrobot for the treatment of retinoblastoma [69]. To proceed, they developed a bilayer structure consisting of an MNPs layer of PEGDA (poly(ethylene glycol)diacrylate) and iron oxide nanoparticles, as well as a therapeutic layer of gelatin/PVA and PLGA–DOX (poly(lactic-co-glycolic acid)–doxorubicin) drug particles. Upon the application of an AMF at the target point, the therapeutic layer dissolved to deliver drug particles, the MNPs layer being retrieved using a magnetic field. *Ex vivo* bovine vitreous and *in vitro* cell experiments highlighted the potential for the vitreous migration of the proposed system and the therapeutic effect against retinoblastoma Y79 cancer cells.

4. Therapeutic properties of magnetic nanosystems

As previously mentioned, MNPs can cause cancer cells death by inducing a local increase in heat when submitted to an external AMF. After multiple preclinical studies, MNP-MH treatments have been translated to clinical trials in the early 2000s for the treatment of glioblastoma (clinical trial DRKS00005476; in combination with radiotherapy) and prostate cancer (clinical trial NCT0203344) using NanoTherm (*i.e*. aminosilan-coated MNPs). Research are still going on and in 2019, MagForce USA, Inc. has successfully completed stage 1 of its clinical study on the focal ablation of intermediate-risk prostate cancer, and the next stage is under preparation [83].

X-ray is a high energy radiation with strong tissue-penetrability, which has been widely used for cancer therapy, including radiation therapy (RT). However, the nonspecific absorption of X-rays combined with the particular tumor microenvironment leads to severe damages to normal tissues. Rapid advances in nanotechnology provided great opportunities for the development of functional nanoparticles and strategies to address these problems. If most of the studies focused on high-Z particles (typically gold, platinum, gadolinium, …) as a consequence of their higher X-ray absorption cross section, other nanomaterials with distinctive chemical properties have recently emerged as novel radiosensitizers to enhance RT through other mechanisms [84]. Fenton's reaction occurs in almost all iron-related vital processes, H_2O_2 produced by the cell metabolism is catalyzed by Fe^{2+}/ Fe^{3+} to form highly reactive hydroxyl radical ($OH^{\bullet}$) or superoxide radical ($O_2^{\bullet-}$). These ROS can further cause oxidative damages to DNA, membranes, and other vital cellular components. If healthy cells keep redox homeostasis, excessive production of ROS could overwhelm this homeostasis, resulting in irreversible permanent damage to the cells and eventually lead to cell apoptosis. Recent reports [85] suggest that the uptake of iron oxide nanoparticles can lead to a concentration increase of intracellular unbound iron, which may result in cell injury or death, especially when combined with additional therapies, such as radiation-therapies. In a recent study, Russell *et al*. highlighted the radiosensitizing properties of MNPs in combination with 225 kVp X-rays for 6 different cancer cell lines *in vitro* (using clonogenic assays), as well as *in vivo* by intratumoral injection to H460 lung xenograft mice [86]. Significant increase in double-strand breaks alongside with decrease in clonogenic cell survival evidenced the radiosensitization effect caused by MNPs. For the *in vivo* experimentation, all subgroups showed a significant decrease in the rate of tumor growth suggesting that MNPs alone impaired the ability for the tumor cells to grow and divide, the impact on the tumor being more pronounced when radiation were applied on MNPs treated groups.

Another study [87] reported that iron oxide nanoparticles have an anti-tumor effect by inducing the polarization of pro-inflammatory macrophages in tumor tissues. *In vitro*, they evidenced that Ferumoxytol, a clinically approved MNPs formulation, increased the transcription of the proinflammatory Th1 response mRNA in macrophages, promoting caspase-3 expression in adenocarcinoma cells. *In vivo*, it was found that Ferumoxytol significantly inhibited the growth of subcutaneous adenocarcinoma in mice and could also prevent liver metastasis.

