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Magnetic targeting for site-specific drug delivery: applications and clinical potential

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Background: Magnetic vehicles are very attractive for delivery of therapeutic agents as they can be targeted to specific locations in the body through the application of a magnetic field gradient. The magnetic localization of a therapeutic agent results in the concentration of the therapy at the target site consequently reducing or eliminating the systemic drug side effects. *Objective*: The aim of this review is to provide an update on the progress made in the development of the magnetic targeting technique addressing characteristics of the magnetic carriers and limitations of the current targeting magnet systems. *Methods*: This review discusses fundamental requirements for the optimal formulation of the magnetic carrier, current applications and potentially new approaches for the magnetically mediated, site-specific localization of therapeutic agents, including drugs, genes and cells. *Results/conclusion*: More efficient targeting magnetic systems in combination with prolonged circulation lifespan and carriers' surface recognition properties will improve the targeting efficiency of magnetic nanocarriers and enhance therapeutic agent availability at the molecular site of agent action. The main future magnetic targeting applications were categorized emphasizing the most promising directions and possible strategies for improving the magnetic targeting technique.

Keywords: cell delivery, drug delivery, gene delivery, magnetic field gradient, magnetic nanoparticles, site-specific targeting

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1. Introduction

The use of magnetic micro- and nanoparticles as therapeutic agents has increased exponentially over the past decade [1]. Their intrinsic magnetic properties enable these particles to be used in numerous medical applications (**Table 1**) such as: i) hyperthermia agents, where the magnetic particles are heated selectively by the application of a high frequency magnetic field (e.g., in thermal ablation or hyperthermia of tumors [2,3]); ii) magnetic carriers that can be directed by means of a magnetic field gradient towards a certain location, such as in the case of the targeted drug delivery [4,5]; iii) tissue engineering [6-8]; and iv) magnetic contrast agents in magnetic resonance imaging (MRI) [9]. The applications of magnetic particles to MRI [10-13], magnetically induced hyperthermia [14] and tissue engineering [15,16] have been covered in recent reviews. This paper will focus on the current status (state-of-the-art) of the magnetic guidance of therapeutic vectors, discussing the requirements for the magnetic carriers, applications and potentially new approaches for the magnetically mediated, site-specific localization of therapeutic agents, including drugs, genes and cells.

Magnetic particles develop magnetic polarization and magnetophoretic mobility when an external magnetic field and field gradient are applied [17]. Therefore, by means of a selective application of a magnetic field gradient to a desired area, active ingredients bound to or incorporated in these particles can be successfully carried to the desired site of action with a relatively high accuracy, minimum

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Table 1. Medical applications of magnetic NPs.

Application	Approach
Therapy	Delivery of a localized therapeutic effect: Hyperthermia (thermal ablation) Radionuclide therapy
	Delivery of a therapeutic agent: Drugs (small molecules, peptides, proteins) Nucleic acids (DNA, siRNA, oligonucleotides, viruses) Cells (endothelial precursor cells, stem cells, blood cells, immune cells)
	Tissue engineering: Patterned cell assemblies Mechanostimulation Directional cell growth Construction of template-based cell structures
Diagnosis	*In vivo* MRI contrast agents

surgical intervention and maximum dose [18]. In this way regional therapy efficacy may be improved by increasing local therapeutic material levels, while systemic drug biodistribution and undesired toxic side effects may be decreased or eliminated [19,20].

In this approach, the particles carrying the therapeutic entity (drug, radionuclide, gene or in some cases these particles loaded into cells for cell delivery applications) are injected systemically or delivered locally via catheter and a strong, high-gradient magnetic field (usually created by an internally implanted or an external permanent magnet) is used to capture the particles as they flow through the bloodstream or move through tissue. Once the magnetic carrier with the therapeutic agent is captured at the treatment site, depending on the application, the particles then release the therapeutic agent or deliver a localized effect (irradiation from radioactive micro- or nanoparticle or hyperthermia with magnetic particles) [21]. Drug release usually occurs by diffusion or takes place through mechanisms involving enzymatic activity or changes in physiological conditions, such as pH, osmolarity, or temperature [17]. Drug release can also be magnetically triggered from the drug-containing magnetic particles [22-24].

2. Magnetic carrier characteristics

The crucial role of the magnetic carrier in drug targeting imposes a number of requirements on these particles, based on physical or biological reasons. These requirements are summarized in **Table 2**.

2.1 Superparamagnetism

The property of superparamagnetism is one of the important requirements for magnetic carriers. Materials magnetize in response to the external magnetic field in different ways. Paramagnetic materials magnetize very weakly along the external field direction. Diamagnetic materials magnetize in the direction opposing the external field. Ferromagnetic and ferrimagnetic materials magnetize strongly in the direction of the external field and tend to retain their magnetization when the external field is removed [25]. The force applied on magnetic particles during targeting is proportional to their magnetization and, therefore, one generally prefers to have the largest possible magnetization. However, since ferro- and ferrimagnetic materials have remnant magnetization (remanence in magnetic literature) even when the field is removed, they tend to interact magnetically and stick to each other, forming aggregates [26]. These aggregates can cause accidental embolisms and, therefore, ferro- and ferrimagnetic particles are generally avoided in targeting applications.

Superparamagnetic particles are typically chosen as a compromise between the desire to achieve strong magnetization and the desire to avoid particle aggregation. In fact, these particles are often made up of ferro- or ferrimagnetic materials such as iron oxide (i.e., magnetite [Fe_3O_4=Fe_2O_3 ·FeO] and maghemite [γ-Fe_2O_3]). So, why don't these nanoparticles have remnant magnetization?

The reason is thermal fluctuations [1]. The effect of thermal fluctuations makes the probability of observing a particular magnetization state, roughly speaking, proportional to $e^{\frac{VU_{\text{int}}-V\vec{M}\cdot\vec{B}}{kT}}$, where U_{int} is the energy density associated with internal interactions such as exchange and anisotropy, $\vec{M}$ is the magnetization, $\vec{B}$ is the magnetic flux density of the external field, V is the particle's volume, k is the Boltzmann constant, and T is the absolute temperature. In the absence of the external field, the magnetostatic energy $-V\vec{M}\cdot\vec{B}$ of the particle is zero and the time-averaged magnetization depends only on the relative magnitude of VU_{int} and kT. Near room temperature and in the absence of an external field, particles of sufficiently small volume V experience fast thermal fluctuations which randomize their magnetization, making its time-averaged value zero. As a result, such particles have no effective remanence and do not interact magnetically, causing aggregation. However, as the external field increases, the particles' overall potential energy $VU_{\text{int}}-V\vec{M}\cdot\vec{B}$ begins to exceed the energy of thermal fluctuations kT and particles develop strong magnetic moments. When the field is sufficiently strong, their time-averaged magnetization is as high as the magnetization of the bulk material from which the particles are made. For iron oxide nanoparticles, for example, this occurs at a relatively weak field around ~ 0.2 Tesla (26). Thus, superparamagnetic particles do not have remanence (just like their paramagnetic counterparts), but have strong magnetic moments in the presence of the external field (just like the bulk ferro- or ferrimagnetic materials).

