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Superparamagnetic Iron Oxide Nanoparticle-Based Delivery Systems for Biotherapeutics

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Abstract

Introduction—Superparamagnetic iron oxide nanoparticle (SPION)-based carrier systems have many advantages over other nanoparticle-based systems. They are biocompatible, biodegradable, facilely tunable, and superparamagnetic and thus controllable by an external magnetic field. These attributes enable their broad biomedical applications. In particular, magnetically-driven carriers are drawing considerable interest as an emerging therapeutic delivery system because of their superior delivery efficiency.

Area covered—This article reviews the recent advances in use of SPION-based carrier systems to improve the delivery efficiency and target specificity of biotherapeutics. We examine various formulations of SPION-based delivery systems, including SPION micelles, clusters, hydrogels, liposomes, and micro/nanospheres, as well as their specific applications in delivery of biotherapeutics.

Expert opinion—Recently, biotherapeutics including therapeutic cells, proteins and genes have been studied as alternative treatments to various diseases. Despite the advantages of high target specificity and low adverse effects, clinical translation of biotherapeutics has been hindered by the poor stability and low delivery efficiency compared to chemical drugs. Accordingly, biotherapeutic delivery systems that can overcome these limitations are actively pursued. SPION-based materials can be ideal candidates for developing such delivery systems because of their excellent biocompatibility and superparamagnetism that enables long-term accumulation/retention at target sites by utilization of a suitable magnet. In addition, synthesis technologies for production of finely-tuned, homogeneous SPIONs have been well developed, which may promise their rapid clinical translation.

Keywords

biotherapeutics; magnetic field; penetration; retention/accumulation; superparamagnetic iron oxide nanoparticle (SPION); targeting

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Declaration of interest
The authors declare no conflict of interest.

1. Introduction

Biotherapeutics including therapeutic cells, protein/peptides, and nucleic acids are highly target-specific through processes such as antibody-antigen interactions and antisense oligonucleotide-target mRNA hybridization, and function in a relatively well defined manner compared to conventional chemical drugs [1]. These desirable properties could significantly improve therapeutic effects while lessening adverse effects. Accordingly, they have been extensively studied as a new class of drugs for treating cancers and autoimmune diseases over last decades [2-4]. However, application of this technology is hindered by lack of effective methods to protectively transport these biotherapeutics to disease sites. This is largely due to the intrinsic physicochemical characteristics such as physical/chemical stability, degradability of protein/peptides and nucleic acids that make their delivery rather difficult [5, 6]. For example, proteins can be easily degraded by proteinases in serum and self-aggregated via noncovalent interactions. DNAs and RNAs are susceptible to nucleases in serum and acidic microenvironment, which could dramatically shorten their biological half-life [7]. As biotherapeutics are hydrophilic with relatively high molecular weight, their delivery to and accumulation at target sites such as tumors can be difficult. Furthermore, negatively charged nucleotide-based drugs cannot penetrate cellular membranes and thus their poor intracellular delivery is a serious concern. One approach to overcome the biological barriers is to encapsulate biotherapeutics in micro- or nano-material formulations. In design of these formulations, considerable care must be exercised to minimize damage to biological macromolecules and prevent loss of their biological activities by physical stress [8, 9]. Another approach is to prepare specialized vectors according to the types of biotherapeutics for appropriate delivery. Recently, a wide range of carriers harnessed with targeting ligands or stimuli-responsive moieties that are responsive to external stimuli such as lights, ultrasounds, and magnetic fields have been developed for targeted delivery of biotherapeutics [10, 11]. Among these carriers, superparamagnetic iron oxide nanoparticle (SPION)-based delivery systems have drawn considerable attention because of their unique features such as biocompatibility and superparamagnetism that makes them detectable by MRI for monitoring treatment response and enables magnet-driven drug delivery and release [12-17].

This review covers recently-developed magnetically-driven delivery systems, their unique characteristics, and their applicability for delivery of biotherapeutics. Since methods for synthesis of SPIONs and use of SPIONs as MRI contrast agents for diagnosis have been extensively reviewed [18, 19], this review focuses on the SPION-based formulations that are specific to delivery of biotherapeutics. Magnetic nanoparticles dispersed in organic solvent and aqueous solutions can be loaded within liposomes, micelles, hydrogels, and micro/nanospheres during formulation. Physicochemical properties of SPIONs including particle size, surface charge, magnetization, and environmental conditions such as the strength and duration of the exterior magnetic field, are all critical for the success of magnet-based delivery systems [20]. First, we examine recent formulation strategies for modification of SPIONs including particle clustering and encapsulation within hydrogels, liposomes, micelles, and micro-/nano-spheres. Second, we discuss the considerations to be taken into account in design of SPION-based carriers for the delivery of specific biotherapeutics

including cells, proteins/peptides, genes, and viruses. Further, we examine several commercial magnetic nanoparticles for delivery of biotherapeutics. Finally, we provide perspectives in the future directions of magnetically triggered, SPION-based carriers for biotherapeutics, and their potential clinical applications.

2. Advantages and considerations in design of magnet-based carriers

A magnetically-driven delivery system uses SPION as carriers to facilitate fast extracellular, intracellular, and site-specific/targeted delivery of biotherapeutics under the influence of an external magnetic field (Figure 1) [21]. As shown in Figure 1a, administered SPIONs can be stacked up in the area with strong magnetic field upon application of an external magnet. In this way, biotherapeutics coated on or mixed with SPIONs can be guided to localize and be retained in specific tissue or organ sites. This may improve target specificity and therapeutic efficacy [22]. A study has shown that most magnetic nanoparticles are taken up by cancer cells in vitro in 15 min in the presence of a magnetic field, which could significantly reduce time for transport of genes and proteins into cells (Figure 1b) [23]. An external magnetic field can also accelerate cellular uptake of nanoparticles in 2D and 3D culture systems *in vitro* [23, 24]. In a 3D hydrogel-cell (human fibroblast cells) model, the external magnetic field enabled the deep penetration of magnetic nanoparticles coated with cell-penetrating peptides up to a depth of ~700 μm in collagen gels at a rate of 33 μm/h, which mediated intracellular uptake of magnetic nanoparticles into fibroblast cells even in deep areas (Figure 1c). Hydrogels crosslinked with magnetic nanoparticles can be compressed by movement of magnetic nanoparticles toward the magnetic field, which could trigger specific release of encapsulated biotherapeutics as shown in Figure 1d [25]. Magnetic fields are safe and are widely used in clinics. In a previous study, a static magnetic field as strong as up to 10 tesla did not show a negative impact on cell growth and cell cycles for 4 days in mammalian chinese hamster ovary CHO-K1 cells [26]. However, in vivo safety of magnetic field of > 10 tesla is yet to be demonstrated. Magnetically-driven accumulation of SPIONs can be a promising treatment for localized diseases like solid tumors but less so for disseminated diseases. It is known that only less than 5% of administered nanoparticle can reach tumor sites after systemic injection due to loose tumor vasculature in tumors, a phenomenon known as an enhanced permeation and retention (EPR) [27, 28]. External magnetic fields can enhance the accumulation of magnetic particles in tumors by the EPR effect. In a more recent study, significantly-elevated accumulation of SPIONs was observed in a mouse tumor model in the presence of magnetic field for targeted delivery of therapeutic protein, interferon gamma [29]. The study showed that magnetic particles accumulated in tumors under the influence of the external magnetic field were 6–10 fold more than those accumulated without magnetic field assistance. SPION-based carriers also allow magnetic resonance imaging-guided drug delivery, which could enable pre-selection of patients according to extent of target site accumulations by visualizing biodistribution of drugs after administration and following high efficient treatment in clinics [30].

