Focus Article

Open challenges in magnetic drug targeting

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The principle of magnetic drug targeting, wherein therapy is attached to magnetically responsive carriers and magnetic fields are used to direct that therapy to disease locations, has been around for nearly two decades. Yet our ability to safely and effectively direct therapy to where it needs to go, for instance to deep tissue targets, remains limited. To date, magnetic targeting methods have not yet passed regulatory approval or reached clinical use. Below we outline key challenges to magnetic targeting, which include designing and selecting magnetic carriers for specific clinical indications, safely and effectively reaching targets behind tissue and anatomical barriers, real-time carrier imaging, and magnet design and control for deep and precise targeting. Addressing these challenges will require interactions across disciplines. Nanofabricators and chemists should work with biologists, mathematicians, and engineers to better understand how carriers move through live tissues and how to optimize carrier and magnet designs to better direct therapy to disease targets. Clinicians should be involved early on and throughout the whole process to ensure the methods that are being developed meet a compelling clinical need and will be practical in a clinical setting. Our hope is that highlighting these challenges will help researchers translate magnetic drug targeting from a novel concept to a clinically available treatment that can put therapy where it needs to go in human patients.

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INTRODUCTION

Magnetic drug targeting refers to making therapy magnetically responsive, so that it can be manipulated inside the body by external magnets, and thus focused to disease locations such as deep tissue tumors. In the first human trials of magnetic drug targeting,[1] the chemotherapy drug epidoxorubicin was attached to 100 nm diameter bio-compatible iron-core particles, these particles were administered systemically, and an external magnet was used to concentrate the therapy to inoperable but shallow tumors (Figure 1(a)). In these human safety trials, blood sample HPLC (high-performance liquid chromatography) and magnetic resonance imaging (MRI) measurements showed that the magnet removed about half of the particles from blood circulation and collected them to the vicinity of the tumor.[1,2] It was found that all patients tolerated the magnetic drug delivery procedure and that peak epidoxorubicin concentrations in blood plasma were much reduced for patients with magnetic drug targeting as compared to patients who received conventional systemic epidoxorubicin applications. Since a single magnet can only attract magnetic particles,[3–5] these trials were restricted to treating tumors near the skin surface.

Today, 18 years later, although there have been significant advances in the field, we are still a long

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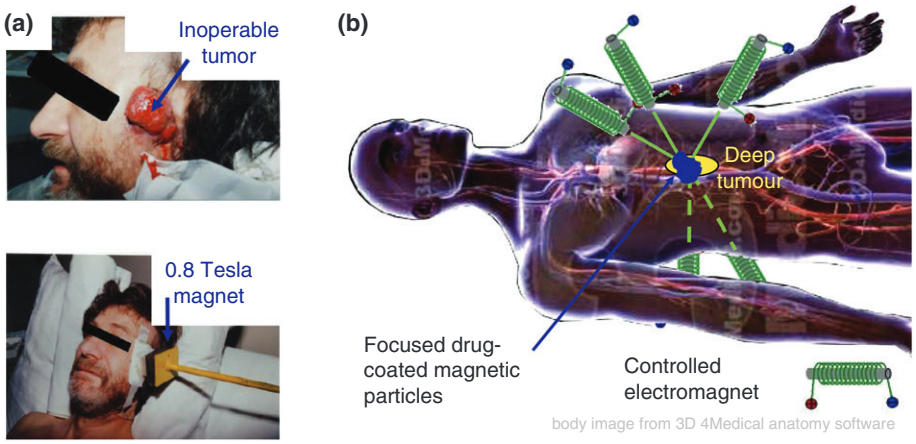

FIGURE 1 | (a) The first human trials in magnetic drug targeting.[1] Epidoxorubicin-coated magnetic nanoparticles were administered systemically to advanced head and neck and breast-cancer patients, and a single permanent magnet was held near inoperable but shallow tumors to concentrate the chemotherapy. (b) A goal in magnetic targeting is to use magnetic fields to focus therapy precisely to any desired target in the body, for example to a deep tumor as illustrated. Currently there are no magnetic systems that can achieve this kind of precise and deep focusing.

way from being able to magnetically direct therapy to wherever it needs to go in human patients—to deep targets (Figure 1(b)), to thousands of metastases, safely to targets in the brain, and to disease targets behind cellular and tissue barriers or anatomical obstructions. The majority of prior studies have been restricted to small animals, there have been only a small number of human clinical trials,[1,6–9] and the critical issue of scale-up to human dimensions is still open. Our goal in this article is to identify open challenges in magnetic drug targeting that must be solved so that it can safely and effectively target a broad range of human diseases.