2.2 Nanoparticle size considerations

When the carrier moves through viscous fluid, the drag force scales as ~ d, carrier's diameter. The magnetic force acting on the carrier is given by $\vec{F}=V_m(\vec{M}\cdot\nabla)\vec{B}$,

Table 2. Magnetic nanoparticle (MNP) requirements related to site-specific targeting.

MNP requirements	Physical/biological reason
Sufficient magnetic moment	To experience as large as possible magnetic force to overcome drag and yield forces
Superparamagnetism	To prevent agglomeration, avoiding embolism
Biocompatibility	To prevent toxicity, enhance cell survival and reduce inflammatory responses
Biodegradability	To improve clearance of the carrier material from the body and, in some cases, provide control over drug release
Capability as a carrier for drugs/genes	Reversible drug binding for some applications Contains and releases drugs and genes in a defined and controlled way to sustain drug/gene concentration and maximize its therapeutic effect
Structural stability	To allow delivery of the therapeutic agent after magnetic localization in the desired site
'Stealth' and functional surface characteristics	Prolong the blood half-life of the carriers. Improve the colloidal stability at the physiological conditions and prevent agglomerations and reduce toxicity Enhance the recognition, adhesion and internalization within targeted cells
Reproducible sizes and shapes, relevant for clinical applications	To efficiently control properties of the formulation To allow the optimal balance between magnetic responsiveness and circulatory time
Reproducible and scalable methods to allow mass production	To allow large scale fabrication of carrier with controllable physicochemical properties and sufficient shelf-life

where V_m is the total volume of the magnetic material embedded in the carrier, which is typically a composite particle containing superparamagnetic nanoparticles as inclusions. Assuming that V_m scales as $\sim d^3$ suggests that one would want to maximize the carrier diameter. A similar conclusion can be reached when considering carrier movement in soft tissue where yield stress imposes a threshold value of magnetic force, below which carrier movement may not occur at all.

However, there is a trade-off between the need for larger magnetic force and the need for an appropriate physiological response of the body, which generally requires smaller particles. Large particles (larger than 1 – 2 micron) can physically irritate the surrounding tissue and embolize small blood vessels and capillaries [27-29]. It is difficult to inject suspensions of such particles through a catheter and their half-life time of circulation is substantially low. Once the carriers enter the bloodstream, opsonization processes activate the reticulo-endothelial system (RES) response. Circulating mononuclear phagocytes (monocytes) clear the carriers to the liver, spleen and bone marrow, where the resident cells (Kupffer cells [KCs] in the liver) capture them prior to degradation. Particles smaller that 4 μm are eliminated by cells of RES, mainly in the liver (60 – 90%) and spleen (3 – 10%). Particles larger than 200 – 250 nm are usually filtered off by the spleen at the inter-endothelial cell slits (IES) in the walls of venous sinuses [30,31]. Particles up to 100 nm are mainly phagocytosed though liver cells (openings in the endothelium of liver cells are between 100 and 150 nm). The general trend is that the larger the particles are, the shorter their plasma half-life time [32].

For vascular applications, where the magnetic carrier is injected systemically, the ideal size of an engineered long-circulatory carrier is suggested to not exceed 200 nm [30]. For other applications, where the particles are delivered inside the tissue (i.e., for cancer applications), the size must be determined in each particular case, based on the tissue properties. The size of the carriers has also an effect on endocytosis. Rejman and co-workers reported that the size itself of (ligand-devoid) particles can determine the pathway of entry to the cells [33]. An interesting approach to controlling the size of polymeric magnetic particle formulations designed for gene delivery was demonstrated by Chorny and co-workers. In these studies poly(lactic acid) (PLA)-based magnetic nanoparticles (MNPs) were prepared by the modified emulsification–solvent evaporation method [34]. The size of the particles ranged between 180 – 400 nm and was controlled by adjusting the ratio of the organic solvents (chloroform/tetrahydrafuran [THF]) used in the emulsification step. The inclusion of the water-miscible solvent (THF) in the organic phase resulted in a decrease in particle size, as its gradient-driven distribution into the aqueous medium provides additional energy, resulting in the formation of smaller sized organic phase droplets and consequently smaller nanospheres [35].

2.3 Nanoparticle surface characteristics

The surface characteristics of nanoparticles also play a very important role in their colloidal stability, endocytosis rates and blood half-life time. The surface charge of nanoparticles is represented by the electric potential at the slipping plane of the double layer, so-called zeta-potential (ζ), or in other words it is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. Electrostatic stabilization of the magnetic carrier requires high surface charge or high zeta-potential values. The surface charge is dependent on the electrolyte concentration in the *in vitro* medium and additionally, on the adsorbing plasma proteins *in vivo* [36]. Therefore, changes in zeta-potentials relative to a given critical value of the particulate system might result in aggregation and precipitation of the particles. Endocytosis is also affected by the surface charge of the carriers. Negatively charged particles are electrostatically 'repulsed' from the negative cell membrane surface, thus slowing the endocytosis. Positively charged particles are strongly attracted to the cell surface membrane, promoting the endocytosis process. However, it was observed that the endocytosis index *in vitro* is minimal for the carriers with zeta potential close to zero [37]. It is generally agreed that strong positive and negative surface charges decrease the circulation time of the nanoparticles. In such cases, particles undergo phagocytosis, resulting in clearance from the blood mediated by the cells of RES of liver and spleen [38].

Initial nanoparticle (NP) surface characteristics are usually dictated by the chemical structure of the NP shell matrix and a stabilizing agent used to formulate NPs. However, the NP surface can be further modified with molecules that either mask NPs from being opsonized by blood proteins or that confer NPs with the cell-specific recognition element. Therefore, the circulation half-life time of the carriers might be altered by introducing specific molecules on their surface [39]. A common approach to prolong the circulation time consists of grafting carriers with polyethylene glycol (PEG). With this technique, sterically stabilized carriers (as oppose to electrostatic stabilization by charge) prevent protein binding (opsonization) and macrophage recognition. In recent years so-called 'stealth' nanoparticles have been developed by several research groups and have been shown to be undetectable by the mononuclear phagocyte system (MPS) [40-45]. These stealth nanoparticles have also shown a prolonged half-life in the blood [46]. The modification of the magnetic nanoparticles with PEG and folic acid protected them from phagocytosis, promoted particle stabilization and improved their cell internalization [47]. The introduction of folic acid on the surface of magnetic particles also allowed them to specifically target cancer cells, since the folate receptor is frequently over-expressed on the surface [48].

2.4 Biodegradability and biocompatibility of NPs

Another key requirement is the biodegradability of the magnetic carrier and biocompatibility of its degradation products. Water-based magnetic colloids (ferrofluids) stabilized (peptized) by various polymers such a polysaccharides, typically dextran and PEG, are usually used as contrast agents in MRI, or for tumor or cell hyperthermia [49]. For drug targeting applications, the most suitable systems are perhaps polymer-based nanoparticles consisting mostly of the superparamagnetic crystals embedded into a polymeric shell. In these systems the main components are magnetite, polymer and stabilizer, which all ideally should satisfy the biocompatibility criteria.