For successful delivery of biotherapeutics using magnetic carriers, several criteria should be met. First, magnetic carriers should be stable and remain constant in size and magnetic property during the course of treatment [31]. Second, magnetization of the SPION-based carrier must be sufficient in response to the applied magnetic field. At the same time,

exterior magnetic fields in terms of magnetic flux density and permeability should be optimized to be strong enough to mediate penetration of biotherapeutics across the biological barriers and sufficient accumulation at target sites while remaining safe to normal tissue [32]. A wide range of SPION-based carriers have been investigated, including surface-modified SPIONs, SPION micelles, SPION clusters, SPION-encapsulated hydrogels, SPION liposomes, and SPION micro/nanospheres (Figure 2). Proper formulation of SPION using diverse materials like polymers and lipids not only increases the rate of response to external magnetic fields and the yield of accumulation/retention of biotherapeutics at target sites, but also enables simultaneous delivery of multiple drugs and functional agents incorporated in one carrier.

3. Formulations of SPION-based carriers

3.1 Surface modification

Various synthesis strategies have been established to modulate the size, morphology, and homogeneity of SPIONs [18, 19, 33]. Surface modification of magnetic nanoparticles could endow versatility for diverse applications and efficient drug delivery. Chemical and physical stability of magnetic nanoparticles can be improved via surface modification [34]. SPIONs can be chemically destabilized by oxidation in air and lose magnetism. This can be prevented by surface coating [35, 36]. In addition, due to large surface area–to–volume ratio, nanoparticles can easily aggregate when exposed to salts and serum proteins [37, 38]. To prepare stable SPIONs and reduce the clearance through the reticulo-endothelial system (RES) during circulation in vivo, hydrophilic polymers including poly(ethylene glycol) (PEG), dextran, heparin, chitosan, and poly(vinyl alcohol) (PVA) are immobilized onto SPION via coordination of metal irons on the surface of SPIONs (Figure 2a) [31, 39-43]. Steric stabilization of SPIONs can be achieved by modifying its surface with a steric layer such as nonionic macromolecules, which is resistant to exterior electrolytes in a wide range of pH conditions [44]. On the other hand, SPIONs surface-modified with ionic molecules for electrostatic stabilization could be more susceptible to exterior electrolytes than sterically stabilized SPIONs. However, it should be noted that the excessive steric hindrance of the surface-modified SPIONs can also hinder their interaction with target cells or tissues resulting in poor intracellular uptake, compared to SPIONs modified with cationic materials like polyethylnenimine. Not only the stability in physiological conditions but also the physicochemical properties of SPIONs such as surface charge and hydrodynamic size can be modulated by surface modification. The size and surface charge of SPIONs may affect their affinity to target cells as well as stability and pharmacokinetics in vivo [15, 20, 45-47]. In particular, the particle size determines the fate of the particle when confronted with biological barriers such as kidney filtration, phagocytosis, and extravasation from tumor vasculature after in vivo administration [48]. For example, nanoparticles with a size of 10–100 nm can escape from the clearance by liver as well as renal filtration [14]. Thus, the coating material and density are crucial for successful in vivo delivery of biotherapeutics. Surface coatings also serve to provide functional groups for further conjugation of targeting ligands and therapeutics.

Once functional groups are adopted onto the surface of SPIONs, biotherapeutics can be attached onto the surface of SPIONs either covalently or noncovalently (Figure 2) [49-52]. In addition, targeting agents such as stimuli-responsive moieties and ligands specific to target tissues or cells can also be immobilized onto the surface of SPIONs [53, 54]. In our previous study, SPIONs were decorated with PEI modified with an acid responsive moiety, a citraconic anhydride, for siRNA delivery in acidic conditions such as tumor microenvironments [49]. Targeting peptides like chlorotoxin for glioma cells, Arg-Gly-Asp (RGD) peptide for endothelial cells, and antibodies like Herceptin for various cancer cells can be chemically conjugated to surface-functionalized SPIONs for targeted delivery of plasmid DNA and siRNA [33, 52, 55]. However, it should be noted that surface coating with polymers may slightly reduce the saturation magnetization of the nanoparticle [56].

3.2 Micelles

Micelles are spontaneously assembled nanostructures composed of surfactant molecules when their concentrations are higher than the critical micelle concentration (CMC). Hydrophobic drugs are conventionally incorporated in cores of micelles via hydrophobic interactions for delivery [57]. In the same way, magnetic nanoparticles in nonpolar solvent can be loaded in the hydrophobic cores of micelles, while hydrophilic biotherapeutics can be attached on the surface of micelles (Figure 2a). Amphiphilic block copolymer, poly (hexafluorobutyl methacrylate (HFMA)-g- (methoxy poly(ethylene glycol) monomethacrylate (PEGMA), was synthesized by free radical polymerization. Magnetic nanoparticles prepared by iron precipitation in alkaline solution were encapsulated within micelles composed of amphiphilic poly(HFMA-g-PEGMA) during oil-in-water emulsion and solvent evaporation process [58]. The size of resultant magnetic nanoparticle-incorporated micelles (magnetomicelles) was over 100 nm, which showed decreased magnetization due to the surface modification with amphiphilic polymers compared with oleic acid coated magnetic particles with a size of 10 nm. However, in another study, there was negligible loss of the saturation magnetization of magnetic nanoparticles encapsulated within pluronic F-127 micelles, compared with polyacrylic acid-coated iron oxides [59]. The differences in saturation magnetization between bare and micelle-encapsulated magnetic nanoparticles might depend on the amount of magnetic nanoparticles within micelle cores. If the amount of magnetic nanoparticles loaded in micelles is large enough so that the relative amount of the polymer becomes trivial such as in clustered magnetic nanoparticles, the saturation magnetization of magnetic micelles might remain largely unchanged. Thus, the amount of SPIONs per micelles is critical for providing sufficient magnetization and subsequent magnetic response.

3.3 SPION Clusters

Several structural parameters, size and shape of the particle in particular, can dictate the magnetic properties of SPIONs, that is critical to the success of magnetically driven delivery of biotherapeutics [35]. Colloidal magnetic nanocrystals with a size of ~10 nm have low magnetization, which could hinder effective and prompt response of the nanocrystals to external magnetic fields [60]. To design nanocrystals with high magnetization, the size of the crystal can be increased only up to 30 nm before it loses superparamagnetism [60]. As an alternative approach, assembled SPIONs can provide great advantages over single SPIONs

because of significantly-elevated magnetic responses and retained super-paramagnetic property. Assembly of magnetic nanocrystals via a polymeric crosslinker like poly(acrylic acid) (PAA) or polyethyleneimine (PEI) can produce particle clusters with a size over 100 nm (Figure 2a). The type of the crosslinker used is determined by the biotherapeutics to be delivered. In a previous study, the coordination of carboxyl groups in PAA with iron of magnetic crystals enables the preparation of PAA-coated magnetic clusters through iron precipitation in alkaline solution. The prepared 174-nm nanocrystal assembly exhibited approximately 10^4-fold higher magnetic moments per particle than a nanocrystal with a size of 8 nm [60].

