Review

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Regenerative Medicine

Advanced cell therapies: targeting, tracking and actuation of cells with magnetic particles

Regenerative medicine would greatly benefit from a new platform technology that enabled measurable, controllable and targeting of stem cells to a site of disease or injury in the body. Superparamagnetic iron-oxide nanoparticles offer attractive possibilities in biomedicine and can be incorporated into cells, affording a safe and reliable means of tagging. This review describes three current and emerging methods to enhance regenerative medicine using magnetic particles to guide therapeutic cells to a target organ; track the cells using MRI and assess their spatial localization with high precision and influence the behavior of the cell using magnetic actuation. This approach is complementary to the systemic injection of cell therapies, thus expanding the horizon of stem cell therapeutics.

Keywords: cell tracking • magnetic actuation • magnetic targeting • MRI • regenerative medicine • SPION

John J Connell[‡,1], P Stephen Patrick[‡,1], Yichao Yu[1], Mark F Lythgoe[§,1] & Tammy L Kalber*[,§,1]

[1]UCL Centre of Advanced Biomedical Imaging, Division of Medicine, University College London, Paul O'Gorman Building, 72 Huntley Street, London, WC1E 6DD, UK

*Author for correspondence: Tel.: +44 (0) 207 679 0810; t.kalber@ucl.ac.uk

[‡]Authors contributed equally

[§]Joint senior authors

Cell therapy is one of the most exciting and promising areas for disease treatment and regenerative medicine [1]. However, the degree of success that many of these regenerative cell-based therapies will achieve in the clinic relies on several challenging factors, including efficient delivery and retention of therapeutic cells in the target organ; monitoring the safety and efficacy of the therapy; and obtaining and maintaining a therapeutic cell phenotype [2]. To address these needs, this review will present emerging and established magnetic particle-based techniques for targeting, imaging and stimulating cells *in vivo*, and discuss the potential benefits of their application alongside clinical cell-based regenerative medicine therapies. We will provide a guide for improving cell delivery and retention using magnetic targeting, as well as reviewing established methods to image magnetic particle-loaded cells for assessment of cell delivery, and the innovative use of magnetic actuation to stimulate and control a specific cell phenotype. Finally, we hope to enable informed decisions as to which technology to use and fuel conversations between researchers in the field of regenerative medicine and magnetic cell-based technologies.

Magnetic targeting

Magnetic targeting has emerged over the past 15 years as a method to improve the delivery and retention of transplanted therapeutic cells within a target organ. Preclinical reports demonstrate a 1.5–30-fold improvement in cell delivery and retention above nonmagnetically targeted control experiments [3–8], which in several disease models translates to a significant increase in therapeutic effect (see Table 1) [6,9–15].

Magnetic targeting relies on two main procedures: first labeling the cells with magnetic particles, and second the application of a magnetic field over the target body region to attract and retain the labeled cells after injection (Figure 1). The first of these procedures was originally developed preclinically to enable detection of transplanted cells with MRI, in order to noninvasively confirm their anatomical position *in vivo* [18]. A number of US FDA-approved magnetic particles (superparamagnetic iron oxide nanoparticles, or

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Table 1. Relevant preclinical magnetic targeting studies *in vivo* (unless otherwise stated) using human cells and US FDA-approved or commercially available magnetic particles using various delivery methods.

Cell type	Particle type	Area targeted	Therapeutic effect	Uptake improvement versus nontargeted cells	Ref.
Mesenchymal stem cells	FluidMAG-D (ChemiCell)	Leg vasculature	Reduced restenosis (by half)	Fourfold	[6]
	Feridex® (Tanabe Seiyaku)	Knee joint cartilage *ex vivo*	Growth of cell layer in targeting group only	Improved (nonquantitative)	[16]
	Feridex (Berlex Laboratories)	Liver	N/A	Twofold (15 days post-injection)	[4]
	Resovist/ Ferucarbotran (Bayer Healthcare)	Damaged skeletal muscle	Improved healing and reflexes	Three- to 20-fold	[15]
Human umbilical vein endothelial cells	TransMAG and CombiMAG (ChemiCell)	Denuded carotid artery	N/A	Targeted cells retained. No retention of control cells (nonquantitative)	[17]
Cardiosphere-derived stem cells	Feraheme® (AMAG Pharmaceuticals)	Heart (myocardial infarction)	Improved left ventricle ejection fraction, improved repair	Threefold	[14]
Endothelial progenitor cells	Feridex/Endorem® (Guerbet)	Carotid artery (ischemia)	N/A	5.4-fold (24h post-injection)	[8]
Neural stem cell line (HB1.F3 immortalized)	Feridex (Advanced Magnetics)	Brain (Ischemia)	25% reduction in infarct volume	Sixfold	[11]
Human mononuclear cells	Feridex/Endorem (Guerbet), BioMAG (Bangs laboratories), FluidMAG (ChemiCell)	Vascular bifurcation phantom	N/A	1.5-fold	[5]

N/A: Not applicable.

SPIONs) have been used for this purpose prior to their use for magnetic targeting [19], as well as non-FDA-approved SPIONs [20]. The same particles therefore have a dual role in allowing cells to be targeted to specific organs using magnetic fields, and imaged to confirm successful delivery with MRI [21–23]. The imaging of SPION-labeled cells has been a relatively more active area of research than magnetic targeting, and will therefore be discussed in more depth later in this review. As the field of magnetic targeting is still emerging, we will discuss, in some detail, the types of magnetic particle suitable for magnetic targeting, the various types of magnetic device that can be used for this purpose and the relevance of magnetic targeting to clinical regenerative medicine therapies.

Magnetic particles

Of all magnetic particles that are available, SPIONs are the most practical choice for magnetic targeting for a number of reasons including high magnetic moment, affordability, availability, biocompatibility, tunable cellular uptake and low toxicity [24,25]. To date around 20 different magnetic particles have been used to demonstrate magnetic targeting in cells, and around two third of these utilize commercially available, and/or FDA-approved particles (see Table 1). Among the SPIONs that are available, the specific choice of particle type is influenced by three general considerations of cell uptake, toxicity and magnetic properties.