2.4.1 *Iron oxide*

Iron oxide (magnetite) presents low toxicity and is well tolerated in the human body. Iron oxide is usually eliminated or degraded into iron and oxygen by the liver (Kupffer cells [KCs]) and spleen macrophages (cells of the RES). After phagocytosis, intracellular iron oxide is degraded by heme oxygenase and the resulting iron is then bound to transferrin (responsible for safe iron transport), ferritin (the major iron storage protein) or other iron binding proteins, and will be exocytosed out of the cell [50]. Studies in rats have demonstrated that hepatocytes quickly take up the iron-rich ferritin released by the KCs [51]. Ferritin may also be released by macrophages in the spleen, resulting in ferritin delivery to the hepatocytes via the portal vein. The pharmacokinetics and toxicity of iron oxide were studied by Weissleder and co-workers [52]. After approximately one day, the dissolution of the superparamagnetic particles in the liver and spleen was observed. As evidenced by radiotracer studies, iron was still present; however, it was no longer in the crystalline form necessary for superparamagnetic behavior. Bioavailability, the incorporation of molecular iron into hemoglobin after administration of radiolabeled iron oxide, was evident from the incorporation of radioactive iron into hemoglobin. Twenty percent of the iron was found in hemoglobin 14 days after i.v. administration. No acute or subacute toxic effects were detected by histologic or serologic studies in rats or beagle dogs who received a total of 3000 µmol Fe/kg, 150 times the dose proposed for MR imaging of the liver. Magnetite is one of the iron oxides approved by the FDA for *in vivo* use [53]. Magnetic fluids have also demonstrated good cardiovascular tolerance. Their infusion has been shown not to change blood pressure, heart rate, or respiratory rate [54].

2.4.2 *Polymeric matrix*

The nature of the polymer matrix used to prepare polymeric magnetic nanoparticles for drug delivery is another important property of the magnetic carrier. Commonly, the magnetic carriers designed for *in vivo* applications consist of magnetic nanocrystals (10 – 20 nm in size) incorporated into various biocompatible, non-toxic preformed synthetic polymers such as poly(D,L-lactic-co-glycolic acids) (PLGA) [55-60], PLA [35,61,62] poly(e-caprolactone) (PCL) [63,64] and poly(alkylcyanoacrylate) [65,66]. The natural polysaccharides

(i.e., dextran [67-69], alginate [70,71] and chitosan [72-75]) are also very attractive coatings of magnetic crystals because of their biocompatibility. However, these natural polymers are not optimal matrices for the drug delivery carriers due to their water solubility and lack of mechanical strength. Even after crosslinking of these polysaccharides to prevent their breaking down in aqueous media, they are still mechanically week. Additionally, these coatings tend to be porous and display non-specific adsorption [76].

Perhaps the most suitable preformed polymers for the preparation of magnetic drug carriers are synthetic polyesters, for example PLGA and PLA. In addition to their good histocompatibility, biodegradability and non-toxic byproducts, these polymers have been found safe for human use, as has been extensively documented during the last three decades. Several drug delivery systems designed with polyesters have been FDA approved and commercialized [77-84]. Another advantage of these polyesters is their flexibility in tuning the rate of biodegradation that might affect the drug release profiles employing the following strategies: i) altering the chemical composition by increasing the glycolide mole ratio in the copolymer increases the rate of biodegradation; ii) the rate of diffusion across PLA and PLGA nanoparticles can be adjusted by varying the molecular weight of polymers – the lower the molecular weight, the faster the degradation rate; and iii) the PLA derived from D,L-lactic acid is less ordered than that from L(+) lactic acid and therefore the former will degrade faster [84].

2.5 Nanoparticle formulation methods

Quintanar-Guerrero and co-workers have summarized a number of useful techniques and mechanisms (i.e., emulsification evaporation, solvent displacement, salting out and emulsification diffusion) related to formation of biodegradable non-magnetic nanoparticles from preformed polymers [34]. These techniques might be applicable to the formulation of the magnetic nanoparticles as well. One of the most frequently employed methods to produce PLGA or PLA nanoparticles is emulsification solvent evaporation [85]. In this technique, the organic phase containing the polymer is emulsified with an aqueous phase to obtain an oil-in-water (o/w) emulsion (a single emulsion) and the polymeric nanoparticles are formed after solvent evaporation [35,58,86]. Lipophilic drugs and lipophilic magnetite (stabilized with an amphiphilic agent, usually oleic acid) are incorporated by dissolving them in the organic solvent along with the polymer. For hydrophilic drugs the water-in-oil-in-water (w/o/w) emulsification solvent evaporation is used, dissolving the drug into the inner water phase of the double emulsion. In the w/o/w process, the water soluble drug is dissolved or dispersed in an aqueous phase, which is then emulsified in an organic solvent containing the dissolved polymer. Then this primary (w/o) emulsion is dispersed in a second aqueous phase containing an appropriate emulsifier (stabilizer) and forms a double (w/o/w) emulsion. Following the complete removal of the organic solvent, the polymer solidifies, resulting in usually hollow particles or capsules. The magnetite can either be included in its lipophilic form (coated with oleic acid) into an organic phase or in its hydrophilic form (coated with PEG/poly(acrylic) acid [PAA] [87] or peptized in water [55]) into the inner water phase. However, the overall magnetite inclusion efficiency using double emulsion methodology is expected to be significantly lower compared to a single emulsion method. Peptization in water is not a suitable approach since it will result in the agglomeration of magnetite crystals and an inhomogeneous distribution within the particle. All the processing and formulation factors must be controlled since they influence the stability of the emulsion and consequently the particles size, surface morphology, loading efficiency and drug release pattern of the carrier [87-90].

2.5.1 *Stabilizing agents for polymer-based formulations*

In the first step of the polymeric nanoparticle preparation, an emulsifier (stabilizer) is usually used to stabilize the emulsion. In most cases, the stabilizers influence the properties of the formulated particles. The type and the concentration of the stabilizer selected can affect the particle size [34]. Being present at the boundary layer between the organic phase and the aqueous phase during the emulsification step, the stabilizer can also be partially incorporated on the particle surface, modifying the particle surface properties, that is surface charge (zeta-potential) and mucoadhesion [91,92]. Drug release rate, biodistribution, mucoadhesion and cellular uptake can all be influenced by the type and concentration of the stabilizer used [93].

Poly(vinyl alcohol) (PVA) and albumin have been preferentially used as colloidal stabilizers in many studies. PVA has been shown to be an excellent stabilizer to prepare biodegradable non-magnetic and magnetic nanoparticles using a variety of techniques. Furthermore it is one of the few stabilizers that prevents nanoparticle aggregation during post-preparative steps (e.g., purification and freeze drying), enhancing the yield of dry nanoparticle product without addition of other adjuvants [94]. However, PVA is still not approved for i.v. administration. Albumin has been proposed as a biodegradable substitute for PVA. Its structure seems to be stable after microfluidization, and evaporation procedures employed in preparation of nanoparticles and the immunogenicity of the albumin adsorbed on nanoparticles is the same as that of native albumin solution [95,96]. However, the source (human or bovine) and the purity of this protein are aspects that could limit its use for *in vivo* applications.