To fabricate homogeneous SPION clusters, an oil-in-water emulsion method has also been used [23]. A catechol-functionalized PEI was utilized as a crosslinker, where the catechol groups bind to the surface of SPIONs via a metal-oxygen coordination, and the cationic polymer, PEI, mediates outstanding complexation with nucleic acids. By changing the molar ratio of the crosslinker (catechol-functionalized PEI) to SPIONs, homogeneous SPION clusters with a size of approximately 150 nm were prepared. The clustered magnetic nanocrystals exhibited magnetization 3-fold higher than non-clustered and polymer-coated magnetic particles. The enhanced magnetization might be attributed to the smaller amount of polymers encapsulated in the clusters during the cluster preparation than the amount needed for surface coating of each SPION. As expected, ~20% of reduced magnetization was shown in SPION clusters due to reduced portion of SPIONs per total amount after polymer encapsulation. Thus, in preparing SPION clusters, both crosslinking density and molecular weight of polymers are critical to the resultant size and saturation magnetization per weight of produced clusters. Incubation of cells with clustered SPIONs in a magnetic field for 15 min has been shown to successfully mediate intracellular uptake of siRNAs and induce the subsequent gene silencing in prostate cancer cells (PC-3 cells) *in vitro*. In Figure 3a, SPION clusters linked by PEI (PMNC) showed over 50% of target GFP gene knockdown only in the presence of external magnet at a SPIONs/siRNA weight ratio of 32, while less than 20% of gene knockdown was observed by PEI coated SPIONs (PMNP) under the same condition. Considering that the surface charge of PMNP (39.1 ± 2.0 mV) was higher than that of PMNC (30.3 ± 3.4 mV), increased saturation magnetization by 3-fold via agglomeration is critical for gene magnetofection in vitro. Higher magnetization of clustered SPIONs, compared to polymer coated single SPIONs, might be attributed to less amount of polymer used in the cluster formulation. This result suggests that clustered cationic SPIONs may be preferable to cation-coated single SPIONs for mangetofection of genes in vitro.

3.4 SPION in hydrogels

Hydrogels have been extensively investigated and utilized for drug delivery and tissue engineering for a long time due to their tissue compatibility and well-established physical properties such as degree of swelling [61]. On-demand modulation of swelling degree in hydrogel systems can radically affect the loading efficiency and release profile of drugs, which is crucial for targeted drug delivery and sustained release. Accordingly, stimuli-responsive moieties such as pH-sensitive and thermal-responsive polymers have been adopted to hydrogel systems [62]. As one of stimuli-responsive materials, SPION can be

embedded in hydrogels for magnetically triggered swelling or shrinkage for drug delivery using an external magnetic field (Figure 1d).

To incorporate SPION into hydrogels with a micro- or nano-size, SPION should be well dispersed in aqueous solution via surface modification with hydrophilic macromolecules. Once hydrophilic polymers like PEG and polysaccharides, and surfactants like pluronic F-127, effectively stabilize SPION in aqueous solution, the SPION can be loaded in hydrogels via one of two methods (Figure 2a). First, SPION can be encapsulated within hydrogels by mixing with monomers followed by polymerization. Second, SPION can infiltrate into hydrogels during swelling. In a previous study, SPIONs with a size of 7 nm were prepared by a thermal decomposition method and siRNAs were encapsulated via pH-responsive volume transition of nanogels [63]. To create pH-responsive polymeric hydrogels, monomers including 2-vinyl pyridine (2-VP) and divinylbenzene (DVB) were polymerized by addition of azobis(isobutyl-amidine hydrochloride) (AIBA) initiators. Hydrogels incorporated with magnetic nanoparticles showed prompt response to external magnetic signals, enabling efficient intracellular delivery of and subsequent gene silencing by siRNAs.

Magnetic nanoparticles can be loaded in hydrogels for on-demand delivery of therapeutic proteins and cells in the presence of a magnetic field in vitro. Carboxyl groups of alginate were chemically linked to adipic acid dihydrazide (AAD) as a crosslinker using 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) to prepare hydrogels with stable structures even after lyophilization and rehydration. Pluronic F127-coated magnetic nanoparticles were loaded in alginate gels [25]. After freeze-drying, the alginate gels were incubated in aqueous solution with small-molecule drugs, proteins, and cells for encapsulation. Due to the presence of superparamagnetic particles in hydrogels, biotherapeutics incorporated in the hydrogels, including mouse mesenchymal stem cells, could be specifically delivered to a target site after transplantation *in vivo*, directed by an external magnetic field.

3.5 Liposomes

Liposomes are well-established nanocarriers for drug delivery [64]. Liposomes have been conventionally prepared by thin-film hydration procedures and reverse-phase evaporation methods [65]. Liposomes have several features, including biocompatibility and good cellular permeation, that make them favorable for delivery of chemical drugs and biotherapeutics [66]. In particular, both hydrophilic and hydrophobic molecules can be simultaneously encapsulated in liposomal cores and embedded in liposomal membranes, respectively. Thus, SPIONs coated with both hydrophilic and hydrophobic materials and loaded with biotherapeutics can be incorporated in and delivered by liposomes (Figure 2a). In a previous study, magnetic nanoparticles coated with palmityl-nitroDOPA have been successfully embedded in liposomal membranes [67]. In this study, magnetic nanoparticles significantly elevated the temperature of liposomal membranes by exposure to a high frequency alternating magnetic field, which ruptured the liposomes and selectively released the encapsulated drugs. Magnetic nanoparticles coated with hydrophilic polymers can be loaded within the core of liposomes or attached to the surface of liposomes via ionic interactions.

Commercial cationic iron oxide nanoparticles (CombiMAG, Chemicell GmbH) has been complexed with N,N″-dioleylglutamide (DG)-based lipoplexes/siRNA via electrostatic interactions for magnetically-driven delivery of therapeutic siRNA against Mcl1 gene [68]. By measuring the amounts of transfected cells with fluorescence-labeled siRNAs using flow cytometry, the investigators demonstrated that the exposure of CombiMAG/lipoplexes/siRNA complexes to a magnetic field for 15 min significantly increased the efficiency of intracellularly delivered siRNA by 2–5-fold in various types of cancer cells studied, including human lung cancer, breast cancer, and cervical cancer cells. Although cationic liposome/siRNA complexes could be continuously delivered for several hours during incubation, cationic liposome/siRNA complexes combined with SPION significantly reduce the time of transfection and the amount of siRNAs required for target gene silencing down from several dozens of nM to 8 nM in vitro. This fast transfection and gene silencing at low doses by SPION-based liposomes can be very beneficial to *in vivo* application.