CHOOSING MAGNETIC CARRIERS, FOR SPECIFIC CLINICAL NEEDS

A wide variety of magnetic carriers have been demonstrated and proposed in the literature. Drugs and gene therapy have been attached to magnetic nanoparticles,[10–16] bio-compatible microscopic or nanoscale capsules have been filled with both drugs and magnetic materials,[17,18] and live cells have been cultured in media that contains magnetic particles so that the cells injest the particles and can then be manipulated by magnetic fields.[19,20] Multiple excellent review articles are available that describe progress and challenges in developing safe and effective magnetic carriers.[9–11,21–24] These carriers differ in their properties (size, shape, flexibility, coatings, magnetic loading, and drug loading), experience different size forces for the same applied magnetic field, and encounter different motion resistance for different bodily fluids, barriers, and tissue types. One carrier type does not fit all clinical needs, and thus there is a need to select, design, and implement carriers that are matched to specific clinical indications. The particles that are best for targeting of deep tumors after systemic administration are not necessarily best for crossing the blood–brain barrier (BBB) or for safely penetrating the window membranes to reach inner ear diseases.

For the situation where magnetic particles are administered systemically into the blood stream and external magnets are meant to capture and collect them to a desired target (as in the original Lübbe clinical trials,[1] Figure 1(a)), a first question is whether the applied magnetic field is sufficient to hold particles against blood flow at the target region. We investigated this question in Refs 25, 26. Based on physical first principles (particle diffusion and convection by blood, and magnetic drift), we computed the distribution of particles in and around small and large blood vessels, located at any depth in the body, for the range of magnetic field strengths used/anticipated in magnetic drug targeting, and we compared our predictions to all then-available *in vitro* and *in vivo* experimental data. We collapsed the large magnetic delivery design space (particle size, magnet size, shape, and strength, blood vessel depth and flow velocity) to three essential nondimensional parameters, and computed the parameter region where the applied magnetic field could hold particles against blood flow. This analysis answered which particles could or could not be captured by an applied magnet. It predicted in which blood vessels (with which diameter, depth, and blood velocity) which particles could be held magnetically against blood flow, and it matched till-then available

experimental data. For example, we were able to predict the depth of particle focusing observed by MRI in the Lübbe clinical trials. Next questions for carrier behavior in blood flow include expanding the analysis to cover nonspherical carriers (e.g., rods, wires, shells, cubes, triangles, etc.,[27–29]), as well as extending the analysis to living cells (e.g., stem cells) loaded with magnetic materials.

To optimize therapy delivery into tissues targets, the next steps are to better understand carrier transport and penetration under magnetic forces through vessel walls and into tissues (e.g., across the blood–brain barrier), through various tissue types (liver, muscle, fat, brain, etc.), and across anatomical barriers (skin, ear window membranes, eye sclera, etc.). In Ref 30, based on breast-cancer patient autopsy data and numerical simulations, we predicted that magnetically shifting nanoparticles would allow them to reach thousands of poorly vascularized liver metastases, which otherwise would not be reached effectively by nanotherapy (Figure 2(a)). Normal and cancerous liver resistance to carrier motion was represented according to two commonly used mathematical models—the Renkin pore model[31] and the fiber-matrix model[32]—and we found an optimal particle size for shift effectiveness. Too small particles (<10 nm diameter) would not experience sufficient magnetic force to move effectively through the liver (because magnetic forces scale with particle volume). However, if particles were too big (>400 nm) they would encounter too much tissue resistance. Our optimal particle size prediction (see Figure 2(b) below) must now be tested against animal experiments, and to that end we have initiated a program to measure magnetically induced motion of different particle types in animals and freshly excised tissue samples.[33] In addition to size, our preliminary data indicates that particle surfaces and coatings are key parameters. For example, chitosan-coated particles move better than starch particles of the same size and magnetic loading through freshly excised rat liver tissue. Thus there is a need to select both carrier size and coatings to enable the most effective magnetic delivery of therapy to target tissues.