To address the limitations of PVA, Vandervoort and co-workers have compared a number of biocompatible polymers with PVA using a 2^2 full factorial design study. The polymers evaluated as potential stabilizers in this study were cellulosic derivatives including methylcellulose (MC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC), as well as gelatin

type A and B, carbomer and poloxamer. The influence of the concentration of PVA and the polymers tested on particle size and zeta potential value was evaluated before and after freeze-drying of the prepared particles. It was found that nanoparticles were obtained with most polymers when they were used in combination with PVA. Leaving PVA out of the formulation in most cases increased the size of the particles over 1 µm. The authors concluded that most polymers tested are not able to stabilize the emulsion as well as PVA does except the poloxamer and carbopol, which can be considered as valuable alternatives for PVA [93]. Other studies have also evaluated a number of biocompatible polymers as possible stabilizers, including poly[2-(methacryloyloxy)ethyl phosphorylcholine] [97], poly(ethylene glycol)-oligo(aspartic acid) hybrids [98], and *N*-succinyl-*O*-carboxymethylchitosan [99]. However these polymers have been used to stabilize the iron oxide nanocrystals to obtain magnetic colloids (ferrofluids) only and have not been tested as emulsion stabilizers for preparation of the polymeric magnetic nanoparticles.

2.5.2 *Solid lipid magnetic NPs*

Solid lipid magnetic nanoparticles (SL-MNP) have been described as an alternative to polymeric carriers. Lipophilic drugs are easily included in the internal phase of the solid lipid particles [100-102], while in the case of hydrophilic drugs, it was shown that they can be also incorporated through becoming 'lipophilic' by formation of complexes [103,104]. Muller and colleagues prepared SL-MNP by the high pressure homogenization method where the solid lipid phase incorporating magnetite was repeatedly ground and melted to achieve a homogeneous dispersion of the magnetite in the lipid matrix. In this study, the toxicities of the SL-MNP and polymeric MNP (PLA/PLGA and polystyrene) were compared and the SL-MNP were proved to be the least cytotoxic preparation [105]. In more recent studies SL-MNP were prepared using a two-step method, where a warm micro-emulsion of lipid/magnetite/drug phase is dispersed into cold water. Using this approach, solid lipid particles incorporating magnetite [106] and both magnetite and drug (ibuprofen) [107] were reported. Although solid lipid-based systems are less toxic than the polymeric nanoparticles and can be used to deliver drugs orally, topically, or via the pulmonary route, the concerns regarding their stability still remain.

3. Magnet systems for targeting applications

Magnet system requirements for targeting applications are completely dictated by the equation $\vec{F} = V_m(\vec{M} \cdot \nabla)\vec{B}$ for the magnetic force. To maximize the force the magnet system should, on the one hand, generate a field which is sufficiently strong at the location of the carrier to maximize the induced carrier magnetization $\vec{M}$. On the other hand, the magnet system should generate strong field gradients at the carrier's location. No magnetic targeting of carriers could possibly occur with a permanent uniform magnetic field, no matter how strong it is. Although magnetic systems designed to achieve both sufficient magnetizing field and strong field gradients might differ in details and exact parameters, the fundamental properties of magnetic fields permit to describe roughly the main constraints and to estimate the magnitudes of the expected fields and field gradients.

Let us consider a situation where a magnet which generates a certain maximum flux density strength B_{max} on the surface of its poles and has its largest pole dimension equal to D. Consider the behavior of the field and its gradient as a function of distance z away from the pole closest to the carriers. Roughly speaking, the magnet looks like a dipole from very far away because both of its poles contribute to the field in opposite ways. Therefore, a long way from the magnet both the field and its gradient will be too weak to be useful. However, in close proximity to the pole, that is $z << D$, the magnetic field is nearly uniform, that is $|\nabla B| \approx 0$, and nearly equal to the field at the pole, that is $B \approx B_{max}$. In this case, although the carriers will get magnetized, they will experience a weak force because the gradient is too weak. When $z \approx D$, the field diminishes roughly as $B \sim \frac{D^2}{z^2} B_{max}$, while its gradient decays roughly as $\nabla B \sim \frac{D^2}{z^3} B_{max}$.. In some intermediate range $0 \leq z \leq D$, the field may decay as $B \sim \frac{D}{z} B_{max}$ and its gradient will behave was $\nabla B \sim \frac{D}{z^2} B_{max}$.. This range is probably the most useful for magnetic targeting applications. Carriers located in this range will experience a field whose magnitude is some fraction of B_{max}, typically around $0.1 - 0.5\ B_{max}$, while the gradient will be roughly a similar, although often somewhat larger, fraction of $\frac{B_{max}}{D}$.

For example, when a permanent magnet material, such as samarium cobalt (SmCo) or neodymium iron boron (NdFeB), is employed to construct the targeting system, the maximum flux density at the surface of its pole might be about 0.5 Tesla (higher values are possible, but not in orders of magnitude). In its intermediate range, this magnet might generate a carrier magnetizing field around 0.1 – 0.2 Tesla. If the characteristic dimension of its pole is around $D = 0.1$ meters, a field gradient of about 2 – 5 Tesla/meter can be expected. It might be useful to compare these numbers to those found in typical Magnetic Resonance Imaging (MRI) systems. Typical MRI uses static field magnitudes of about 1.5 – 3 Tesla. MRI gradient coils employed for imaging generate gradients of the order of 30 – 100 milli-Tesla/meter. In most practical situations, where iron oxide-based carriers are employed, the carrier magnetization saturates above about 0.2 Tesla. Therefore, the magnetic field need not be much higher than this at the location of the carrier. However, gradients around 5 Tesla/meter are not strong enough for many applications involving carriers whose size is smaller than 1 micrometer. To increase the gradients, one has to either increase B_{max} or decrease the characteristic dimension D and, therefore, the useful targeting range. For example, if the useful range and characteristic

pole dimension are reduced to 1 millimeter, the gradient of the permanent magnet will be in the range of 200 – 500 Tesla/meter.

Magnet systems for magnetic targeting that have been proposed or employed so far fall into two main classes. One is the class where magnets external to the body provide both a carrier magnetizing field and field gradients for targeting. The other is one where a combination of external magnets and magnets (or magnetizable devices) proximal to the target location are employed. In the second class of systems, the external magnet would typically provide the carrier magnetizing field, while the proximal magnet (or magnetizable implant) will have small characteristic features and a short range in order to provide the largest possible field gradients for targeting [108,109].

Numerous examples of magnetic systems of the first type can be found in the literature. These are the systems employed by FeRx, Inc. in their clinical trials [110,111] (discussed below), by Lubbe and co-workers [112-114], by researchers working on aerosol delivery [115] and many others. One interesting example is the use of MRI for both targeting and monitoring drug delivery [116-119]. The main disadvantages in using the MRI magnets is that only limited control over the direction of the magnetizing field is possible, and the gradients are relatively weak, making it difficult to target particles smaller than a few micrometers in diameter. Potential advantages of this system are the combined targeting and imaging capability, as well as flexibility in the direction and time-variation of the field gradients. Examples where combinations of external and proximal magnets are employed can be found in work related to re-stenosis treatment, where cardiovascular stents are employed to generate locally strong field gradients [120,121]. Cell targeting studies have also been carried out using similar magnet systems [62] (discussed below).