3.6 Micro-/Nano-spheres

The shape and size of nanoparticles are tightly connected to the pharmacokinetics after administration *in vivo* [69]. Magnetic nanoparticles surface-modified with hydrophobic or hydrophilic moieties can be incorporated in micro/nanospheres via conventional single or double emulsion methods, respectively (Figure 2a) [70]. Biotherapeutics can be embedded within micro/nanospheres during formulations. In addition, micro/nanospheres surface-modified with cationic materials can be conjugated with biotherapeutics like proteins and DNA/RNA via ionic interactions (Figure 2b). Magnetic nanoparticles encapsulated within micro-/nanospheres can be manipulated to generate desired pharmacokinetic behaviors and deliver therapeutics to target tissues and cells based on the physical properties of the nanoparticles. In a previous study, microspheres with a size of 32 μm, composed of polyesters and SPIONs, showed 3.5-fold higher accumulation of iron in liver tumor tissue than in normal liver tissue after hepatic arterial infusion and exposure to magnetic fields for 20 min. This increased accumulation of irons in tumors elicited a significantly reduced tumor volume through arterial embolization hyperthermia in rabbit models [71].

Nanospheres may be used for tumor targeting and intracellular delivery of biotherapeutics via enhanced permeation and retention (EPR) effect after systemic administration [72]. Magnetic nanoparticles prepared by alkaline precipitation have been coated with oleic acid so that they could be dispersed in organic solvent mixture (chloroform and tetrahydrofuran) [73]. The hydrophobic polymer, poly(D,L-lactide) (PLA), were mixed with oleic acid coated magnetic nanoparticles for oil-in-water emulsion. The surface of SPIONs incorporating PLA nanoparticles was coated with PEI for ionic adsorption of plasmid DNA. Although the magnetization of each particle was similar, iron oxide-loaded PLA nanoparticle with a large size of ~375 nm exhibited greater intracellular uptake and gene delivery efficiency than other nanoparticles with a smaller size of ~185 and 240 nm in the presence of magnetic field, probably due to greater magnetic responsiveness of particles and the different cellular uptake mechanisms for particles of different sizes. However, values of saturation magnetization of SPIONs incorporating PLA nanoparticles were approximately 3 emu/g, which is extremely low compared to oleic acid-coated SPIONs and clustered SPIONs with saturation magnetization of ~66 emu/g and ~54 emu/g, respectively [23]. This is attributed

that loading percentage of SPIONs in PLA nanoparticles (relative percentage of magnetite weight per total particles weight) is only around 10%. Thus, comparable magnetization of SPIONs-incorporating PLA nanoparticles to other SPION-based carriers cannot be expected despite their own advantages like simultaneous loading of biotherapeutics within nanoparticles.

4. Applications of SPION-based delivery systems for Biotherapeutics

4.1 Cell therapy

Recently cell-based therapies using stem cells and immune-related cells have been actively investigated [74]. To boost efficacy of these therapeutic cells, they have been genetically engineered in vitro. However, most primary cells and stem cells have shown poor transfection efficiencies. To improve the gene transfection efficacy, magnetic nanoparticles have been utilized to increase the cellular uptake of therapeutic genes loaded on the nanoparticles and greatly improve gene transfection efficacy [75-77]. Another challenge in cell-based therapy is low retention of injected therapeutic cells at target sites. For example, intracoronary delivery of therapeutic cells for cardiovascular treatment is limited by low cell delivery efficiency despite its convenience. Labeling therapeutic cells including stem cells with magnetic nanoparticles by attaching to cell surfaces and internalization of magnetic particles within cells have been exploited as a novel method to localize and/or retain transplanted cells at target sites.

Diverse SPION-based carriers have been investigated to modify therapeutic cells [78-80]. Human endothelial progenitor cells (EPC) were labeled with FDA-approved and commercialized SPION, Endorem (Feridex; ferumoxides, Guerbet) by incubation for 1–24 hrs at an iron concentration of 3.9 pg per cell [78]. In this study, magnetically responsive CD133-positive EPC were administered in the common carotid artery through embolectomy catheter in rat carotid artery injury model. In this in vivo model, an external magnetic field applied for 12 min notably enhanced EPC retention at injury site by 5-fold compared to EPC accumulation in the absence of the magnetic field. Interestingly, prolonged exposure of magnetic field could affect cell viability in cell study. As the exposure time to magnetic field (neodymium iron boron permanent disk magnet, 1.195T, Magnetic Applications Ltd.) increased to 24 hrs, cells with high content of magnetic nanoparticles exhibited poor viability, which might be attributed to mechanical stress of intracellular organelles by magnetic force [79]. Thus, the appropriate exposure time to the magnetic field should be determined to localize therapeutic cells on target locations without toxicity. As an alternative approach to label therapeutic cells by attaching magnetic nanoparticles to cell membrane, magnetic nanoparticles were endocytosed in cells. For enhanced intracellular uptake, Feridex complexed with cationic polymer poly-L-lysine by simple mixing successfully labeled mesenchymal stem cells (MSC) [80]. A significantly higher amount of magnetically responsive MSC accumulated in the target organ, liver, under the influence of an external magnetic field after intravenous injection, compared to the case without the intervention of magnetic stimulation.

In previous studies, commercialized superparamagnetic microspheres with a size of 0.9 μm (Bangs Laboratories, Inc ™) were incubated with cardiosphere-derived cells for 24 hrs to

label the cells [81, 82]. The number of superparamagnetic microspheres per cell was 500. In a rat model, cells labeled with superparamagnetic microspheres and exposed to a 1.3 tesla magnetic field at ~1 cm above the heart showed retention 4-fold greater than cells that were not exposed to the magnetic field. The magnetic field not only improved the short-term (24 hrs) retention, but also the long-term (3 weeks) engraftment. Furthermore, this magnetic targeting did not induce any embolic injury or compromised the therapeutic efficacy of cardiosphere-derived cells.

To label bovine aortic endothelial cells, oleic acid coated SPIONs prepared by an iron-base precipitation method were incorporated within nanoparticles composed of poly(D,L-lactide) (PLA) via the emulsification-solvent evaporation. The surface of iron oxide-PLA nanoparticles were then stabilized with bovine serum albumin (BSA) to prepare stable nanoparticles with a size of 270 nm in aqueous solution [83]. After incubation of iron oxide-PLA nanoparticles for 24 hrs in the presence of a magnetic field with a gradient of 32.5 T/m (LifeSep ™ 96F, Dexter Magnetic Technologies), approximately 160 pg of iron oxide were internalized into a cell. Cells labeled with magnetic nanoparticles showed successful translocation toward the magnetic field *in vitro*. Cubic magnetic nanoparticles coated with polylysine (PLL-MNP) with a diameter of ~70 nm were attached onto the cellular membranes and taken up to cytoplasm after 24-h incubation [84]. Magnetically responsive SH-SY5Y cells (human neuroblastoma cell line) containing magnetic particles showed significantly enhanced migration toward an external magnetic field (1.41 T) compared to non-labeled cells.

4.2 Protein/peptide delivery

The protein/peptide drugs are most advanced biotherapeutic drugs with a large potential market. Currently, over 100 protein/peptide drugs including antibodies, insulin, and human growth hormone are in clinical use [3, 6]. SPIONs can be used to mediate intracellular delivery of therapeutic proteins/peptides and increase their accumulation at target sites using a magnetic field. Chlorotoxin with 36 amino acids is a potential therapeutic peptide under clinical trials for treatment of glioma [85]. Chlorotoxin on SPIONs was shown to perform multivalent interactions with glioma cells, which significantly increased their cellular uptake compared to free chlorotoxin. Accordingly, chlorotoxin coated SPION inhibited invasion of glioma cells up to 98% at 3 μM of chlorotoxin while free chlorotoxin can stop only 45% of cell invasion at a same condition, which might be attributed to the better suppression of MMP-2 surface expression [86].