Ma *et al* [88]. combined iron ions Fe^{2+}/Fe^{3+} released from iron oxide nanoparticles, with cisplatin prodrug to achieve a synergistic effect through maximizing the intracellular ROS (Figure 4). These nanocarriers can enhance Pt and Fe internalization in the cancer cells after tumoral NP accumulation. The loaded cisplatin(IV) prodrug can be rapidly reduced to toxic cisplatin that subsequently formed Pt-DNA adducts and also activated NOXs, which triggered a cascade reaction to form H_2O_2. Metabolized NPs released excess labile iron ions that catalyze H_2O_2 decomposition into highly toxic ROS within cancer cells; this results in fast oxidation and deterioration of cellular membranes. By taking the full advantage of Fenton's chemistry, the authors highlighted tumor site-specific conversion of ROS generation induced by released cisplatin and Fe^{2+}/Fe^{3+} from iron-oxide nanocarriers with cisplatin(IV) prodrugs for enhanced anticancer activity but minimized systemic toxicity.

Yu *et al* [89]. proposed a strategy to stimulate the Fenton reaction triggered by an exogenous circularly polarized magnetic field (MF) to enhance the ferroptosis-like cell death-mediated immune response. The system consists of hybrid core-shell vesicles (HCSVs) prepared by loading ascorbic acid (AA) into the core and a poly(lactic acid-co-glycolic acid) shell