4. Development of magnetic targeting and current applications

Medical applications of magnetic drug targeting and magnetic guidance have been studied for more than 50 years. In one of the first applications, Gilchrist injected 20 – 100 nm-sized maghemite particles into lymph nodes near surgically removed cancer for selective inductive heating [122]. Turner and Rand combined Gilchrist's radiofrequency heating method with embolization therapy where the ferromagnetic iron–silicone microspheres bearing the radioactive agent (P^{32}) were injected to achieve the arterial vascular occlusion of hypernephromas by obstructing the tumor vascular tree [123]. In 1960 Freeman proposed that magnetic particles could be transported through the vascular system and concentrated in a specific part of the body with the aid of a magnetic field [124]. In 1963, Meyers described the use of metallic iron particles in the lymphatic and vascular system of dogs as a contrast and isotopic agent. Meyers and co-workers were able to accumulate iron particles intravenously injected into the leg vein of dogs, using a large, externally applied horseshoe magnet [125]. The use of magnetic guidance was first proposed by Frei and his colleagues who designed a catheter tip with an attached micro-magnet to ease the difficulty of directing a paraoperational device catheter through the tortuosities of the intracranial vasculature [126]. An external magnetic field could be used to control the intravascular catheter. This concept was extended to applications such as the steering of a magnetic tipped catheter [127] and endovascular treatment of intracranial aneurism using iron particles [128,129].

The use of magnetic particles for the delivery of chemotherapy has evolved since the late 1970s. Zimmerman and Pilwat in 1976 proposed to load red blood cells simultaneously with a cytotoxic drug (methotrexate) and para- or ferromagnetic substances to obtain organ-specificity for any selected site of the body through magnetic targeting [130]. Subsequently, a number of investigators introduced the idea of using magnetic particles to act as therapeutic drug carriers in order to target specific sites in the body [131-133]. Widder and others developed magnetic microspheres bearing cytotoxic drugs. The magnetic carrier loaded with drug was injected into the subject either intravenously or intra-arterially. High-gradient, external magnetic fields generated by bipolar rare earth permanent magnets were used to guide and concentrate the drugs at the tumor locations. In the 1980s, several authors further developed this strategy to deliver different drugs using ethylcellulose-based microcapsules [134] and albumin-based microspheres [135-140]. In 1994, Hafeli and colleagues prepared biodegradable poly(lactic acid) microspheres that incorporated magnetite and the beta-emitter ^{90}Y for targeted radiotherapy and successfully applied them to subcutaneous tumors [141,142].

4.1 Magnetic drug delivery

The initial approaches described above utilized particles in the micron-sized range. Magnetic nanoparticles were probably used for the first time in animal models by Lubbe and his colleagues [113]. In the first part of their study, various concentrations of the magnetic fluid were tested in rats and immunosuppressed nude mice with regard to subjective and objective tolerance. In the second part, the same parameters were evaluated after administration of the ferrofluid to which epirubicin (4′-epidoxorubicin) was chemically bound. Two forms of therapy with the magnetic fluid were tested: tumor treatment by mechanical occlusion with the ferrofluid in high concentrations and magnetic drug targeting using small amounts of the ferrofluid as a vehicle to concentrate epirubicin locally in tumors. It was found that the ferrofluid caused no major laboratory abnormalities (including weight and behavior, hematological and blood chemical values, histology and survival) and there were no intolerances with the epirubicin-bound ferrofluid. Both forms of treatment led to complete tumor responses

within 14 days (in terms of the tumor's volume regression) in an experimental human kidney as well as in a xenotransplanted colon carcinoma model [113]. In 1996, a first Phase I clinical trial was carried out by the same group in patients with advanced and unsuccessfully pretreated cancers or sarcomas using ferrofluid with reversibly bound epirubicin. The main objective of this study was to determine a potential toxicity of the treatment with increasing concentrations of epirubicin while the amount of the ferrofluid that had been administered to the patients remained constant (0.5% of the estimated blood volume). All patients tolerated the magnetically targeted drug test dose well without anti-emetic or other supportive therapy. Based on magnetic resonance tomographic techniques, pharmacokinetics and the histological detection of magnetite, the ferrofluid was shown to be successfully directed to the tumors in 6 out of 14 patients. However, in that first trial, a significant portion of the particles not targeted to the tumor site accumulated in the liver, although without causing apparent adverse effects [114].

A second Phase I/II clinical trial was conducted by the start-up company FeRx, Inc. (founded in 1997). In their studies on 32 patients with unresectable hepatocellular carcinoma (HCC), carbon-coated iron particles of 0.5 – 5 μm bearing adsorbed doxorubicin (magnetic targeting carrier bound to doxorubicin [MTC-DOX]) were directly delivered to the target site via a catheter placed in a sub-segmental branch of a hepatic artery feeding the tumor to be treated. In this study, delivery was targeted to a single lesion in a specific hepatic segment, using a small, externally positioned magnet (5 kGauss or 0.5 Tesla) to create a localized magnetic field within the body over the tumor site. The physical force created by the magnetic field induced transport (extravasation) of the magnetic carriers through the vascular wall, leading to localization and retention in the tissue at the targeted site. MRI results showed that 28 days post-delivery the particles remained in the targeted site with no redistribution. Angiography showed no significant arterial embolization, and no study-related deaths occurred. The localization of the MTC-DOX to the tumor was achieved in 30 out of 32 patients (n = 24, single treatment; n = 6, two treatments; and n = 2, three treatments). The analysis of 20 of the tumors in 17 patients showed that 15 tumors had remained stable or reduced in size, and only five had progressed. The conclusion from this trial was that intra-arterial administration of the doxorubicin containing magnetic carriers in either single or multiple treatment cycles had no clinically significant toxicities [110].

Later in a similar trial, four patients from the larger group of 33 patients enrolled in previous Phase I/II trials of MTC-DOX of inoperable HCC agreed to participate in investigating the safety and efficacy of an MTC-DOX. In this study, tumors were characterized and treated by both conventional angiography and MRI during a single treatment session. Angiography helped to characterize the major hepatic artery branches supplying the neoplasm. It was also used for catheter guidance, delivery of MTC-DOX and evaluation of the target vessel patency after the injection of carriers. The MRI component was used to complement the angiography and helped to define tumor size and localization before MTC-DOX administration. After MTC-DOX was delivered, MRI enabled immediate intra-procedural evaluation of the location of unaffected tumors, the size of the treated regions, and the extent of the affected normal parenchyma. The results suggested that that the carrier/drug complex was well targeted to the tumor sites, with the final fraction of treated tumor volume ranging from 0.64 to 0.91. It was also found that the fraction of affected normal liver volume ranged from 0.07 to 0.30 [111].