For delivery of proteins to brain tumors, heparin-coated iron oxide nanoparticles (Chemicell) was complexed with PEI (1.2k) conjugated with a model protein, beta-galatosidase (β-gal), via ionic interactions, which resulted in a nanocomplex with a size of ~176 nm [87]. In a rat intracerebral glioma model prepared by implantation of 9L cells, the protein-iron oxide complex successfully accumulated at tumor sites in the brain, mediated by a magnetic field (0.35 T) for 30 min after injection of the complex via insertion of catheter into carotid artery. The activity of β-gal in the brain after magnetic targeting was 4.7-fold higher than that in the brain without magnetic exposure.

4.3 Gene delivery

Nucleic acid drugs are negatively charged at physiological condition because of the presence of phosphate group [5]. For gene delivery, SPIONs were modified with cationic lipids and polymers, which facilitate interactions with anionic nucleotides and with cellular membranes. Thus, most conventional carriers for gene delivery are cationic magnetic nanoparticles [88, 89]. As shown in Figure 3, cationic nanomaterials including surface-modified SPIONs and cationic SPION clusters can be attached to siRNAs via ionic interactions, which mediates fast sedimentation and local accumulation *in vitro* and *in vivo*, respectively. Fast accumulation and long-term retention of SPION/gene complexes can significantly enhance intracellular uptake and subsequent genetic process like gene expression and RNA interference. Oleic acid-coated magnetic nanoparticles in chloroform were coated with cationic lipids via hydrophobic interactions. Cationic lipids including DOTAP, DC-6-14 (O,O′-ditetradecanoyl –N-(a-trimethlammonioacetyl) diethanolamine chloride), TMAG (N-(a-trimethylammonioacetyl didodecyl-d-glutamate chloride), have been coated on magnetic nanoparticles to prepare various lipid-coated magnetic nanoparticles formulations (LipoMag) [89]. LipoMag composed of DC-6-14 and DOPE (dioleoylphosphatidylethanolamine) exhibited superior siRNA delivery to commercial magnetic nanoparticle, Polymag, showing over 60% of target epidermal growth factor receptor (EGFR) suppression *in vivo* by application of an external magnetic field. PEI-coated SPION with a size of 200 nm prepared by custom made magnetic nanoparticles by Chemicell (TransMAG^{PEI}) showed successful DNA delivery into the cytoplasm via endocytosis by magnetic exposure for 10 min [90]. Since plasmid DNA should be delivered into nucleus for gene expression, nanoparticles must be designed to mediate nuclear localization of plasmid DNA after transfection. PEI-PEG-chitosan copolymer was coated onto the surface of SPIONs for plasmid DNA delivery [91, 92]. For RNAi processing, siRNAs should be delivered into cytoplasm. After intracellular uptake, nanoparticles should be released from endosome to cytoplasm. To do this, endosome-breaking materials including fusogenic peptides and lipids can be adopted onto nanoparticles for facile intracellular localization. Polyarginine, polylysine, and PEI were conjugated to PEG immobilized SPIONs to attach siRNAs via ionic interactions and escape from endosome [93]. The cationic liposomes composed of N,N-dioleylglutamide were complexed with siRNAs to form lipoplexes, which subsequently mixed with cationic iron oxide nanoparticles (CombiMAG) [68]. In the presence of an external magnetic field, intracellular uptake of lipoplexes/iron oxide nanoparticles mostly occurs within 30 min, while intracellular delivery by conventional lipoplexes takes more than 3 hrs *in vitro*. Synthetic siRNAs can also be covalently conjugated onto surface modified SPIONs by adoption of functional groups like amines and thiol groups to the 3′ and 5′ end of sense and antisense strands [94]. In a recent study, plasmid DNA expressing small hairpin RNA (shRNA) targeting type 1 insulin-like growth factor receptor (IGF-1R) was successfully delivered using PEI-coated SPIONs (combiMAG) and lipofectamines *in vivo* to suppress endogenous IGF-1R (Figure 3b) [95]. After intravenous administration of plasmid DNA complexes with PEI-coated SPIONs and lipofectamine and incubation for 72 hrs, approximately 2-fold higher gene suppression was observed with magnetofections as compared to that with lipofection.

Recent research demonstrated that plasmid DNA can be successfully accumulated at target site by magnetic stimulation *in vivo* [96]. In this study, plasmid DNA was mixed with PEI-coated magnetic nanoparticles (Chemicell) at a SPION/DNA weight ratio of 10, and subsequently administered to each lung as microdroplets (3.5 μm) through nebulizer for magnet-guided pulmonary delivery in a mouse model. The magnetic field was only exposed to right lung, which resulted in approximately 2.5-fold and 2-fold increase in deposition of SPIONs and DNA, respectively, in right lung, compared to left lung that was exposed to the magnetic field. A high concentration of SPIONs in the droplet (2930 SPIONs/droplet) may increase the response to the external magnet as a result of greater magnetic moment. However, the study also showed that the magnetic flux density declined rapidly as the coil tip was moved away from the target site and when the distance between the target and magnetic source was greater than 10 mm, negligible magnetic flux was observed. This result suggests that magnetic fields strong enough to induce the accumulation of SPIONs beyond this distance may need to be explored for application in humans [97].

4.4 Virus

Therapeutic viruses have been vigorously studied for cancer treatment, and some candidate oncolytic viruses are under clinical trials [98]. However, several challenges remain, including random transduction without specificity, nonspecific immune responses, and low transfection efficiency for therapeutic cells like stem cells, depending on the type of virus [99, 100]. To enhance transduction efficiency, viral surfaces are modified with cationic polymers and lipids via ionic interactions because the net charge of a viral particle surface is negative, approximately −20 mV [100-102], which is not preferable for facile interaction with anionic cellular surface. Adenoviruses have been complexed with commercial magnetic nanoparticles AdenoMag (OZ Biosciences) via electrostatic and hydrophobic interactions for magnetically-driven delivery, which showed significantly increased transduction for cells poorly expressing Coxsackie-adenovirus receptor (CAR) on cellular surface like NIH-3T3 cells (mouse fibroblast cells) [103]. Approximately 3.5-fold higher gene transfections were observed via magnetofection for NIH-3T3 cells compared with transfection without cationic polymer coated virus (polybrene-virus mixture). Heparin-coated magnetic nanoparticles (fluidMAG-Heparin, Chemicell) can be bound to adeno-associated virus via receptor-ligand interactions due to structural similarity of heparin to heparin sulfate proteoglycan [104]. In the presence of a magnetic field, heparin-coated magnetic nanoparticles/adeno-associated virus complexes were successfully taken up into PC12 cells, which mediated elongation of neurites.