For directing magnetic nanoparticles through tissue barriers, e.g., through the ear-drum to reach middle ear infections without ear-drum puncture or through the eye sclera to treat the retina,[34,35] it is also still an open question which particle sizes and coatings are best. To answer these type of questions, we believe there should be reproducible standardized experimental methods to characterize the transport of magnetic carriers through live tissue, to measure which carriers move most effectively yet safely through blood vessel walls, different tissue types, and across barriers.

The BBB is of particular interest as it can prevent or limit therapy from reaching brain tumors and other brain diseases (Figure 3(a)).[36–43] Unlike in other organs (e.g., liver, spleen, etc.), endothelial cells that separate the blood stream from brain tissue are tightly attached to each other, minimizing free passage of substances between blood and the brain.[36,37] Additionally, efflux transporters (e.g., P-glycoprotein) located in the membranes of these cells actively pump out most drugs that arrive.[38] Thus, to deliver therapy to the brain requires strategies to safely and effectively bypass the BBB, as illustrated in Figure 3(b).

A potential approach to pass through the BBB is to mimic the active transport mechanisms by which natural body substances (e.g., nutrients) and natural body carriers (e.g., lipoproteins, exosomes) travel from the blood into the brain.[36,40] This 'Trojan horse' approach leverages the presence of specific channels in cells of the blood–brain barrier.[36,37] Alternatively, transport can be mediated by binding to specific receptors on endothelial cells, which triggers uptake at the blood interface, transport across the barrier, and release at the brain interface (transcytosis[36,41]). Magnetic forces can potentially help therapies cross the BBB. Recent studies in cell cultures and rodents have shown that magnetic nanoparticles of different types (silica, PAMAM dendrimers, liposomal, etc.) can traverse the BBB for gene transfection or drug delivery.[44–49] Use of magnetic carriers for therapy delivery into the brain requires special attention to safety. Carriers that contain magnetic materials, and that also have protective, solubilizing, or molecular targeting coatings, can easily approach or exceed the size limitations of the natural transport pathways shown in Figure 3(b). For safe and effective delivery into the brain, carrier designs should be carefully adapted to physiological variables of blood flow, disease status, and brain tissue architecture.[41]

After carriers have reached their disease target, in the body or in the brain, they must further safely and effectively release or provide their therapeutic payload to target tissues. Items that should be addressed include drug loading and drug release rates from the carriers under physiological conditions, as well as the resulting uptake and elimination of the drug and the body's response to the drug (pharmacokinetics and pharmacodynamics).[9–11,21–24,50,51] Magnetic carrier design should also avoid carrier agglomeration which can block blood vessels and must ensure adequate stability and shelf life to enable regulatory approval and subsequent clinical use.