Based on the initially promising results, a larger multinational trial, designated the MAGNET trial, was designed to enroll 240 patients at clinical sites in North America, Europe and Asia. The primary objective of the study was to detect a clinically and statistically significant increase in median survival time for MTC-DOX treated patients relative to patients treated with intravenously injected doxorubicin, the comparator arm in this study. However, from an interim analysis, it was determined that the clinical endpoints of the Phase II/III trial could not be met with statistical significance with the product (MagneTarg™, FeRx Inc., USA) manufactured at the time of trial and therefore, the clinical trial was immediately terminated in 2004. Unfortunately the company did not provide any reliable information regarding the possible reasons that led to the failure of these clinical trials. It is still remains unclear whether the targeting of the chemotherapy was inefficient, the release of the chemotherapeutic agent was not adequate or whether there were other reasons that did not lead to the expected improvement in median survival lifespan.

4.2 Magnetic gene delivery

Magnetically mediated gene delivery, usually referred to as 'magnetofection' is a promising technique in which a virus or nucleic acid is reversibly bound to the superparamagnetic carrier that can be then guided to the target site via a high-energy magnetic field. The idea of applying the magnetic targeting principle to nucleic acid delivery was first formulated by Kuehnle and Kuehnle [143] in a US patent application filed in 1994. In 1996, Chan filed a patent application disclosing the use of pulsating magnetic fields to transfect cells with nucleic acids complexed with superparamagnetic nanoparticles pre-coated with cationic lipids [144]. The first scientific paper on associating a genetic vector with magnetic microparticles was published in 2000 by Mah and co-workers, who reversibly conjugated recombinant adeno-associated virus (AAV) with magnetic microparticles, which led to increased vector transduction efficiencies [145]. Plank and colleagues reported on magnetofection and publicly used this term at a scientific conference in 2000 [146]. The work of Hughes and co-workers on magnetically enhanced retroviral nucleic acid delivery was the

first to be published in a peer-reviewed journal [147]. This report was shortly followed by Scherer and colleagues, who demonstrated that magnetofection promotes rapid transfection rates both for *in vitro* and *in vivo* applications [148] and Mah's paper on magnetically enhanced AAV vector-mediated gene delivery [149].

The efficiency of non-viral gene delivery systems critically depends on providing an optimal local concentration of the genetic vector to the target site and overcoming a number of barriers, including rapid degradation by extra- and intracellular endonucleases [150,151] and restricted nuclear entry in non-dividing cells [152,153]. The magnetically mediated gene delivery approach allows very efficient and rapid (within minutes) concentration of the genetic vector near the surface of cellular membrane *in vitro*, minimizing the exposure of the nucleic acids towards extracellular enzymatic degradation. It is of note that conjugation of adenoviral vectors with magnetic carriers enabled transduction of a number of cell lines that express little or no coxsackie-adenovirus receptor (CAR). This receptor, together with the $\alpha_v\beta$-integrin receptor system, is responsible for viral uptake through endocytosis. This result indicates that viral vectors associated with magnetic carriers may allow receptor-independent cell entry and thus extend the host tropism to non-permissive cells [148].

For successful expression and processing, the genetic vector must be reversibly bound to the magnetic carrier to be able to be transported into nucleus, the final destination. A number of strategies can be implemented including cleavable linkers, electrostatic interactions or incorporation of the vector within a degradable matrix, which releases the gene vector as the matrix is broken down. Although the use of target-specific linkers is an elegant approach to the attachment of target gene vectors, it is not always easily achievable. One of the alternative approaches for binding nucleic acids to the surface of the magnetic particles is the employment of a physical method based on electrostatic interactions. A popular choice for this approach is the cationic polymer polyethylenimine (PEI) in its branched or linear form. This polymer has proven to be an effective transfection agent in both *in vitro* and *in vivo* settings. Due to its high cationic charge density, this macromolecule is able to effectively condense DNA and form nanometer-sized particles taken up by endocytosis. In addition, since every third atom in the polymer is protonatable amino nitrogen, PEI acts as a buffer preventing endosomal acidification and protecting DNA from lysosomal nuclease degradation and facilitating endosomal escape to the cytoplasm by a 'proton sponge' mechanism [154]. Combining these useful features of PEI and magnetic nanoparticles, a number of studies have shown the potential of the magnetically driven plasmid DNA delivery in cell culture [148,155]. However, despite promising results showing high gene transfer efficacy in some cell types under optimized conditions, inefficient transfection was demonstrated with this approach in association with toxicity in contact inhibited cells [156]. Furthermore, iron oxide nanoparticle aggregation on DNA complexation in a salt-containing medium resulted in a loss of colloidal stability [157-159]. To address these limitations Chorny and co-workers investigated a DNA delivery concept combining a biodegradable solid phase polymeric carrier with magnetic targeting. In these studies the biodegradable magnetite-laden nanoparticles were formulated with controllable size and surface modification with PEI oleate ion-pair complex for DNA binding. The MNP efficiently bound, protected and delivered plasmid DNA in the presence of serum and allowed for high transfection efficiency in contact-inhibited cultured vascular cells [35].

Although the PEI is a very efficient transfection agent, it is a non-degradable macromolecule that possesses high cytotoxic effects, depending on its concentration in the cell nucleus, where it may interfere with critical cell functions [160-162]. Therefore, focusing research efforts on the development of biodegradable polymeric alternatives to PEI, such as poly(β-amino esters) [163,164] or quaternary ammonium polysaccharides [165-168] in combination with magnetic biodegradable carriers may tremendously improve the performance of the magnetic carriers in gene delivery applications.

In another recent study, superparamagnetic iron oxide nanoparticles (SPIONs; 50 nm) coated with 25-kD branched PEI were used in the form of an aerosol for targeted delivery of plasmid DNA (pDNA) to the lung [115]. Calculations in this study demonstrated that even with optimized magnet design, the resulting magnetic forces would not be sufficient to efficiently guide physically independent SPIONs because of their small magnetic moment. However, it was calculated that if a multitude of SPIONs are assembled in an aerosol droplet ('nanomagnetosol') of an average diameter of 3.5 μm, this would lead to an increased magnetic moment of the assembly, which would result in aerosols guidable by technically feasible and medically compatible magnetic fields and field gradients. The authors designed and built an electromagnet with biconical coil tip architecture to create a high magnetic field flux gradient of $\nabla B \geq 100$ T/m in close proximity to the magnet tip. Two experiments were performed. The first experiment was conducted using a toracotomy where the lungs were exposed and the magnet tip was positioned maximally close the lung. The results indicated an eightfold higher accumulation than in the opposite lung. In the second experiment the lungs were not exposed and the magnet's tip was therefore positioned at a distance from the actual target tissue. In this case the results indicated a 2.5-fold higher accumulation compared to the control lung. Although the amount of deposited pDNA did not differ between the left and right lungs in the absence of the magnetic field, a twofold higher dose of pDNA was detected in the magnetized right lung than in the unmagnetized left lung. These results demonstrated that nanomagnetosols can be used successfully for targeted aerosol delivery of pDNA to the lung. To reduce deposition in the untargeted lung

lobe, the authors suggested, based on their calculations, the positioning of a bypass magnet close to the main bifurcation of the trachea. The gradient field from this magnet will result in a force to direct the major part of the particles to the desired lung lobe. A second target magnet positioned downstream will trap and retain the particles at the target site [115]. However, scaling up the magnetic gradient field to address the size of the human lung represents the major challenge in translating this technology to humans, although high field gradient electromagnets for use in magnetic drug targeting in pigs are already available [169,170]. Therefore, the proposed magnetic targeting of nanomagnetosols might have a great potential for targeted inhalation therapy in the future.