Cell uptake

First of all, particles must enter the cell in sufficient quantity. This is affected by cell type and size, culture conditions and interacting variables such as particle size, charge and chemistry of the coating. While macrophages will readily take up particles with a neutral charge, nonphagocytic cells such as stem cells more efficiently take up positively or negatively charged particles [5,24]. In nonphagocytic cells, particles below 200 nm typically show greater uptake than larger particles, with several studies reporting an optimum size between 20 and 100 nm [24,26–27]. For this reason, most magnetic targeting studies have used particles within this range, however, much larger particles such as Bangs' particles

(0.9 μm and above) can also be taken up efficiently by several nonphagocytic cell types if they are positively charged [26], and have been successfully used for magnetic targeting applications with both cardiac-derived stem cells and endothelial cells [3,10,13,28].

Toxicity

To retain the usefulness of a cell therapy, the particle should be nontoxic to the cell at the concentrations needed for magnetic targeting. Magnetic particle toxicity varies considerably between particle and cell type, and depends on the particle coating, aggregation, stability and other factors such as interaction with the cytoskeleton [29–31]. Insufficient coating or the release of iron into the cell from particle breakdown [30,32] can produce hydroxyl radicals (Fenton reaction) which cause DNA damage, lipid peroxidation and protein oxidation [33]. Therefore increasing the particle coating or reducing its breakdown when internalized within a cell can greatly reduce toxicity [29,34]. One approach to monitoring particle breakdown uses Förster resonance energy transfer-compatible fluorescent dyes conjugated to the particle coating to detect disassembly in real time *in vivo* [35]. Though this approach is not compatible with many currently marketed magnetic particles, it demonstrates that this noninvasive form of quality control can be incorporated into the design of new particles. Furthermore, caution should be taken before using iron-labeled cells in therapies for diseases with an iron component to their pathogenesis. For example, it has been shown in a model of multiple sclerosis that iron-labeled therapeutic cells can aggravate symptoms compared with otherwise identical nonlabeled controls that reduce symptoms [36]. Despite these concerns, a number of preclinical and clinical studies have used iron-loaded therapeutic cells without loss of either migratory or regenerative capacity [37–39].

Magnetic properties

The suitability of a particle for magnetic targeting is not directly related to its ability to produce contrast on MRI. As the majority of SPIONs were designed to produce contrast on MRI, and not for magnetic targeting, not all particles are effective for both purposes. Though a comprehensive comparison has not been made for commercially available SPIONs to assess their suitability for magnetic targeting, FluidMAG (ChemiCell) was shown to have improved magnetic targeting results over Feridex (Endorem) owing to increased uptake into cells and better magnetic properties [5,6].

Further, when considering rapid translation to the clinic, previously FDA-approved particles might be preferable. Of these Feridex (Endorem) [4,8–9,11,40], Resovist (Ferucarbotran) [15,41–42] and Feraheme® (ferumoxytol) [14], have all been used successfully for magnetic targeting. To date a variety of non-FDA-approved commercial magnetic particles, including the large 0.9 μm particles from Bangs laboratories [3,10,13,28], FluidMAG [6,12,17], as well as a range of noncommercially produced particles [43–51] have also been used for magnetic targeting. To achieve optimal results, particle uptake and targeting efficiency needs to be tested for a number of magnetic particles for each cell type prior to use *in vivo,* and Table 1 may provide researchers with an initial starting point for their experiments.

Magnetic targeting devices

The type of magnetic device used to target the cells will change depending on the body location to which the cells are to be targeted. The majority of studies have used externally placed magnets, which are preferable to implanted magnets for clinical applications as the use of surgical procedures and the risk of infection is avoided. A number of different types of external magnets have been demonstrated to efficiently target cells: permanent magnets [3–4,8,10–11,13–15,17,41–42,44,47–49] including a cylindrical Halbach array (Figure 2) [6], electromagnets [16,52] and in an emerging application an MRI system [5], see also Table 2. These approaches each have advantages and disadvantages depending on the body region to be targeted, and will be discussed in further detail below.

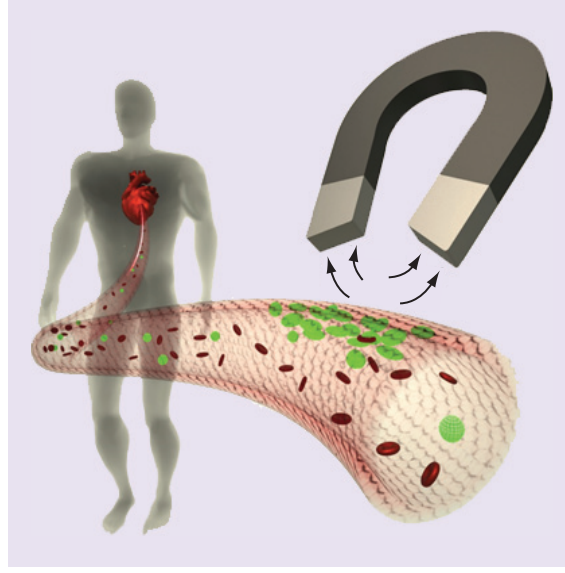

Figure 1. Schematic representation of magnetic targeting. Therapeutic cells are loaded with superparamagentic iron oxide nanoparticles and injected into the body. Delivery and retention are improved by placing a magnetic field at the site of interest toward which passing cells are steered. Reproduced with permission from © Panagiotis Kyrtatos (2011).