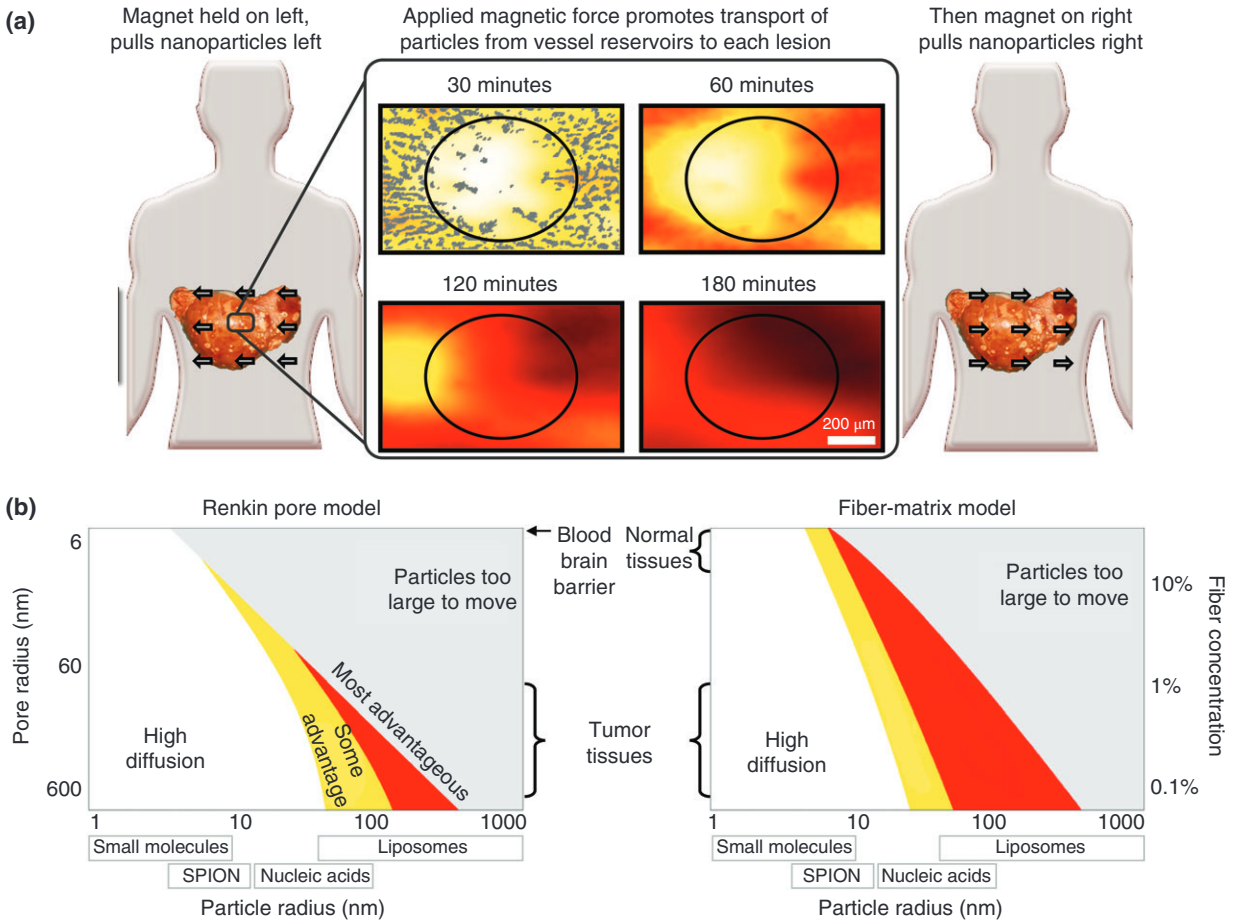

FIGURE 2 | The magnetic sweep concept to reach hundreds of poorly vascularized metastatic tumors. In human autopsy studies of breast-cancer patients who died from their disease, we measured vascularization in and around hundreds of micro-metastases (top middle panel: tumor marked by the black oval, blood vessels marked in gray). A magnet on either side of the patient could pull nanoscale magnetic carriers from the surrounding well-vascularized normal liver into each poorly-vascularized micro-metastasis. Our simulations indicate that there is an optimal nanoparticle size: big enough to react to the applied magnet, small enough to move effectively through liver tissue. (Reprinted with permission from Ref 30. Copyright 2011 Dove Medical Press Ltd)

To answer the type of magnetic drug delivery challenges posed above, as a research community we need to select and optimize magnetic carriers for specific clinical needs, and we must bring those carriers up to a level where they can pass regulatory scrutiny. Rather than continuing to implement new types of magnetic carriers because we can, we should take a step back and ask: which type of carrier is best for this clinical need? And what advances are needed for this carrier so that it can achieve FDA or EMA (European Medicines Agency) regulatory approval? Since different carriers travel differently through blood and tissue types, and since size, shape, and coatings can change magnetic forces and resistance to motion by orders of magnitude[26,33,52–54] as well as impact carrier drug release and safety, this is a choice that should be made with care. If only one or two parameters are considered (e.g., particle size and coating), it is conceivable to choose the best type of particle for a particular clinical need by live animal testing—one could imagine testing a 3×3 matrix of cases with three particle sizes and three coatings for a few animals in each group and selecting the best one. However, when we also consider carrier shape, flexibility, targeting coatings, the potential for agglomeration (which is influenced by magnetic field strength and concentration), drug release rates, and using living cells loaded with magnetic materials as carriers, then the design space becomes too large to search with animal studies alone. We need to begin to *understand* how magnetic carrier properties influence their motion and drug release *in vivo*, at least to the degree that we can begin to make sensible judgements about when to use which carriers. Then we need to select a few best candidates and carry out the extensive safety and efficacy animal testing that will enable human trials and regulatory approval.