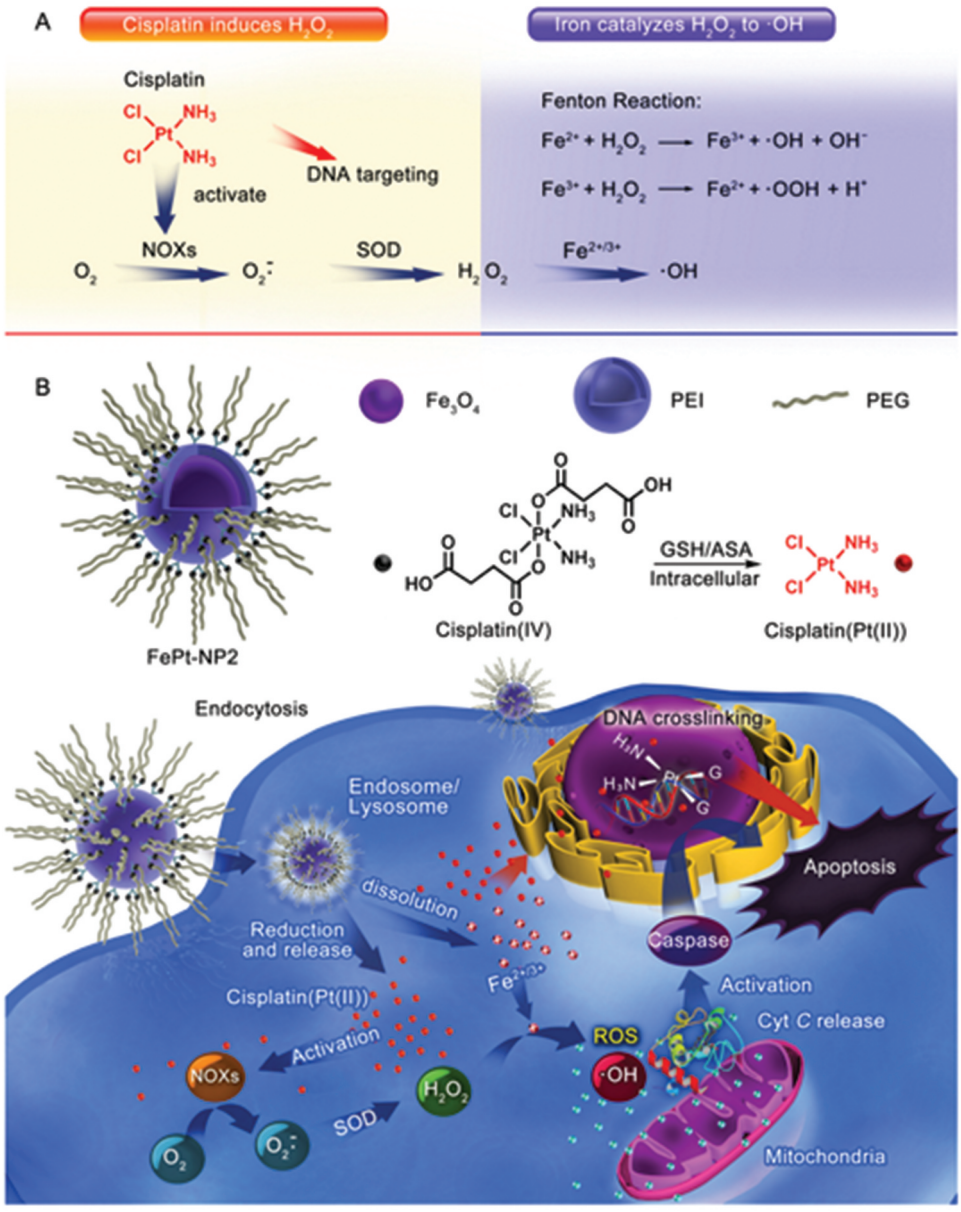

Figure 4. Maximizing cisplatin efficacy by constructing self-sacrificing iron-oxide nanoparticles with cisplatin(IV) drugs FePt-NP2 for synergistic actions. (A) Cisplatin activates NOX, which catalyzes formation of superoxide and H_2O_2 from O_2; iron catalyzes the Fenton chemistry to turn H_2O_2 into highly toxic ·OH. (B) Construction of self-sacrificing iron oxide nanoparticles with cisplatin(IV) prodrug (FePt-NP2) circumvents the endocytosis of cisplatin into the cells. In this way, excess ·OH are formed, which results in fast lipid and protein oxidation and DNA damage, as well as apoptosis via the ROS/Cyt C/caspase-3 pathway. Reprinted with permission from [88] Copyright 2017 American Chemical Society.

incorporating iron oxide nanocubes. The MF-triggered release of the AA leads to the increase of ferrous ions by the redox reaction between the AA and the nanocubes. Oxidative stress induced by the Fenton reaction led to calreticulin exposure on tumor cells, resulting in dendritic cell maturation and cytotoxic T cell infiltration into the tumor. As the ratio change of ferric to ferrous resulted in R_2* decrease, the MRI monitoring of Fenton reaction was enabled as well as MRI tracking of HCSVs.

5. Protein corona

Advances in nanotechnology have led to the development of functional nanomaterials with promising properties for the biomedical field. Controlled drug delivery is one of these promises. Despite the efforts made and the deployment of sophisticated approaches, it is clear that translation to the clinic is slow. Some reports point to a lack of knowledge of the mechanisms of action as a major factor in this failure. In particular, the formation of a protein corona (PC) on the surface of nanoparticles when submitted to a biological environment (culture medium or organism fluids), although decisive in ensuring their function, is still not sufficiently considered. Indeed, it has been observed that the surface of nanoparticles changes due to the adsorption of biomolecules (essentially proteins) after incubation in a biological fluid [90,91]. Thus, the formation of a PC can have a significant impact on various biological processes, such as targeting, biodistribution, cell recognition or drug release. Moreover, the composition of

the PC is largely influenced by the physicochemical properties of the nanoparticles, *i.e*. their size, shape, surface charge, as well as by the composition of the medium or the exposure time. Generally speaking, the PC is made up of two layers: the so-called « hard layer » made up of high-affinity proteins (*i.e*. apolipoproteins, fibrinogen and albumin), and a « soft layer » made up of very abundant proteins that bind transiently to the surface of the particles through weak interactions (protein–protein interactions with the proteins of the 'hard' layer), making these interactions a dynamic process that involves continuous adsorption/desorption of proteins (Vroman effect) [92]. Depending on the case, a size of about 3–15 nm has been estimated assuming that the protein corona should be formed by several protein layers [92,93]. Based on this observation, it is understandable that the exact structure and composition of the 'soft' corona is largely unexplored, in particular because of the technical challenge of its extraction and isolation.

The NP-PC assembly thus acts as a new biological entity with distinct properties that may have advantages and disadvantages in a biological system. In the context of molecular-targeting applications, the surface density, accessibility, and affinity of the ligand, as well as the expression level of the receptor on the cell membrane are important elements to consider for specific targeting [90]. In addition to interfering with the mechanisms of cell internalization by preventing the particles from interacting with the cell surface [94,95], it has been shown that the protein corona results in the loss of targeting properties of the functionalized nanoparticles, and that the activity of enzymes bound to the surface of the nanoparticles may be altered [92,96]. In addition to these aspects, it has also been shown that the formation of a PC can significantly modulate the release profile of a drug [97], leading in a rather general way to a decrease in the release of the drug (*e.g*. release of camptothecin from SPIONs or paclitaxel bound to albumin) [90], and in some cases even to an increase in the release of the drug from the nanocarrier.