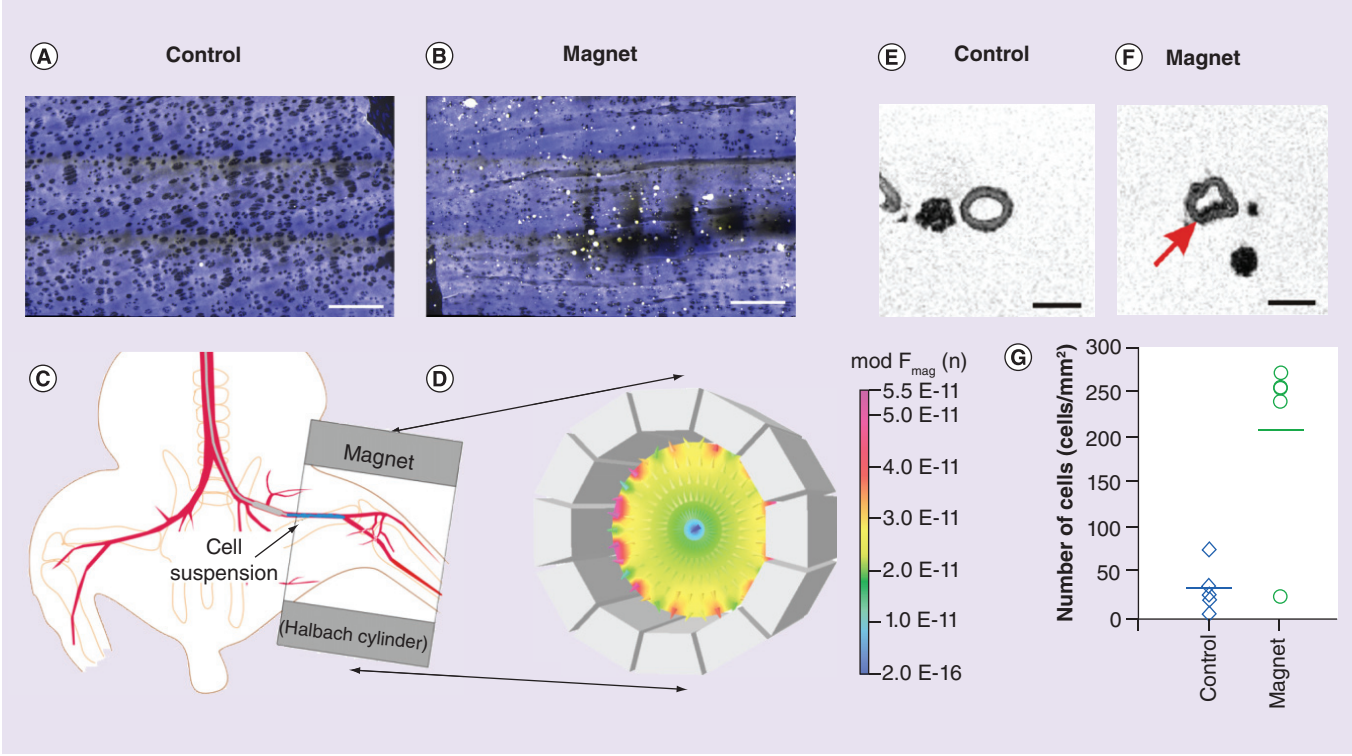

Figure 2. Magnetic targeting of endothelial cells to the leg vasculature using a Halbach Cylinder. (A & B) Confocal microscopy images of magnetically and fluorescently (yellow) labeled cells 24 h after cell delivery without **(A)** or with a magnet **(B)** placed around the leg during cell delivery (scale bar: 100 mm). **(C)** Schematic of mouse anatomy showing placement of Halbach array around the leg, and the location of cell implantation. **(D)** Circular Halbach array with simulation of magnetic field strength and force acting upon cells. **(E & F)** Axial high resolution magnetic resonance images of artery samples 24 h after cell delivery without **(E)** or with **(F)** a magnet (scale bar: 1 mm). Negative contrast on magnetically targeted conditions shows location of superparamagnetic iron oxide nanoparticle-labeled cells in the vessel wall (arrow). **(G)** Scatter plot for number of cells per unit area 24 h after cell delivery (c, n = 5 animals for each group). Adapted with permission from Biomaterials [6] © Elsevier (2013).

Permanent magnets

Permanent magnets are the most portable, and can achieve field strengths above those of small electromagnets of an equivalent size. They also do not require a power supply, or need cooling systems like some electromagnets. The distances over which the magnetic gradients need to be produced for effective magnetic targeting in deeper tissue might limit their use in the clinic to more superficial locations. This has been less of a concern in preclinical studies due to the lower tissue depth of internal organs compared with humans. One solution to this is the use of Halbach cylinders (circular arrays of several permanent magnets in the form of a cylinder, see Figure 2), in which the magnetic field strength can be focused toward the interior of the cylinder for targeting an internal body region such as a limb joint or vasculature [6,53]. This has the advantage over the use of a single permanent magnet in that it can produce a focal 3D magnetic gradient encompassing the internal area of interest. The question is still whether this technology is scalable to the clinical scenario. Initial simulations indicate this is a realistic possibility, and a scalable device has been produced [6], which suggest that permanent magnets would be a feasible method to target peripheral arteries in the human leg [53]; an area that has received a lot of interest in the development of cell therapies especially for treatment of limb ischemia [6,54–55].

Implanted magnets

Implantation of permanent magnets can achieve a greater local magnetic field in deeper tissues than the external use of the same magnet. A small number of studies have used implanted magnets to target cells to the spinal cord [9,40,46], heart [43,51], retina [12] and brain [50]. However, external magnets have also proved successful in targeting to the heart [10,13–14,21–22] and brain [11]. The use of magnetizable stents for targeting cells has only been demonstrated in blood vessels [28,45], though again this area can also be targeted by externally placed magnets [6,8,17]. At this moment in time, it remains to be seen whether the risks associated with surgical implantation are outweighed by the increases in cellular delivery.

Table 2. Summary of anatomical locations to which cells have been delivered using magnetic targeting.