5. Commercialized magnetic nanoparticles for the delivery of biotherapeutics

A broad range of iron oxide-based nano-/micro-particles have been developed and commercialized as carriers or additives for delivery of biotherapeutics by biotech companies including OZ Biosciences and Chemicell (Table 1). To enhance transfection efficiency, the commercialized magnetic nanoparticle CombiMag (OZ Biosciences) has been used as an additive for gene transfection [22, 68, 95]. After mixing transfection reagents with therapeutic gene, CombiMag is added to the mixture, which resulted in 3-fold higher gene

transfection in A549 cells (human lung carcinoma cells) than conventional liposomal transfection using lipofectamine [95]. The polymag™ particles (100–200 nm, OZ Biosciences, France) showed plasmid DNA delivery to NCI-H292 human lung epithelial cells 4-fold greater than the commercial lipid-based carrier, lipofectamine [32]. In addition, lipomag™ particles and Adenomag have been developed for gene delivery and virus delivery, respectively [89, 103]. The diverse fluidMAG particles (Chemicell) are magnetic nanoparticles coated with various polymers including chitosan, starch, and PVA, and lipids [84, 105]. The particle sizes are in the range of 50–200 nm, which can be taken up by cells via endocytosis. Custom-made magnetic particles coated with heparin and PEI by Chemicell have been investigated [87, 90]. Feridex (Guerbet), a dextran coated iron oxide nanoparticle with a size of 80–150 nm, are well known as one of magnetic resonance imaging (MRI) contrast agents [106, 107]. Recently, Feridex has been used to label therapeutic cells including endothelial progenitor cells and mesenchymal stem cells for their targeted delivery [78, 80].

Micron-sized particles have also been developed and commercialized for magnetically-driven cell delivery. Polymeric microspheres with magnetic nanoparticles encapsulated, several micrometers in size, and surface-modified with various functional groups and functional proteins like antibody and streptavidin, were commercialized by Bangs Laboratories, Inc [81, 82]. While magnetic microspheres might not be internalized into cells, they could successfully label therapeutic cells by attaching onto cells, which might enable magnetically-driven accumulation and retention of therapeutic cells at target sites. However, due to the micron-size, applications of these particle systems might be limited to targeted delivery of therapeutic cells and genes only for immune cells.

Not only magnetic nanoparticles but also types of magnetic fields are important for magnetically-driven delivery of biotherapeutics. According to a recent study, polymag/DNA exposed to an oscillating magnetic field exhibited 2-fold higher gene transfection without notable cytotoxicity than those exposed to a static magnetic field, likely because an oscillating magnetic field could elicit additional lateral motion of magnetic particles [32]. Nanotherics (UK) has developed devices for magnet-medicated transfection using oscillating magnetic fields for enhanced delivery of biomolecules including plasmid DNA, siRNA, and viruses. Using these devices, gene transfection efficiency for mouse embryonic fibroblasts by magnetic nanoparticles was increased by approximately 20% compared to those driven by static magnets [108]. The investigators showed that the transfection efficiency increased as the oscillating frequency increased, and no cytotoxicity was elicited. This is probably due to mechanical stimulation by an oscillating motion of SPIONs within cells in response to external oscillating magnetic field. However, the use of oscillating magnetic fields in vivo has yet to be demonstrated for clinical application. A recent study showed no adverse effects on mice when they are repetitively exposed to 7 Tesla high-strength magnetic field [109], which promises the clinical potentials of SPION-based carriers.

6. Expert Opinion

With the outgrowth of biotherapeutics in research and for their potential wide-range applications in clinic, development of specialized delivery carriers for them is urgently needed. SPIONs have shown great promise in this regard. In particular, their prompt magnetization in response to an external magnetic field can be used to mediate local accumulation/retention and facilitate the penetration of biotherapeutics through tissues and cells both *in vitro* and *in vivo*. This enables targeted delivery of therapeutics to disease sites and minimizes the side effects to normal tissues/organs, leading to safer and more effective treatment while saving time and cost. To endow SPIONs with more desirable properties for delivery of biotherapeutics, a number of carrier formulations have been developed including surface modified SPION, SPION clusters, SPION-encapsulated hydrogels, SPION liposomes, SPION micelles, and SPION micro/nanoparticles. Versatile SPIONs can be prepared by surface modifications with diverse materials, and directly attached to biotherapeutics such as siRNAs and peptides. Incorporation of SPIONs within cores of clusters and micelles significantly increases magnetization per carrier, allowing much more prompt and significant response to external magnetic fields. Simultaneous incorporation of SPIONs and biotherapeutics in hydrogels can enable timely and on-demand release of biotherapeutics through hydrogel swelling or shrinkage triggered by an external magnetic field. By adjusting size of micro-/nano-spheres encapsulated with SPIONs, both SPIONs and biotherapeutics can be selectively delivered to specific cells and tissues. Micro-sized, SPION-based carriers can be adsorbed onto surfaces of therapeutic cells such as stem cells, while nano-sized carriers can be internalized to intracellular regions. Both of them have demonstrated success in mediating translocation and accumulation of therapeutic cells at target sites by application of external magnetic fields. It should be noted, however, that the inclusion of non-magnetic materials in the carrier formulation can potentially reduce the magnetization and affect the long-term stability and safety of the carrier in vivo. Thus, optimal weight ratio of magnet/non-magnet materials per carrier should be determined before evaluating each SPION-based carrier.

Indeed, significant advances have been made in the past decade in development of SPION-based carriers for delivery of biotherapeutics. Several magnetically-driven carrier systems have found their way into the market. Despite these encouraging achievements, it is yet premature to foresee when and which formulation of SPION-based carriers will finally win its way into the clinic. Much work needs to be done before the laboratory results can be translated to the clinical setting. From reported studies, research has largely remained on in vitro experimentation, and only recently, a few in vivo studies in small animal models are reported. There is no comparison study of differently formulated carriers in terms of efficiency and safety. In cell cultures and small animal models, magnets can be placed tightly to target cells or tissues, and the magnetic field can be easily confined in a small area. In clinic, however, this is not a common situation, and a large magnet placed distant from the target tissue may be required for noninvasive delivery. This may impose a challenge in construction of proper magnets for clinical use and in magnetic field configuration such as the type, focal area, and strength of the magnetic field. In addition, it is unclear if high magnetic strength necessary for effective delivery is safe in the clinical setting.

Magnetically-driven delivery is mainly targeted at treating solid tumors and may be less effective for treatment of highly invasive cancer cells such as glioblastoma multiform cells. For the invasive cancer cells, a combination of magnetically-driven delivery and ligand-based targeting may prove more effective. This can be done by incorporating additional targeting ligands that are specific to the disease cells into the carrier. Unlike magnetic-driven delivery, these ligands work at the cellular level and provide a higher level of target specificity. For further fine-tuning of magnetically-driven delivery, SPION-based carriers can also be combined with various moieties responsive to other external stimuli such as ultrasounds, temperatures, pHs, and lights, to elevate the efficiency of targeted delivery and enable programmed delivery of biotherapeutics. Furthermore, investigation on functionality of SPION-based carriers should be expanded for a broader range of tissues/organs and diseases to provide insight for their clinical translation. In the near future, SPION-based carriers may serve as effective carrier systems for localized delivery of biotherapeutics without significant side effects and as a monitoring modality to evaluate the therapeutic effects by MR imaging.

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Article highlights

- Iron oxide-based carrier systems can be potentially used for efficient and targeted delivery of biotherapeutics by application of an external magnetic field.
- Diverse formulation strategies of iron oxide-based nanoparticles allow fine-tuning features of carrier-biotherapeutics constructs in terms of magnetization, physicochemical properties, release profile, and delivery specificity.
- The formulation of iron oxide-based carriers is designed based on the type of biotherapeutics.
- Iron oxide-based nanocarriers show fast delivery of biotherapeutics and enhanced accumulation/retention at target sites under the influence of magnetic fields.
- Appropriately-formulated iron oxide-based carrier systems have great potential for prompt translation from laboratory to clinic.