Regarding the importance of PC formation/composition on the biological fate of nanocarriers, it seems obvious that a deep understanding of the mechanisms of interaction between NPs and body fluids components is mandatory. As plasma composition affects NP's corona profiles, one may expect that « sex-based » differences should be observed when studying nanocarriers biological behavior, as it has been shown that the composition of PC on the NP surface are strongly affected by sex-specific paracrine factors [98,99].

Among the strategies used to limit protein adsorption on nanoparticle surfaces, their functionalization with polyethylene glycol (PEG) chains has been widely documented. However, although PEGylation has been shown to effectively reduce protein adsorption, it cannot completely remove it [90]. Furthermore, contrary to initial assumptions, PEG can actually elicit immune responses that limit the targeting of PEG-conjugated agents in patients. Other hydrophilic polymers have been proposed as alternatives to PEG, such as polysaccharides and polybetaines. In this context, Debayle [100] has shown that coating nanoparticles with zwitterionic polymers allows a partial to virtually complete removal of the hard and soft protein corona.

6. Other medical applications of MNPs

Even if most of the studies implying the use of magnetic nanocarriers focused on cancer therapy, several authors reported the potential usefulness of such nanosystems for other less conventional therapeutic applications. As an example, Kannan *et al*. evaluated [101] the antimalarial efficacy of artesunate-modified iron oxide nanoparticles in wild and artemisinin-resistant *Plasmodium falciparum* strains. The artesunate-modified NPs were highly efficient (in the $1/8^{th}$ concentration of artesunate IC_{50}) with significant damage to macromolecules mediated through enhanced ROS production. Similarly, preclinical *in vivo* studies signified a radical reduction in parasitemia (≈ 8–10-fold reduced dosage of artesunate) even for artemisinin-resistant parasites.

Nanoparticles can have enzyme-like activity that can be controlled by tuning their physicochemical properties. This kind of nanomaterials are generally known as nanozymes [46] and have been successfully used for different biomedical applications including diagnosis, antibacterial activity, or tumor therapy. Recently, Qin and colleagues [102] reported the synthesis of iron oxide NPs-based nanozymes for the inactivation of the influenza virus. In that study, authors used the peroxidase and catalase properties of MNPs to induce lipid peroxidation of the viral envelope ultimately triggering the neutralization of influenza A viruses.

Tuberculosis (TB) is an infectious disease that affects millions of people worldwide. Miranda *et al* [103]. proposed the development of a magnetically responsive microparticulate system for pulmonary delivery of an anti-TB drug candidate. Microparticles (MPs) were developed based on a cast method using calcium carbonate sacrificial templates and incorporated superparamagnetic iron oxide nanoparticles to concentrate MPs in *alveoli* and enable drug release on demand upon actuation of an external alternate magnetic field (AMF). Their physical and aerodynamic properties favored alveoli deposition and phagocytosis by alveolar macrophages. Additionally, the developed macroparticles present a pH sensitive drug release profile, showing increased but sustained release rates in acidic pH conditions of phagosomes. In addition, when an AMF was applied, a tenfold increase in drug release was observed suggesting that an external AMF can modulate drug release profile.

Armijo et al. evaluated susceptibility and inhibitory properties of iron-oxide nanoparticles with and without attached drug (tobramycin) against *Pseudomonas aeruginosa* PAO1 communities [104]. The NP-drug conjugates were prepared using EDC/sulfo-NHS crosslinking of the aminoglycoside antibiotic tobramycin with alginate. Bacterial biofilm cultures were grown for 60 days to more closely model an established infection. Positive inhibition of bacterial growth was observed for uncapped and alginate-capped iron-oxide NPs, which was not the case for PEGylated NPs, suggesting that the capping agent plays a major role in enabling bactericidal ability of the nanocomposite. The observations made suggest that the alginate-coated nanocomposites investigated in this study have the potential to overcome the bacterial biofilm barrier, possibly by simple diffusion, due to the favorable solubility of the alginate-coated NPs within the alginate biofilm. Magnetic field application increases the action, likely via enhanced diffusion of the iron-oxide NPs and NP-drug conjugates through mucin

and alginate barriers, which are characteristic of cystic fibrosis respiratory infections.