Cell type	Organ	Animal	Improved delivery	Magnet type	Ref.
Vascular (arteries)	EPCs (rabbit)	Rabbit	No control	External	[3]
	Blood-derived EPCs (porcine)	Pig	Six- to 30-fold	Magnetized stent	[28]
Carotid artery	HUVECs	Mouse	Improved	External	[17]
	Aortic endothelial cells (bovine)	Rat	Sixfold	Magnetized stent	[45]
Vascular (veins)	EPCs	Rat	5.4-fold (1 day)	External	[8]
	MSC (rabbit)	Rabbit	6.2-fold (1 day)	External	[6]
Bifurcation	Mononuclear cells (human)	*In vitro* phantom	1.5-fold	External (MRI scanner)	[5]
Brain	Neural stem cells (human)	Rat	Sixfold	Attached to skull (7 days)	[11]
	EPCs (mouse)	Mouse	Not stated	Implanted	[50]
Eye (retina)	MSC (rat)	Rat	37-fold	Implanted	[12]
Femur	MSC (rat)	Rat	Twofold	External	[41]
Heart	Cardiosphere-derived cells (rat)	Rat	Two- to three-fold	External	[13]
	Cord blood EPCs (human)	Rat	Tenfold (24 h)	Implanted and external	[43]
	Cardiosphere-derived cells (rat)	Rat	Fourfold (24 h); threefold (3 weeks)	External	[10]
	Cardiosphere-derived stem cells (human)	Rat	Threefold	External	[14]
	MSC (porcine)	Pig	Threefold	Implanted	[51]
	MSC (rat)	Rat	Fourfold	External	[42]
	MSC (rat)	Rat	2.7-fold	External	[21]
	Endogenous stem cells	Rat	Twofold	External	[22]
	MSC (rat)	Rat	3.9-fold	External	[23]
Hind limb	EPCs (human)	Mouse	1.9-fold (21 day)	External	[47]
Knee joint (cartilage)	MSC (rat, human)	Rabbit, pig, human joints *ex vivo*	Improved (nonquantitative)	External	[16,52]
Liver	MSC (human)	Rat	Twofold (15 days)	External (jacket)	[4]
Skeletal muscle	MSC (human)	Rat	Three- to 20-fold	External	[15]
Skin	MSC (mouse)	Mouse	30-fold	External (6 h)	[48]
Spinal cord	Bone marrow stromal cells (rat)	Rat	30-fold (1 day)	Implanted	[9,40]
	MSC (rat)	Rat	3.7-fold (1 week)	Implanted	[46]

Experiments performed *in vivo* unless stated otherwise.
EPC: Endothelial progenitor cell; HUVEC: Human umbilical vein endothelial cell; MSC: Mesenchymal stem cell.

Electromagnets

Electromagnets can produce much higher field strengths than permanent magnets, however, they require a constant power supply, and in the case of large electromagnets must be supercooled to maintain low resistance and prevent overheating. The largest electromagnets typically found in the clinic are present in the hardware used for MRI, and these create strong and tunable magnetic gradients large enough to cover the whole anatomy; the use of this technology for magnetic targeting is known as magnetic resonance targeting (MRT) [5]. Proof-of-principle simulations

have demonstrated that a preclinical MRI system can be used, without further modification, to magnetically target cells [5,7]. This could potentially allow real-time noninvasive targeting of cells to internal body locations that would not be accessible using smaller magnets unless they were implanted. Perhaps most importantly, MRT has the potential to be applied to all experimental and clinical MRI systems, thus opening the possibility for a stem cell delivery system in hospitals with MRI systems. Further experimental confirmation in preclinical models, however, would be required to discover the limitations of this technique.

Magnetic field strength

In the same fashion it is important to consider the type of particle and magnet design, it is also important to consider the strength of the magnetic field. An *in vivo* comparison has shown that the highest magnetic field strength is not always the most effective [42]. In a model of myocardial ischemia, stem cells were targeted to the site of injury using 0.15 T, 0.3 T and 0.6 T magnets. The 0.3 T magnet gave significantly greater cell targeting efficiency than the 0.15 T and 0.6 T magnets as well as the nontargeted control [42]. The reduction in targeting efficiency when using the highest strength (0.6 T) being due to cell aggregation, and the reduction in regeneration due to both the lower delivery of cells and the formation of embolisms due to blood vessel blockage [42]. However, this effect of field strength is likely to vary between organs depending on their depth, the nature of the vasculature and the time the magnetic field is exposed, as higher field strengths were shown to be more effective when the magnet is applied for only a short time frame (e.g., 1 min) [15]. Clearly, the interaction between a magnetic field and the particles is complex and as such needs careful planning.

Magnet choice

Considering the above information, there are two general approaches to selecting and positioning a magnetic device to achieve effective targeting. The first of these involves simulating different magnetic fields and the fluid forces to which the labeled cells will be subjected after injection, and optimizing the parameters to be used based on these predictions. This approach has yielded successful results in a number of studies, however, a certain amount of technical expertise is required [5–8,53]. The second option involves testing a number of magnetic field strengths, magnet types or positions of magnet placement *in vivo*, to experimentally determine the conditions that achieve the best results. In practice, a combination of both methods including some simple simulations of forces together with a small number of experimental variations would be advised in initial pilot experiments.

Clinical relevance

If magnetic targeting is to be used in the clinic, it must be shown that it is achievable with human cells, that these cells retain their regenerative properties and that an increased therapeutic effect is seen above nontargeted therapies. Table 1 summarizes the preclinical magnetic targeting studies that have used human cells and commercially available particles. A lot of interest surrounds the use of mesenchymal stem cells (MSCs) for cell therapy, due to their numerous regenerative properties [56] and MSCs feature in every disease category in ongoing clinical trials [2]. These include treatments for myocardial infarction, arthritis, cartilage damage, bone fracture, degenerative disc disease and skin ulcers – all of which occur in regions that can be magnetically targeted to improve delivery (see Table 2). As both human and rodent MSCs have been shown to take up a number of magnetic particle types without adverse effects on their viability or regenerative capacity [15,21,37,41–42,48], this has allowed magnetic targeting to improve their delivery to these areas of clinical interest (see Table 2). Broadly, magnetic targeting enables us to increase the number of injected cells at the site of interest. This may result in the following additional benefits to both the researcher and patient, including reduced cost in cell production as fewer cells are needed; reduced risk to the patient (vascular occlusion, teratomas, off target effects); fewer repeat injection procedures; reduced invasiveness of implantation and targeting to difficult-to-access areas (e.g., stroke, in which magnetic targeting of intravenously administered neural stem cells to the brain improved delivery and reduced the infarct volume [11]).