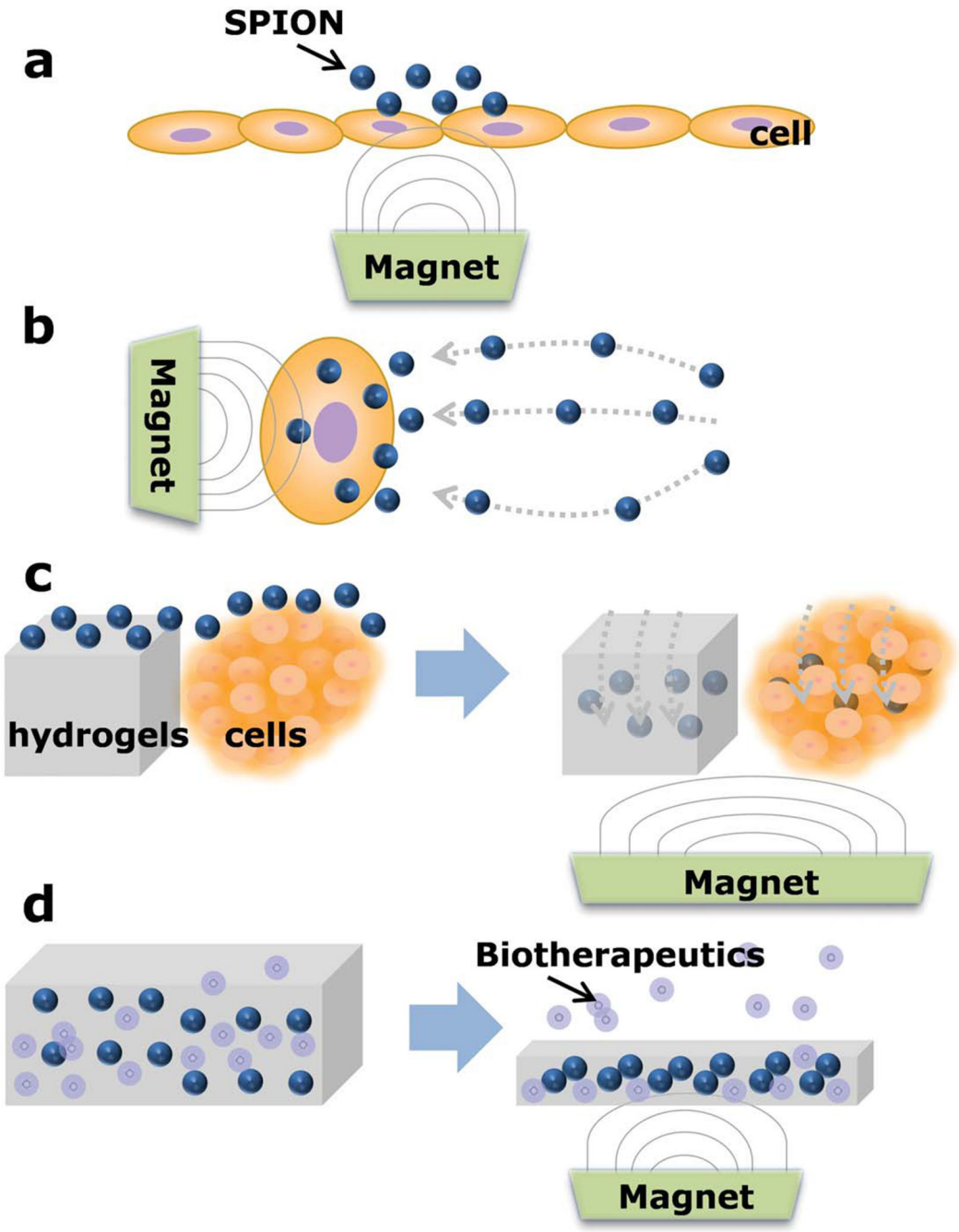

Figure 1.

Schematic representation of magnetically-driven delivery of biotherapeutics. (a) Local accumulation/retention of SPIONs at target sites (tissues and cells) under the influence of an external magnet. (b) Fast sedimentation and effective intracellular delivery of SPIONs. (c) Penetration of SPIONs into various 3D constructs such as hydrogels and cell aggregates. (d) Magnetically-triggered release of biotherapeutics via shrinkage of a hydrogel containing SPIONs and the biotherapeutics.

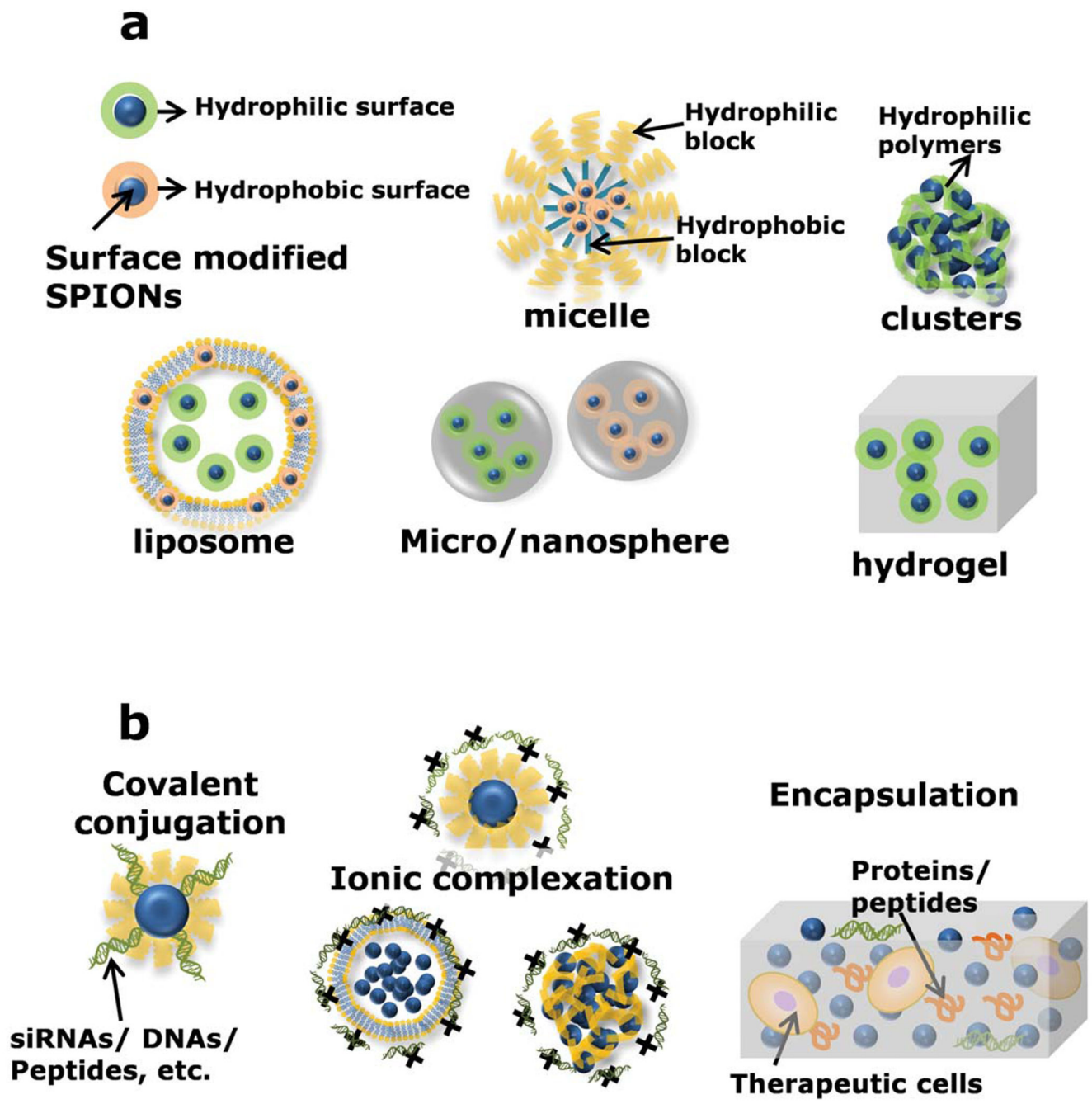

Figure 2.