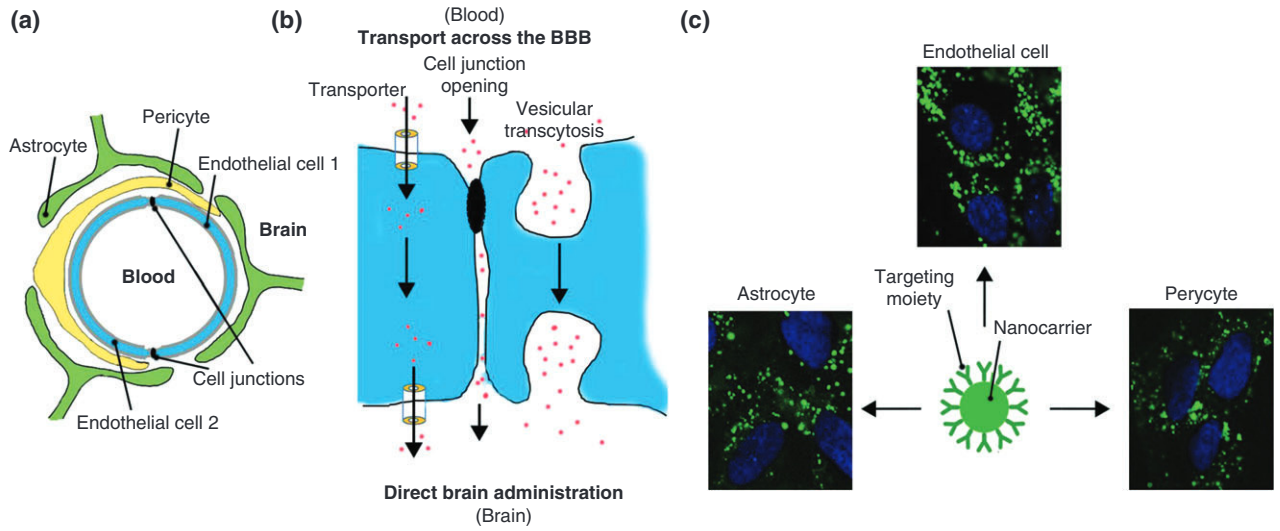

FIGURE 3 | Transport across the blood–brain barrier (BBB). (a) Schematic representation of a blood capillary vessel in the brain. Endothelial cells surround the vessel lumen and seal the passage into the brain by tight cell–cell junctions. Pericytes and astrocytes surround the endothelial lining, further tightening the barrier. (b) Delivery of therapeutics into the brain can be achieved by direct administration through the skull, or by using therapeutics that will cross the BBB. The later involves temporary disruption of the BBB cell–cell junctions (paracellular route) or transport across endothelial cells (transcellular route), including passage using transporter protein channels or vesicular transcytosis. (c) Nanoparticles coated with ligands which can bind to receptors of vesicular transcytosis (ICAM-1 is shown in this example) results in active uptake by cells of the BBB, including endothelial cells, astrocytes, and pericytes.[42]

IMAGING OF CARRIERS AND THERAPY, IN REAL-TIME

To magnetically direct the therapy to the right place, in most cases it will be necessary to be able to visualize where the therapy is versus where the disease targets are located. Thus effective (real-time and deep) imaging of magnetic carriers is a key need, and is also a research challenge. In the Lübbe clinical trials, magnetic nanoparticles were imaged immediately after patient treatment by MRI. Accumulation of particles in and around the tumor disrupted the MR signal and was visible as an extinction phenomena.[2] However, it was not possible to magnetically treat and image at the same time because the permanent magnet shown in Figure 1(a) would have interfered with MRI operation. Nor was it possible to quantify the amount of magnetic particles delivered to the tumor by the disruption of a MR signal.

Two emerging methods have the potential to image magnetic carriers deep *in vivo* and in real time. Magnetic particle imaging (MPI) exploits the nonlinear magnetic response of super-paramagnetic and paramagnetic nanoparticles under strong (>3 T/m) magnetic field gradients.[55–59] It creates a magnetic field node point within the imaging location using two external coils, then additional driving coils apply a time-varying magnetization to which particles near the node point will respond. Finally, additional sensing coils interpret the particle magnetization response and infer the particle concentration at the node point. MPI was specifically designed for imaging of magnetic carriers, and has been shown to have sufficient spatial and temporal resolution to resolve particle concentrations in the beating heart of a mouse.[59]