7. Conclusion

Last years have witnessed impressive developments in the application of MNPs as targeted drug delivery systems. Research has been primarily focused on cancer therapy, using both passive and active targeting strategies to improve drug release in specific areas of the body while reducing the adverse side effects of chemotherapeutic drugs. Here, we sequentially discussed recent results concerning the combination between drug delivery and diagnosis provided by the magnetic properties of MNPs, as well as systems enabling drug release through active targeted therapy and magnetically driven targeting, for either cancer treatment but also for other less conventional therapeutic applications. Hence, the ability of MNPs to directly induce therapeutic effects through production of reactive oxygen species was also outlined as a promising strategy for the improvement of cancer treatment through radiation therapy. Drug delivery systems based on various MNPs formulations (monocore systems loaded with drugs, exosome-based MNPs, microscale and nanoscale robots, …) were proved to be advantageous tools to maximize the therapeutic outcome of various drugs. However, while these systems have been shown to be efficient, substantial research has yet to be done concerning the evaluation of the nano-bio interaction between drug carriers and cells in more realistic conditions *that is* dynamic flow rather than static conditions. Moreover, considerations related to the formation of PC and expression of vectors onto the outershell of a carrier are other aspects that should be systematically explored to provide a better understanding and hence allow a meaningful comparison between various drug delivery systems, such as those summarized in this review.

In addition to considerations related to the formation of a PC, one have to mention that the success of a therapy depends on the ability of the carrier/drug to reach the pathological target. In the field of oncology, passive solid tumors targeting with nanoparticles depends on the assumed vascular permeability of the tumor microenvironment (*i.e.* by EPR effect) [18,105,106,107]. Nevertheless, it seems that the amount of particles accumulating in the tumor is of the order of 1% of the injected dose, even for xenograft tumors [92]. A very recent study [108] even suggests that the nanoparticles (gold NPs) do not extravasate passively into the tumor, but rather penetrate by active transport through the endothelial cells. In addition, there is accumulating evidence suggesting the heterogeneous nature of the EPR effect in humans due to the variability of endothelial gaps in different tumors. Thus, it follows that therapies based on the EPR effect are inconsistent because they depend on the type of cancer, the stage of the tumor or the hypoxic condition [18].

In conclusions, the results gathered in this review offer exciting new perspectives in the field of MNPs-based drug delivery systems, additional data regarding the abovementioned aspects should be studied in the upcoming years to fully exploit the high potential of MNPs and develop *in vivo* assays and, subsequently, standardized clinical studies.

8. Expert opinion

The last decades have witnessed the emergence of nanotechnologies as powerful new tools to address the technological challenges of the 21st century, in fields as diverse as electronics, environment or health. Among the materials of particular interest, MNPs continue to be widely studied and exploited because of their intrinsic magnetic properties (*i.e.* superparamagnetism), particularly in the context of medical applications for which their ability to modulate the signal in magnetic resonance imaging continues to attract the interest of researchers. Beyond these applications, the exploitation of the magnetic properties of these agents in the context of specific and controlled drug delivery is a particularly promising line of research, especially for the treatment of cancer. Thus, our growing ability to control the properties of MNPs (size, shape, surface composition) and to conjugate drugs to these systems in an efficient manner (covalent or non-covalent; on the surface or within self-assembled systems) allows the creation of magnetic systems exhibiting drug release profiles in response to specific internal or external stimuli.