4.3 Magnetic cell delivery

Using living cells (erythrocytes, lymphocytes and leucocytes) as drug delivery systems for temporal and spatial drug administration in human therapeutics and diagnosis was conceptually proposed by Zimmerman and Pilwat in 1976 [130]. In order to obtain organ-specificity, they proposed to load erythrocytes with both drug (methotrexate) and para- or ferromagnetic substances. The advantage of using erythrocytes or other autologous cells is due to their biocompatibility, which leads to a more prolonged circulation lifespan of these vehicles. Later, in the 1990s, erythrocyte-based delivery systems have demonstrated the potential to increase drug concentration by many times at the target site under the influence of an external magnetic field [171-173]. Recently, magnetite-loaded erythrocytes were used as contrast agents for MRI to achieve the prolonged circulation time in the bloodstream [174].

One of the first vascular applications where magnetically responsive endothelial cells were used to improve re-endothelialization of arterial surfaces after angioplasty was reported by Consigny and co-workers in 1999 [175]. They found that delivery of endothelial cells loaded with superparamagnetic microparticles in combination with animal rotation and a magnetic field provided nearly circumferential delivery of endothelial cells to the luminal surface of balloon-dilated arteries. The presence of superparamagnetic microspheres in cells did not impede cell adhesion but did decrease cell retention after exposure to a fluid shear. Pislaru and colleagues recently reported on successful magnetic localization of endothelial cells loaded with magnetic particles on synthetic vascular grafts (using embedded permanent magnets) [176] and in stented vessels using a ferromagnetic stent implant [177]. In another recent study, Muthana and co-workers showed that migration of transfected human monocytes across a human endothelial cell layer into a 3D tumor spheroid is markedly increased when cells were pre-loaded with MNPs and a magnetic force was applied close to the spheroid. Furthermore, systemic administration of such 'magnetic' monocytes to mice bearing solid tumors led to a marked increase in their extravasation into the tumor in the presence of an external magnet [178].

These investigations of magnetically targeted cell delivery systems employed magnetic field sources in a sub-optimal manner by using locally applied magnets. Magnetic force is proportional to both the MNP-loaded cells' magnetic moment and the gradient of the external field at the MNP-loaded cell location. Prior work had been limited to using a single source of magnetic field, in which either a locally applied permanent magnet [175,176], or a ferromagnetic medical implant [177] was used to implement the magnetic capture system. Such sources can be designed to increase the MNP-loaded cells' magnetizing field or the field gradient, but not both; thus making it impossible to maximize the fraction of captured MNP-loaded cells by these techniques.

To address the limitations of the single source magnetic field, Polyak and co-workers have implemented a concept of high gradient magnetic fields, as previously used in high-gradient magnetic separations (HGMS) [62]. This concept is fundamentally different than previously developed approaches in that the magnetic force can be maximized by using a modest uniform magnetic field to both magnetize the MNP-loaded cells and produce large local magnetic field gradients within the magnetizable implant. Specifically, a uniform magnetic field is supplied by electromagnetic coils held external to the body, and the short-range high-gradient magnetic fields (HGMF) are produced by the magnetizable wires of an implant (stent in vascular application), for the purpose of maximizing the regional magnetic force on the delivered MNP-loaded cells. A schematic representation of a stented blood vessel (**Figure 1**) shows that magnetically responsive cells are attracted to steel stent struts in a uniform magnetic field due to generated magnetic force ($\vec{F}_{mag}$) that directly depends on the strength of the total magnetic field ($\vec{B}$), high field magnetic gradients ($\nabla\vec{B}_g$) induced on the stent struts and the magnetic moments ($\vec{m}$) induced on MNP-loaded cells by the uniform magnetic field $\vec{B}_o$. The total magnetic field ($\vec{B}$) is a sum of a gradient field ($\vec{B}_g$) of the stent and a uniform field ($\vec{B}_o$). Using this approach Polyak and co-workers have demonstrated that MNP-loaded bovine aortic endothelial cells (BAECs) could be efficiently targeted to magnetizable steel stent wires *in vitro* and *in vivo* [62]. Further studies involving investigations to quantitatively determine the efficacy of the cell targeting, the fate of the off-targeted cells, re-endothelialization efficiency and therapeutic outcomes are necessary to reach any conclusions about the future clinical implementation of this method.

5. Conclusion

The approach of magnetic particle-based delivery of drugs has significantly evolved since the first developments in the early 1970s. Magnetic carriers of increasing sophistication have been developed with properties that include: nano-size dimensions, biocompatibility, controlled magnetic responsiveness, prolonged circulation lifetime, surface stabilization and surface recognition. These developments have enabled

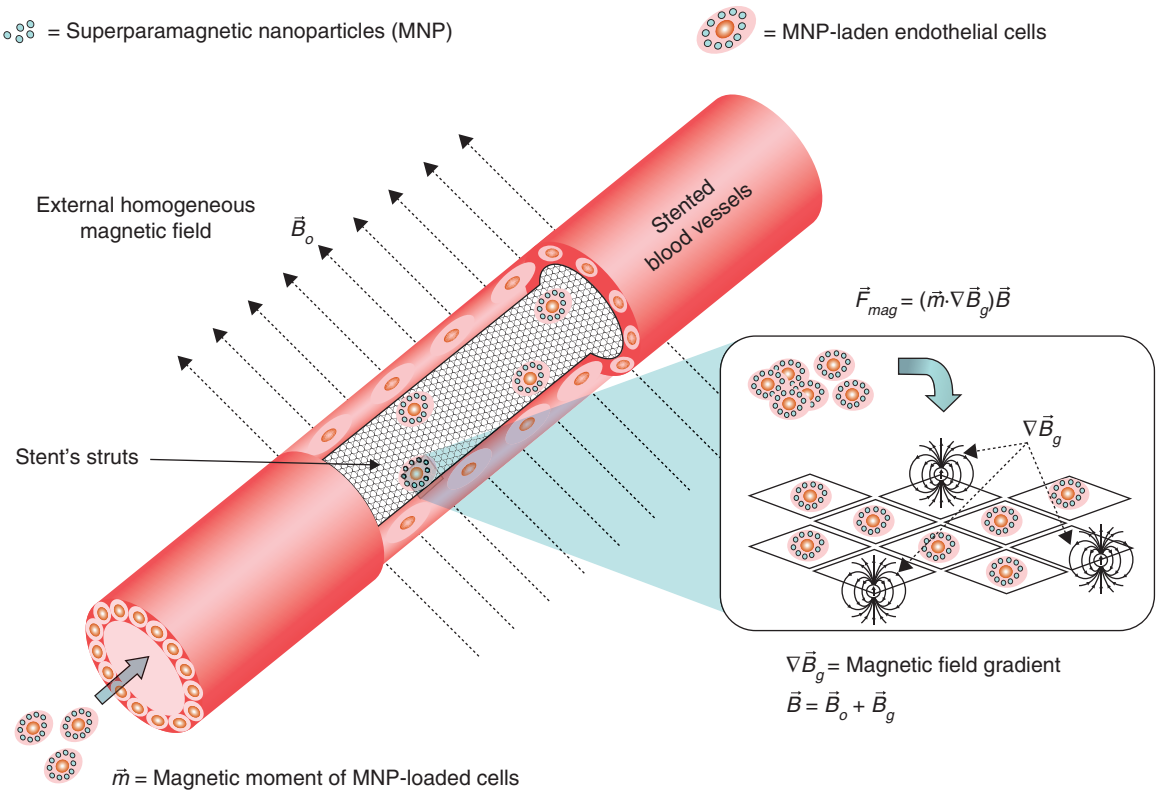

Figure 1. A schematic representation of a stented blood vessel shows that magnetically responsive cells are attracted to steel stent struts in a uniform magnetic field due to generated magnetic force.