The spatial resolution of both MPI and MRI is limited by the strength of the spatial magnetic gradient that can be applied.[60,61] In prior studies, it was thought that peripheral nerve stimulation effects limited the allowable strength of the applied magnetic gradient times the pulse duration to be below a linear threshold (see Figure 6 in Glover[62]). Commercial MRI systems (with millisecond gradient rise times) are unable to achieve microsecond magnetic pulses. Based on Glover this limits their allowable magnetic spatial gradients to ~0.1 T/m. However, recent human trial data from Weinberg has shown that it is possible to eliminate nerve stimulation effects, even at higher field magnitudes, by using ultra-fast magnetic pulses (e.g., with rise times of less than 10 microseconds).[63] Such fast rise times require high voltages and currents that are not readily implemented with conventional MRI, but can be achieved with pulsed-power switching technology and custom high-voltage coils. Instead of the 0.040–0.080 T/m maximum gradients provided by human MRI systems,[64] pulse-power enables ~1 T/m spatial gradients without peripheral nerve stimulation.

Experiments are now underway to interleave imaging and propulsive pulse sequences within the same pulsed-power platform so as to enable real-time image-guidance of magnetic targeting. Such a platform could measure the 3-dimensional anatomic distribution of magnetic carriers multiple times a second, and after each measurement could apply a precisely shaped magnetic field to modify the observed distribution. Using appropriate control algorithms (discussed next) the platform could thus be used to coincide the distribution of therapy with tumor margins while sparing healthy tissue. A reasonable first clinical target would be brain tumors, which are often difficult to treat due to irregular and indistinct margins, and the required extreme attention paid to reducing collateral damage to neighboring structures in healthy brain tissues.

MAGNET DESIGN AND CONTROL, TO REACH DEEP TARGETS

Overall, one of the biggest open challenges in magnetic delivery is precisely targeting deep tissue targets—there are as yet no imaging and actuation systems that can achieve the external-magnet deep-focusing shown in Figure 1(b). To achieve deep targeting requires solution of, at least, four major issues: (1) sufficient magnetic fields/forces deep in the body, (2) real-time imaging, (3) sophisticated control algorithms, and (4) mathematical modeling of carrier motion *in vivo* with at least enough fidelity to enable effective design of the imaging/actuation system and the control algorithms that will determine which magnets to turn on when and for how long. Imaging has already been discussed above, so we now turn to deep forces, mathematical modeling, and control design.

A first reason deep tissue magnetic targeting is difficult is because magnetic fields and forces fall off quickly with distance from external magnets.[65,66] There are two noninvasive ways to improve the situation: improve the external magnets to provide stronger and deeper magnetic gradients or optimizing the magnetic carriers to react more strongly to a magnetic gradient. Optimization of permanent and electro-magnets to increase the strength and depth of magnetic gradients has been reported in Refs 67–73. In our own work, we showed that semi-definite optimization tools could be used to design and implement Halbach-array permanent magnets that provide improved pulling or pushing forces on magnetic nanoparticles.[74] Optimizing magnetic carriers, as discussed in the *Choosing Magnetic Carriers* section, can potentially provide significant improvement in achievable targeting depth. Since magnetic forces are strongly dependent on carrier size, shape, magnetic material properties, and agglomeration, while bodily fluids and tissue resistance forces also depend on size and shape as well as on carrier coatings and potentially mechanical flexibility, it is likely there is significant design freedom to create much more responsive magnetic carriers. As a community, we need to understand enough about carrier motion *in vivo* to design more effective magnetic carriers, and enough about fabrication processes to make them.

To rationally select magnetic carrier designs, and also to implement magnet control algorithms that will drive magnetic carriers to their desired targets, requires understanding and mathematical models at the right level of fidelity and complexity. For magnetic carrier design, it is not feasible to search the large carrier design space by fabricating all-possible magnetic carriers and then testing each of them in animal experiments. Instead, we need to build up predictive-capabilities and mathematical models that will help guide us through the design space faster than animal or tissue experiment. Hence there is a need for simple but at least roughly predictive models that can help tell us what kind of carriers to investigate experimentally.

Mathematical models are also required for dynamic control of magnets to precisely direct magnetic carriers to deep targets. To decide which magnets to turn on when, we need to know, again at least roughly, what each magnet will do to the magnetic carriers *in vivo*. Once a mathematical description is available, then there is a possibility that it can be inverted to decide how to actuate the external magnets to direct the carriers to where they need to go in the patient.[22,75,76] Real-time imaging can greatly aid this process by providing real-time information on where the carriers are in the patient's body, so that feedback control (discussed next) can shift the distribution of carriers from where they are observed to be toward where they should be at each control update time.