Despite the advances in this field and the increasing ingenuity of the proposed systems, it is clear that the use of multifunctional MNPs has not really allowed their integration into the clinical landscape. In order to overcome the many challenges associated with identifying a successful active targeting strategy, it is important to understand the events involved in the transport of the nanocarrier to the delivery site, as well as the response of the organism following the administration of this carrier. Thus, in the context of cancer treatment, the fraction delivered that actually reaches the tumor, the modulation of blood flow time, the control of elimination pathways, the toxicity and biocompatibility of the entire nanosystem, the interaction with plasma proteins and the visualization of the effective release of the drug, are all crucial factors to be considered when designing an MNP formulation of clinical interest. For example, although magnetic targeting can increase the accumulation of magnetic particles in tumor tissue, the intratumoral dose remains less than 2% of the intravenous dose. To this, we can add the uncertainty related to the existence of a EPR effect in solid tumors in humans compared to murine tumor models, which implies a reflection on the relevance of some models used in preclinical studies. Besides strategies involving the use of active targeting processes already discussed in a previous section, the use of a biophysical approach is a potentially interesting approach. For example, it has recently been shown that nanoparticle-loaded microbubbles can improve the delivery and therapeutic effect of nanoparticles (*i.e.* sonoporation [109]) in various mouse tumor models by taking advantage of the cavitation phenomenon [110]. The shape of drug delivery systems is another factor governing the biodistribution and accumulation of MNPs in tumor tissue, preclinical studies demonstrated higher anti-tumor activity of non-spherical nanoparticles due to higher tumor accumulation, hence

suggesting that the next generation of nanocarriers may not be based on spheres [111].

It should be also emphasized that the degree of sophistication of certain systems may limit/prevent their use in the clinic, notably because of their high manufacturing cost. It is therefore important to highlight that the formulation of the device in the research phase should not be very complex because of the high risk of failure when moving from the research laboratory to large-scale production.

Nevertheless, the advent of advanced technologies, such as magnetic particle imaging (MPI), which due to their specificity with respect to MNPs, should provide concrete solutions to constraints encountered with current imaging solutions (such as ensuring the quantitative monitoring of the release of an active ingredient within a diseased tissue). As a result, and due to the continuous evolution of this field of activity, we believe that over the next few decades medical devices based on MNPs will be applied in the clinical field.

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List of abbreviations

AA	Ascorbic Acid
AICAR	5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside
AMF	Alternating Magnetic Field
ART	Artemisinin
BBB	Blood-Brain Barrier
BG	O^6-benzylguanine
CAs	Contrast agents
CT	Computed Tomography
CTX	Chlorotoxin
Cur	Curcumin
DEGMEMA	Diethyleneglycol methyl ether methacrylate
DLS	Dynamic Light Scattering
DTX	Docetaxel
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
EPR	Enhanced Permeation and Retention
FFR	Fast-moving magnetic Field-free Region
FUGY	Fe_3O_4@UIO-66-NH_2/Graphdiyne
GBM	Glioblastoma multiform
GDY	Graphdiyne
GO	Graphene Oxide
HCSVs	Hybrid core-shell vesicles
HER2	Human Epidermal growth factor Receptor 2
hMSCs	Human Mesenchymal Stem cell
MF	Magnetic Field
MG63	Human Osteosarcoma cell line
MGMT	O^6-methylguanine-DNA methyltransferase
MGNP	Magnetic Gold Nanoparticles
MIP	Molecularly Imprinted Polymers
M(i)RNA	Micro-Ribonucleic Acid
MNPs	Magnetic Nanoparticles
MOFs	Metal Organic Frameworks
MPI	Magnetic Particle Imaging
MPs	Microparticles
MRI	Magnetic Resonance Imaging
MTS	Magnetic Seeds
MTX	Methotrexate
NGs	Nanogels
NIR	Near-Infrared optical Imaging
NPC	Nasopharyngeal Carcinoma cells
OEGMEMA	Oligoethyleneglycol methyl ether methacrylate
PC	Protein Corona
PEG	Polyethyleneglycol
PEGDA	Poly(ethylene glycol)diacrylate
PEI	Polyethyleneimine
PLGA	Poly(lactide-co-glycolide acid)
PLL	Poly(L-lysine)
PPBMs	Pine Pollen-Based Micromotor
PPDA	Poly(diallyldimethylammonium chloride)
PS	Polystyrene
PSS	Poly(styrene sulfonate)
PVA	Polyvinyl alcohol
PTFE	Polytetrafluoroethylene
ROS	Reactive Oxygen Species
RT	Radiation Therapy
RTC	Reticulocytes
SMCNC	Superparamagnetic magnetite colloidal nanocrystal clusters
SPNCD	Fe_3O_4@PLGA-DOX
TB	Tuberculosis
TEM	Transmission Electron Microscopy
TMZ	Temozolomide

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