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an expansion of magnetic targeting possibilities to the delivery of conceptually novel therapeutic agents, that is genes and cells. Although clinical trials are few and many obstacles remain to be overcome before this technology can achieve therapeutic success and reach the marketplace, recent advances are quite promising.

While the design of new magnetic drug-carrying vehicles has been the focus of intense research and development over the past two decades, efforts to improve magnetic means of delivery have received less attention. More effective magnetic targeting strategies are required for the successful transition of this technology to the clinic. The more efficient magnetic means of targeting in combination with the prolonged circulation lifespan and carrier's surface recognition properties will improve the targeting efficiency of magnetic nanocarriers and enhance therapeutic agent availability at the molecular site of agent action, consequently reducing the overall systemic side effects and maximizing the therapeutic outcome. While magnetic targeting is not likely to be effective in all clinical situations, advances and further development in this field will be an integrated component of our efforts to provide an alternative or complementary tool for the efficient treatment of a variety of diseases.

6. Expert opinion

Although the technology of therapeutic magnetic targeting has evolved significantly over the last two decades and a number of successful Phase I clinical trials have been completed, there are still no clear successes in clinical translation of this treatment modality. We believe that magnetic targeting will find important clinical uses in the future. However, substantial improvements in both the magnetic carriers and the targeting magnet systems are needed to make magnetic targeting viable. Different types of improvement will most likely be employed in different medical applications. Below we attempt to broadly categorize the main types of applications and to describe possible strategies for improving the magnetic targeting technique.

It is reasonable to split all possible applications into three major categories. The first category includes targeting the vascular system (i.e., the arteries and veins) or any other system for the transport of bodily fluids (e.g., hepatic and renal excretory duct, ureter or urethra). The second category covers targeting of the hollow organs, which might be parts of the digestive system (e.g., esophagus, stomach, intestines or colon) or parts of the respiratory system such as trachea and bronchi. The third category is related to targeting of soft tissues, perhaps to treat malignant neoplasms in organs

like the breast or prostate or for applications such as wound healing, transdermal drug delivery and even orthopedics. In each of the above categories there is a distinct set of challenges and opportunities related to magnetic targeting.

In the case of the vascular applications, rapid circulation and sequestration of the magnetic carriers by parts of the immune or renal systems are the main challenges. Some success has been demonstrated in increasing the concentration of therapeutic agents at targeted sites by using magnetizable implants, such as stents, that produce very high local field gradients to fight the drag forces of rapid flow. Although completely eliminating systemic distribution of the carriers to untargeted locations seems very unlikely, some improvements in the properties of carriers and magnetizable implants employed in these systems might further increase the captured fraction. One opportunity that has not yet been explored in vascular applications is to implement carrier retrieval strategies (most likely based on apheresis) to minimize risks from the untargeted fraction. In this case one would certainly want to ensure that magnetically untargeted carriers are not immediately sequestered by the immune system or RES and remain in circulation beyond the first pass. Although carriers of elongated shapes, needle-like structures or flexible chains of spherical particles are less likely to be sequestered by the immune cells or RES and technology for preparations of such carriers exists [49,179-181], apparently no studies have been performed to investigate such carriers in magnetic targeting.

Aerosol-based delivery to the respiratory system seems to be a very promising use of magnetic targeting because drag forces due to air in the trachea or bronchi are relatively weak. At the same time these pathways do not have components of the protective immune system that can reduce the fraction of the targeted drug. Hence, in these applications the targeting will likely be much more efficient. However, increasing the fraction of targeted carriers versus those that do not make it to the target is still an important concern. Further improvements could be made when targeting implanted devices, like stents, in the airways, because these devices could be made to produce very large localized field gradients. It may also be possible to design magnet systems that pull the carriers away from the sides of the airways in untargeted locations, increasing, for example, the fraction of carriers that reaches the lungs. Potentially, carriers can also be substantially simplified when it comes to aerosol delivery. They do not always need to be solid composite particles, as in the case of soft tissue and blood applications. When magnetic nanoparticles are mixed into the fluid aerosol particles, the overall size of such particles can be enlarged from hundreds of nanometers to few micrometers, since no danger of embolism would exist in this case. Gravitational forces on micrometer particles are too small to prevent them from being suspended in air flow. At the same time, using micrometer-sized particles will significantly increase the magnetic force. Using fluid droplets could also improve the eventual clearance of magnetic material from the body.

The third category of applications, one that involves transporting carriers through tissues, also has many opportunities for improvement and study. Issues that remain very poorly understood are related to the effect of viscoelastic properties of tissues on the carrier transport. It is not at all clear what the optimal carrier size and shape is in this case. For example, smaller carriers may have time to enter some cells through endocytosis and, possibly, move together with more mobile cells. In some cases, it may even be possible to employ cellular motility and to use the magnetic field only to direct the motion. Larger carriers may move through tissues largely via intercellular spaces. For the same amount of magnetic material, elongated carriers or carriers designed as flexible chains of spheres have much better chances of overcoming yield stress in order to start moving. Yet, as far as we know, the use of such carriers has not been explored. It may even be possible to control undulations of flexible carriers in order to create swimming-like locomotion through tissue that does not rely at all on application of magnetic field gradients and employs only uniform magnetic field as a source of energy [182].

Even if locomotion of carriers is difficult to achieve, the use of a time-varying uniform magnetic field superimposed onto the permanent gradient field may dramatically improve transport of carriers through soft tissues and their delivery to the target. We hypothesize that, when applied correctly, time-varying magnetic field could create oscillating force on the carriers which will reduce effective yield stresses which prevent the carriers from moving and, possibly, decrease effective viscous drag in tissues. Alternatively, the magnetic targeting can be enhanced by changing the effective resistance of the biological tissues, for example, by using magnetic carriers with proteolytic surface functionalization to increase carrier's mobility through the extracellular matrix, as was shown *in vitro* [183] or through the inertial cavitation created by ultrasound, as has been shown in transdermal drug delivery [184,185].

In summary, we believe that many opportunities for new developments exist in therapeutic magnetic targeting. Some are related to applications that have not yet been explored. Others are more technological in nature. Since the late 1990s most of the research efforts in the area of magnetic targeting focused on the carrier's development [4,17,19,186-194], often ignoring issues related to the design of targeting magnets and carrier transport mechanisms. This situation is likely to change in coming years as more individuals with an interdisciplinary background become involved in magnetic targeting.

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Declaration of interest

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