Future work is likely to modify the current paradigm of magnetic targeting, adapting the technology for better translation prospects in the clinic. Recently, an alternative magnetic targeting strategy implemented innovations to avoid several barriers to clinical translation, including the need to culture, label and transplant the required cells from an *ex vivo* preparation [22]. Iron-oxide nanoparticles were coated with two antibodies – one to bind the stem cells (anti-CD34), and one to bind to damaged areas of the heart (antimyosin light chain). When these particles were injected intravenously, circulating endogenous stem cells were targeted to the heart by the anitibody-labeled particles, doubling the delivery of cells to the damaged tissue. Furthermore, when a magnet was placed over the heart, the targeting effect was further doubled, and successful delivery was confirmed by MRI. The clinical relevance of this therapy is appreciable from the

significant increases in left ventricle ejection fraction and viable tissue [22]. Second, by using endogenous stem cells the risk of immune rejection is avoided, and the therapy is simplified by not requiring the harvest of donor tissue. By conjugating other disease-specific antibodies to the magnetic particles used in this study, the approach could easily be adapted for treatment of other conditions.

Clinical limitations

Aside from the challenging regulatory requirements that may limit the use of magnetic targeting, the technique may not be applicable to every type of cell therapy. Of the cell therapies in the clinic, the majority use hematopoietic progenitor cells [2], where the main factor influencing success is donor supply and the requirement of precise immunologic donor matching, thus there is not the need for cell targeting to a specific region. Additionally, the need for targeting may be lessened in cells that show some migratory ability, such as that observed in immune cells and MSCs [56]. As magnetic targeting increases the volume of cells to the target site there is the potential for cells to aggregate which could potentially lead to embolization, and therefore this potential complication of magnetic targeting should be thoroughly investigated and overcome before clinical application [23,42]. Finally, of the cell therapies that would benefit from magnetic targeting, the real limitation in their use in patients lies in the manufacture of suitable magnetic devices that possess sufficient field strength and targeting capacity with tunable gradients. However, the strategy employed by MRT might provide the opportunity to target cells using commercially available MRI systems, which are readily available.

Imaging cells using superparamagnetic iron oxide nanoparticles

Delivery and retention of therapeutic cells at the site of interest, and demonstrating this using imaging, is crucial in the development of safe and effective experimental regenerative therapies. As such, longitudinal assessment of the retention of administered cells can influence treatment decisions regarding repeated administration. Imaging of cell therapies is therefore an important factor to consider when designing and using novel regenerative medicines.

As previously mentioned, MRI is highly sensitive to SPIONs and has proven to be a popular approach for the localization of successfully delivered cell therapy to target tissue [57]. The superparamagnetic iron core induces inhomogeneities in the local magnetic field, shortening the T_2 and T_2^* relaxation times. This results in signal attenuation on T_2 and T_2^*-weighted images, producing regions of negative contrast in the location of the nanoparticles (Figure 3). Although gadolinium-based compounds are often used as contrast agents in MRI, and have shown some use in preclinical stem cell tracking studies [58–61], they are considered a poor option for cell tracking, due to their limited sensitivity [38]. More recently, contrast agents based on fluorine have been developed and can be detected with high selectivity (due to the lack of endogenous background signal). Although low signal-to-noise has often limited their use, a recent clinical study has demonstrated their future potential [62]. This review, however, will focus on iron oxide-based cell tracking agents due to their established status [38] and wider varieties of use outside imaging.

An early clinical investigation into the use of SPIONs to detect the spatial location of administered cells immediately highlighted the type of information that could be gained through MRI-assisted cell tracking [63], consistent with prior preclinical demonstrations [64]. Dendritic cells were labeled with a radiotracer (^{111}In-oxine) and a SPION (ferumoxide) and injected into the lymph node. While the radionuclide scans indicated cells present in the region of the draining lymph node, SPION contrast-enhanced MRI indicated that in four out of the eight treated patients, the cells were in fact misinjected into the surrounding muscle or subcutaneous fat (Figure 3). Additionally, in the successful injections migration of the dendritic cells to the surrounding lymph nodes was more detectable with improved spatial resolution. The coupling of high spatial resolution with anatomical information allows for better understanding and therefore leads to more effective treatment. Application of this technique within the field of stem cell tracking has therefore grown considerably, with attempts to answer the critical questions necessary for the successful translation of regenerative medicine: what is the optimal delivery route, how well do the cells engraft and do the cells migrate after injection [65]?

SPION-labeled stem cells have provided some answers to these questions. Successful engraftment has been demonstrated with SPION-labeled neural stem cells with longitudinal MRI in a case study of regeneration of damaged brain tissue [66]. The migration of SPION-labeled cells from the site of injection was suggested in a study assessing the safety of injection of MSCs in patients with multiple sclerosis and amyotrophic lateral sclerosis and also in a study of autologous CD34$^+$ bone marrow cells injected into the spinal cord of patients with chronic spinal cord injury [67,68]. The migration being identified through the negative contrast brought about by the SPION-labeled cells, an effect that would not be known without this technique.

Although the negative contrast (dark spots on images) generated by SPIONs is what enables the

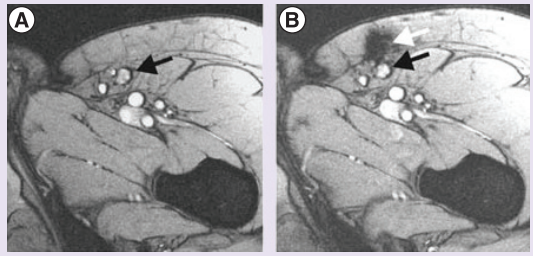

Figure 3. Imaging superparamagnetic iron oxide nanoparticle-labeled cells in a patient with a clinical 3T MRI scanner. (A) MRI before vaccination; the inguinal lymph node to be injected is indicated with a black arrow. **(B)** MRI after injection showing that the hyperintensity of the dendritic cells were not accurately delivered into the inguinal lymph node (black arrow) but in the vicinity, in the subcutaneous fat (white arrow). Reprinted with permission from [63] © Macmillan Publishers Ltd (2005).

detection of cells, the inherent absence of signal can impose limitations on the quantification of SPION concentration and cell number. More recently, sequences have been developed to give a positive signal on magnetic resonance images using off-resonant water frequencies in regions affected by the magnetic susceptibility of SPIONs [69]. Overlaying these images onto anatomical MR images provides the spatial location of the SPIONs but with a positive and quantifiable signal. Alternatively, difference ultrashort echo time imaging has been developed to generate positive contrast from SPIONs. Two sets of images are taken with different parameters and the difference in signal intensities between the two images then quantitatively shows the location of the SPIONs [70] and has been implemented in SPION-labeled islet cells transplanted into the rat liver [71]. The translation into patients is highly convincing, as it has been shown that as few as 120,000 cells in a rat can be imaged using a typical 1.5 T clinical MRI scanner [72]. This number is likely to decrease as improved pulse sequences, labeling techniques and magnetic particles are developed. This potential is illustrated by the detection of a single cell at high field strength in a preclinical *ex vivo* sample (Figure 4) [73].