Precision feedback control of a single magnetic or magnetisable element has already been demonstrated in animals and in patients.[77–83] However, focusing a *collection* (a ferrofluid) of magnetic carriers to a single deep location is more difficult than manipulating a single object because while one particle may be being driven toward its target, the same magnetic field may be driving another particle away from the target. A mathematics result from over 150 years ago summarizes a key challenge to deep tissue focusing. Samuel Earnshaw's 1839 theorem,[22,84] when applied to Maxwell's equations and the magnetic force acting on ferromagnetic nanoparticles,[85–87] shows that no arrangement of external magnets can create a static magnetic trap that will attract all particles to an

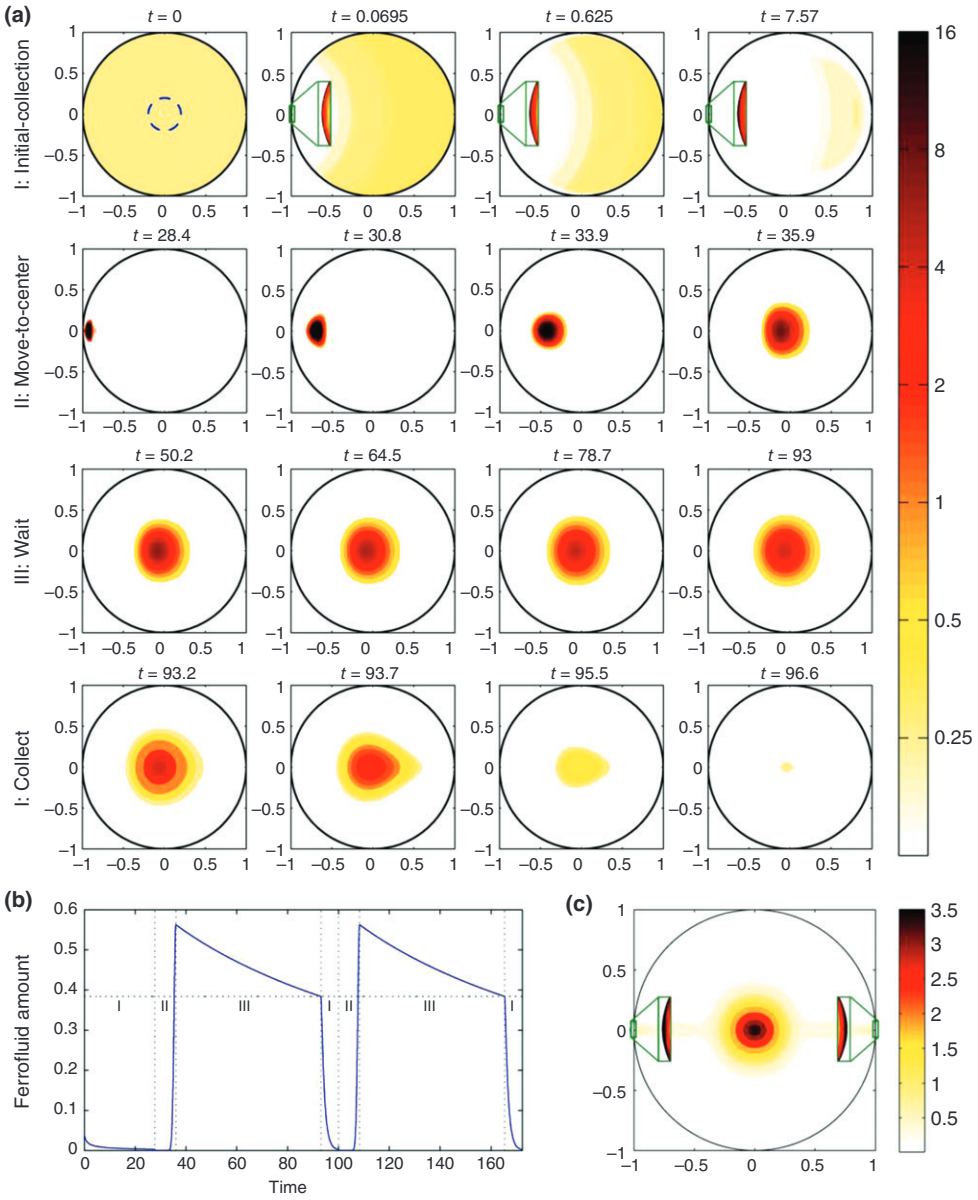

FIGURE 4 | Focusing a ferrofluid to a central target on average in a computer simulation. (a) The concentration of the controlled ferrofluid over time. In phase I, the ferrofluid is collected to the left edge (zooms shown in green boxes). In phase II it is brought to the center with minimal spreading by dynamic control of 8 magnets outside the circular domain (magnets not shown). Then there is a wait step (phase III) and then collection repeats on the right side. (b) The amount of ferrofluid inside the center target at each time. (c) The average ferrofluid concentration. Control achieves a clear hot-spot in the center. (Reprinted with permission from Ref 22. Copyright 2012 IEEE)