(a) Incorporation of SPIONs coated with hydrophilic and hydrophobic materials into carriers. SPIONs with hydrophobic moieties can be loaded within micelle cores, liposomal membranes, and micro/nanospheres. SPIONs with hydrophilic moieties can be embedded within liposomal cores and hydrogels. SPIONs can be also clustered with hydrophilic polymers and stabilized in physiological condition. (b) Methods of incorporating biotherapeutics within SPION-based carriers via covalent conjugation, ionic complexation, and encapsulations. Biotherapeutics including siRNAs, DNAs, and peptides can be attached

onto SPIONs via chemical linkage. Cationic carriers including micelles, SPION clusters, and liposomes can incorporate anionic biotherapeutics like siRNAs via ionic adsorption. Biotherapeutic proteins and cells can be encapsulated in hydrogels for magnet-trigged release.

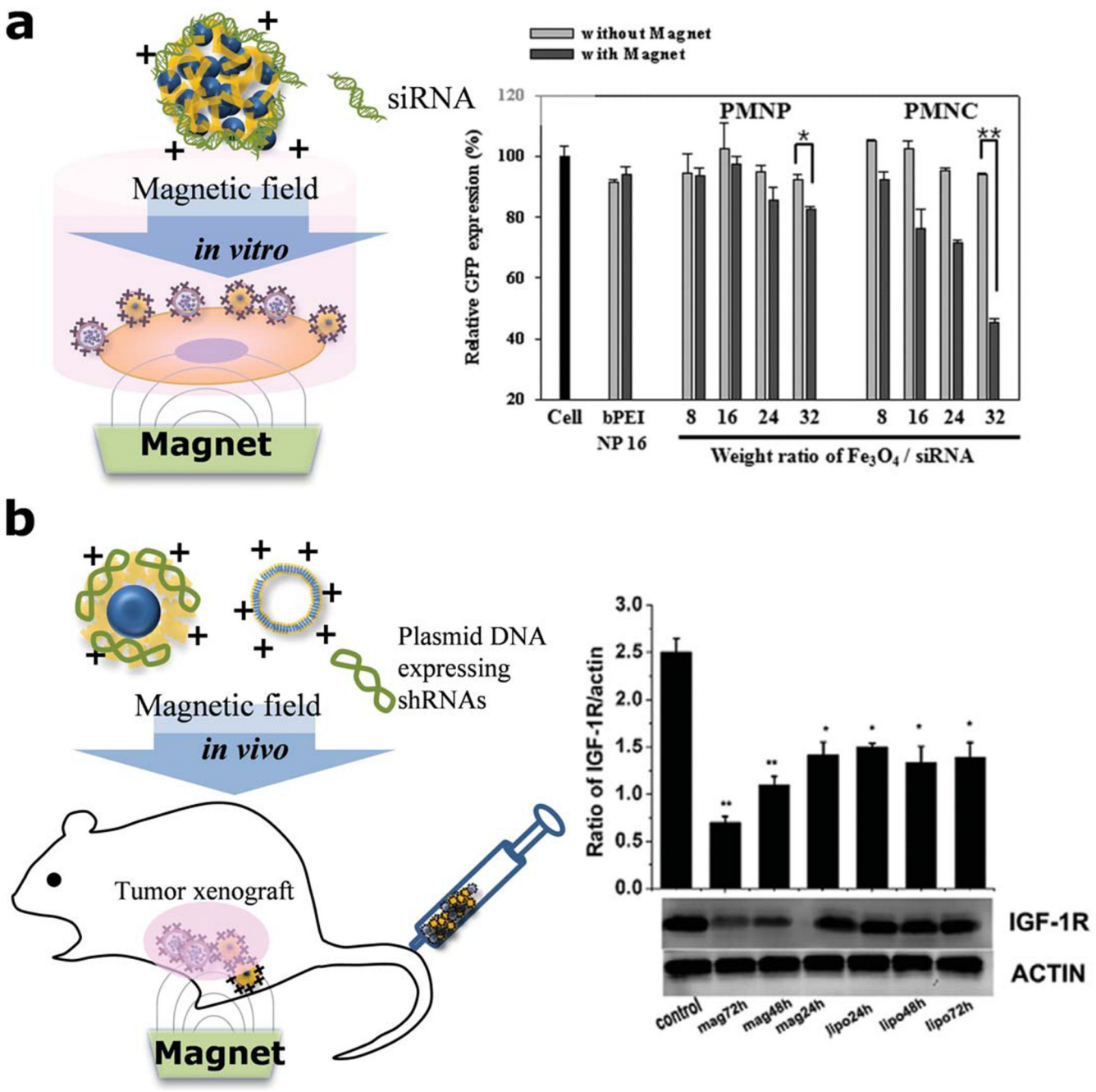

Figure 3.

Magnetically-driven siRNA/shRNA delivery using (a) SPION clusters in vitro and (b) PEI-coated SPIONs (combiMAG) and lipofectamine in vivo. (a) Right panel: Relative expression of GFP proteins as a function of weight ratio of Fe_3O_4/siRNA was analyzed by fluorospectroscopy after magnetofection of PMNP and PMNC for GFP-overexpressing in PC-3 cells (ref 23). b) Right panel: Relative expression of IGF-1R versus combiMag and lipofectamine nanocarriers for shRNA delivery at various time points by Western blot

analysis after intravenous administration of these carriers in A549 xenograft mice to suppress endogenous IGF-1R (ref 95).

Table 1

Commercial SPIONs for drug/gene/cell delivery.

Product name	Company	Size	Surface modification	Applications	Reference
CombiMag, Polymag, Lipomag	OZ Bioscience		variable	Gene transfection (primary neuron, diverse cancer cells)	22, 32, 68, 95
Adenomag	OZ Bioscience		variable	Virus delivery	103
fluidMAG (magnetic nanoparticles coated with diverse materials)	Chemicell	50 ~200 nm	variable	Cell labeling (mesenchymal stem cells)	84, 105
Endorem (Feridex; ferumoxides)	Guerbet	80-150 nm	dextran	Cell labeling (Human endothelial progenitor cells, mesenchymal stem cells)	78, 80
magnetic nanoparticles-encapsulated polymeric microspheres	Bangs Laboratories, Inc	0.5 – 12 μm	variable	Cell labeling (cardiosphere derived cells)	81, 82