As well as the difficulty in quantification of the number of SPION-labeled cells using MRI, other limitations in the technique have slowed their translation to the clinic. SPION labeling is known as a direct labeling technique, where the label is inserted into the cell. The administered cells then replicate and divide, diluting the contrast and lessening its effect. Furthermore, the contrast gives no information on cell viability, as the SPION is retained when the cells die and as such will still be detected. Additionally, dead therapeutic cells and their SPION labels can be phagocytosed by endogenous macrophages and migrated away, causing false positives adding further confounds to confidently assessing cell therapy biodistribution [74]. Areas of the body that are inherently hypointense in signal, such as the lungs, cannot easily be used to identify SPION-labeled cells by MRI as the dephasing of the signal is prevented by the absence of it, although this has been performed [37]. Thus, tracking unwanted migration to the lungs after stem cell therapy may not be possible even with careful examination of the images. One alternative method to avoid the problems of signal dilution and misidentification of cells is the use of reporter genes, which pass on their signal-producing ability to progeny cells, and are available with human proteins or FDA-approved contrast agents [75,76]. Though the use of reporter genes has been pioneered in the clinic using PET [77], human use is not yet widespread due to the further regulatory complications associated with genetically modifying cells. A second alternative is the direct labeling of cells with radionuclide tracers. This has been in use clinically since the 1980s and allows quantitative whole body biodistribution imaging of cells with single-photon emission computed tomography [78]. This method would be especially useful for assessing the proportion of cells that reach the target tissue compared with other areas of the body. Direct labeling of cells with tracers for nuclear imaging has several advantages over SPION-based cell tracking in that cells can be detected in areas of MRI signal void such as the lungs. Signal detection is quantitative and more sensitive, and cell labeling is quick as it does not require active uptake mechanisms. However, the resolution is slightly lower and the toxicity is typically high, resulting in leakage of the tracer and therefore can only be used to image cells directly after injection rather than longitudinally [78].

Tracking of cells for regenerative medicine therapies has, therefore, many powerful uses already demonstrated in patients, however, the greatest benefit might come from a combination of both imaging and targeting to get the full use out of these multimodal particles.

Magnetic actuation

The behavior of a cell can be influenced by magnetic particles in a further application in a process known as magnetic actuation. The field of regenerative medicine has many potential benefits from novel applications involving magnetic actuation including mechanotransduction to control the activation, differentiation, proliferation and migration of stem cells and magnetic nanoparticle-enhanced transfection, and thermal

stimulation to activate insulin release from genetically modified stem cells. These emerging applications can advance the field of regenerative medicine through inducing and enhancing the biological function of cells selectively and remotely.

Mechanotransduction

Mechanotransduction is the initiation of cellular activity via a mechanical force placed on the cell [79] (reviewed in [80]). Mechanical forces sensed by a cell include compression, tension, fluid flow and mag-