interior target. To achieve deep targeting it is necessary to find ways to circumvent Earnshaw's theorem. The most direct way is to exploit the dynamics of particle transport and feedback control to achieve deep focusing in some on-average manner.

In Ref 22 we showed, in simulations, that a collect-at-edge and move-to-center scheme could focus particles on average to the center of a 2-dimensional circular domain (Figure 4). This scheme exploited the edge of the domain to first collect the particles to a focused location. The control algorithm was provided with complete information on the distribution of particles at each moment in time (in other words, we assumed perfect real-time imaging of particle distributions) while it moved the ferrofluid optimally from edge to center. The decisions that the control algorithm made, which told it how to actuate the eight magnets surrounding the circular domain, were based on a mathematical model of particle dynamics due to diffusion and magnetic forces (see Ref 22 for details).

This strategy successfully focused ferrofluid on average to a deep internal target (Figure 4). However, this control result was in idealized simulations, and is a long way from a practical system that can achieve the deep focusing shown in Figure 1(b). There may be other as yet undiscovered ways to bypass Earnshaw's theorem to achieve deep tissue focusing. At present, precisely directing magnetic carriers to anywhere they need to go, including to deep tissue targets, remains a holy grail of magnetic drug targeting, and is an open challenge that will require imagination and collaboration.

CONCLUSION

To advance magnetic drug targeting to the clinic requires solution of key remaining open challenges. There is first a need to develop methods to rationally select and design carriers for specific clinical indications. The magnetic carriers that will be best for targeting deep tumors will not be the same as those most appropriate for traversing the BBB or for noninvasively reaching eye diseases. Owing to the large design space for magnetic carriers (e.g., size, shape, coatings), it is unlikely that carrier selection will be achieved through animal testing alone, instead animal experimentation will have to be combined with effective and predictive mathematical modeling to better search the design space in order to find the most appropriate carrier designs for different clinical indications.

Real-time imaging of magnetic carriers *in vivo* is a major need to enable precise magnetic targeting. In order for a magnetic system to direct therapy precisely to a disease target, the systems controller must be able to 'see' where the therapy is so that correcting magnetic fields can be applied to move the therapy from where it is to where it should be. Without real-time imaging capabilities, magnetic manipulation will remain blind and inaccurate.

To reach deeper targets, in addition to carrier optimization, there is also a need to optimize the design and control of external magnets. Here also the design space is too large to search only experimentally. Mathematical models are necessary to predict how magnetic carriers will move through living tissue under the influence of magnetic fields that are being shaped in time and space. These mathematical models must be built at the right level of fidelity: rich enough to capture fundamental behavior, but simple enough to be computationally tractable and useable for magnet design and control. Optimization and control tools must be implemented that exploit these models to design better magnets (with stronger and deeper forces) and to choose control algorithms for the magnets (to safely and effectively direct magnetic carriers to deep targets in live animals, and eventually in human patients).

Overall, there is a need to move beyond making the carriers we can make and testing them predominantly in cell cultures and small animals, to making the carriers we should make and creating magnetic systems that can precisely manipulate them in large animals, and then in human patients. To translate magnetic carriers from the lab to clinical use will also require regulatory approval, which means rigorous safety and toxicology testing in larger animals in addition to in rodents, before subsequent safety and efficacy trials in human patients. The regulatory framework for such testing is still uncertain, the FDA only recently issued industry guidance for recommended studies to establish the safety of nanomaterials in cosmetic products.[88] To our best knowledge there is as yet no specific FDA guidance available for therapeutic magnetic carriers. Hence there are substantial challenges to translate magnetic targeting from lab demonstrations to a reality for patients. Overcoming these challenges will require significant effort and a genuine collaboration between engineers, mathematicians, chemists, biologists, nanofabricators, and clinicians.

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