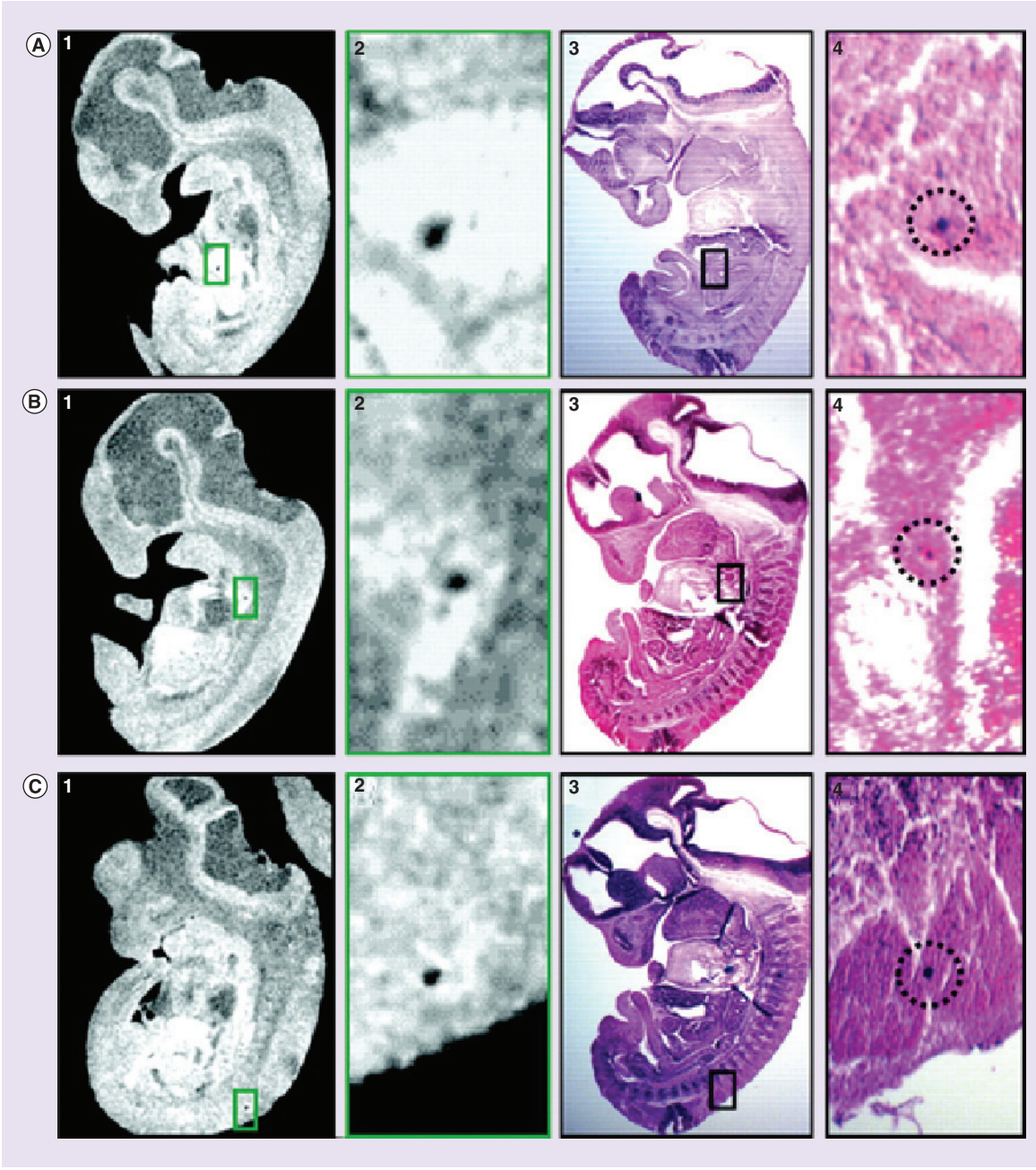

Figure 4. MRI and histology of Bangs particles in E11.5 embryos. (A–C) (A1–C1) Slices from 3D MRI datasets. **(A2–C2)** Expansions of the boxed regions in **A1–C1**, showing susceptibility induced contrast regions in each image. **(A3–C3)** Matching histological sections that correspond to the same plane as the accompanying MRIs. **(A4–C4)** A second set of images of the sections in **A3–C3**, acquired with a higher magnification, approximately representing the boxed regions. The stains from the individual particles are circled to assist visualization in the histology. Shown are single 1.63-μm particles registered to the areas of dark contrast in the MRIs. Each embryo image is almost equal to 6.75 mm from head to tail, both in the MRI and in the histology. All Insets are ×10 magnifications. Reproduced with permission from [73] © National Academy of Sciences, USA (2004).

netism and are transduced through intracellular biochemical signaling which then elicits a cellular response. Physical stress on microtubules can trigger rapid signal transduction seen in Src activity [81] and stem cell fate can be regulated by the physical interactions of integrins and the extracellular matrix [82,83]. Exogenous manipulation of this phenomenon therefore has huge potential in controlling stem cell differentiation to obtain and control therapeutic cell phenotype in regenerative medicine.

Subsequently, research has developed the use of magnetic nanoparticles targeted to mechanosensitive receptors as a tool for controlling the mechanical stimulation of cells [84]. One demonstration of this was through superparamagnetic beads conjugated to antibodies directed against an integrin β1 subunit attached to cells and exposed to a magnetic field. This induced a physical stress on the cells, transduced through the integrins and the actin cytoskeleton, and stimulated expression of VEGF [85] and markers of osteogenic differentiation [86] in MSCs and promoted proliferation of osteoblasts [87,88]. This is similar to what biochemical techniques can achieve, but with selectivity to the target cell.

Mechanosensitive ion channels can be selectively activated through binding antibody-coated magnetic nanoparticles (MNPs) and applying a magnetic field. This technique was originally described with non-targeted collagen-coated MNPs [89,90]. Targeting the stretch-activated potassium ion channel (TREK1) with MNPs allows controlled ion channel activation and has been exploited to trigger biochemical signaling pathways to control differentiation of human bone marrow derived stem cells (hBMSCs) to osteochondral lineage and to increase expression of osteopontin and *Cbfa1* and deposition of various collagens [91]. Furthermore, it has recently been shown that mechanoactivation of TREK1 by functionalized MNPs triggered an increase in mineralization of human MSCs that were implanted into a chick fetal femur osteogenesis model [92].

As well as activation of ion channels, differentiation of MSCs has been demonstrated using mechani-

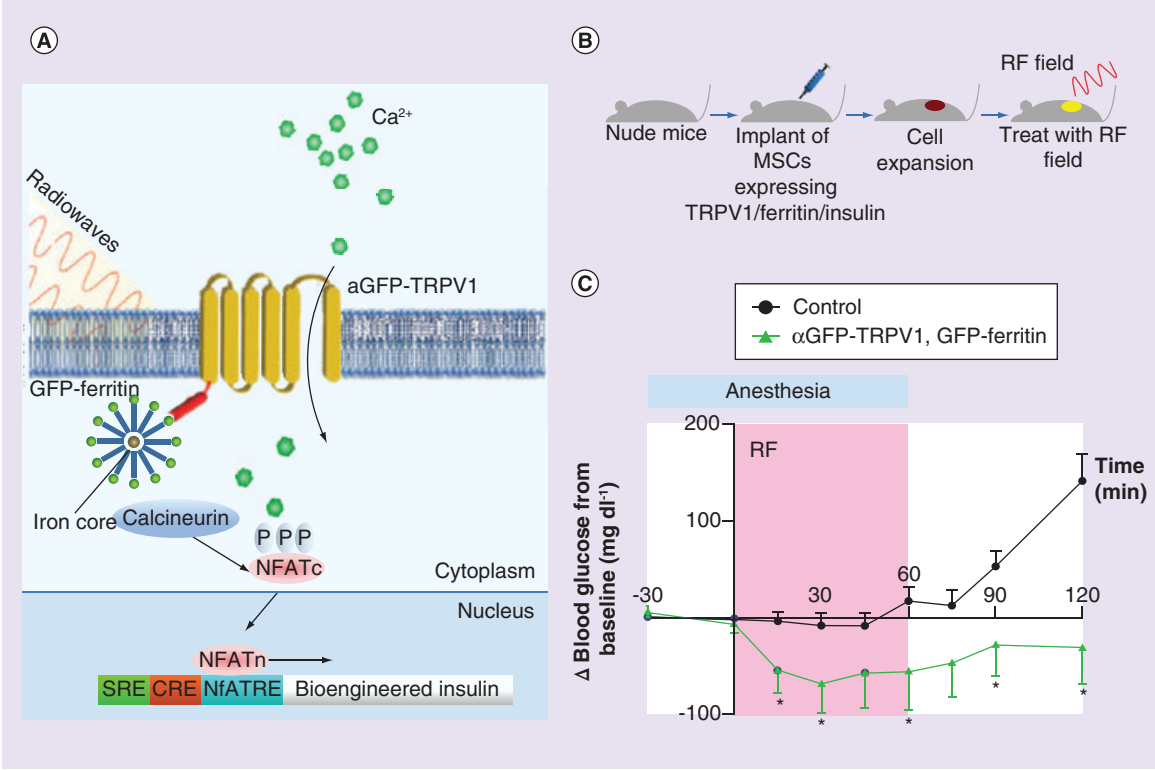

Figure 5. Blood glucose can re be regulated *in vivo* with radiofrequency waves using modified stem cells expressing a magnetic-particle-activated calcium channel and calcium controlled insulin transgene. (A) Schematic showing genetically encoded channel-associated ferritin, (αGFP-TRPV1/GFP-ferritin). **(B)** Schematic for delivery and assessment of effects of radio frequency treatment on blood glucose in diabetic mice with implanted mesenchymal stem cells expressing αGFP-TRPV1/GFP-ferritin and calcium-regulated human insulin. **(C)** Radio frequency treatment of mice implanted with genetically modified mesenchymal stem cells reduces blood glucose compared with that seen in control mice.
MSC: Mesenchymal stem cell; RF: Radio frequency.
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cal stimulation of a receptor tyrosine kinase. PDGFR was stimulated through antibody-coated MNPs within a time-varying magnetic field [93] which caused differentiation into a smooth muscle-like cell lineage.

Thermal stimulation

Thermal stimulation of signal activation relies on similar principles to mechanotransduction, but makes use of thermally activated transmembrane proteins. A slightly different approach to magnetic actuation has been recently demonstrated to regulate insulin release and blood glucose levels. MSCs were genetically modified to express a magnetic iron storage particle (ferritin) together with the heat-sensitive TRPV1 channel and a transgene encoding human insulin under a calcium-sensitive promoter [94]. Upon application of a magnetic field or radiofrequency stimulation, channel opening and calcium influx was triggered by magnetic particle heating, leading to an increase in plasma insulin and a return of blood glucose toward baseline levels (Figure 5). Activation of this same channel (TRPV1) was also achieved in another study, this time using antibody-targeted manganese ferrite-based particles [95]. Here, activation of action potentials was achieved in neurons, and behavioral responses were triggered in nematode worms. This has a possible application in neurological disorders in which brain activity in certain regions is low.

Although these studies are at the *in vitro* or preclinical stage, the principle of remotely controlling specific modes of cell activation offers further options for influencing stem cell differentiation for regenerative medicine alongside or in place of traditional stimulation by soluble factors. Application of these techniques into patients has not yet been assessed, but issues such as magnetic field strength and particle choice will likely be helped by ongoing research into magnetic targeting. Further preclinical work in tissue engineering is also benefitting from magnetic nanoparticles such as tissue scaffolds and grafts [96–101], but will not be focused on here (reviewed in [102]).

Magnetic nanoparticle-enhanced transfection

Transfection of cells commonly involves incubation with a viral vector such that the gene within the payload of the viral vector is inserted into the genome of the host cell. Applications of gene therapy in regenerative medicine are wide ranging and have had huge successes [103–105], though immune response to viral vectors has fatally hampered translation in the past and remained a concern despite the development of safer systems [106,107]. An alternative process for gene delivery has been recently developed through the use of magnetic nanoparticles. First described as a way of enhancing and targeting delivery of recombinant adeno-associated viruses encoding GFP into HeLa cells [108], the approach has been modified to deliver nonviral DNA or siRNA to cells *in vitro* and *in vivo* [109]. By attracting magnetic nanoparticles, coupled to the gene to be transfected, toward a cell, endocytosis of the particle is triggered. The endosome breaks down and releases the DNA which can then be transcribed. Effective delivery of the reporter gene encoding luciferase has been demonstrated in pig ear endothelial cells [109]. Oscillating the magnetic field perpendicular to the cell membrane further increases the transfection efficiency by enhancing endocytosis of the particles [110–112]. Crucially, the process of 'magnetotransfection' can retain the cell surface marker phenotype and avoids the use of electroporation which negatively effects cell viability [112]. Thus, the delivery of genes to stem cells as part of regenerative medicine has been developed by the use of nanoparticles, and opens avenues of research for personalized therapy.

Conclusion

The studies mentioned here describe the developing field of applications for magnetic nanoparticles in regenerative medicine. There is a strong preclinical evidence base providing potential for applications in the clinical setting to enhance the therapeutic outcome of regenerative medicine. Through magnetic targeting, therapeutic cells can be more efficiently delivered to and retained at the target organ which can enhance the therapeutic effect as well as reduce the number of cells required for injection. Successful administration and target tissue accumulation of SPION-labeled cells can be monitored through MRI, giving information about the likelihood of a therapeutic effect that would not be possible with other imaging modalities. Finally, manipulation of the cell phenotype and behavior can be finely tuned with magnetic actuation of cell surface receptors and with magnetic nanoparticle-enhanced transfection of genes of interest. Clearly, regenerative medicine approaches for patient therapy can benefit enormously from the use of magnetic nanoparticles and this innovative field will provide the important tools needed to realize the full potential of stem cell therapy.

Future perspective

Applications of magnetic nanoparticle technologies have great potential in furthering regenerative medicine. Subsequent to some necessary technological advances, the concept of wearable devices, in the form of magnets, could pave the way for long-term cost–effective retention of cells through magnetic targeting. Images combining MRI and SPION-labeled cells have already proved their usefulness in the clinic and, at

some level, widespread use is likely to follow the trend of the increasing use of cell therapies. Finally, the drive for personalized treatment can be met by a MRI system that enables targeting, imaging and actuation of magnetic nanoparticle-enhanced cell and gene therapy. The end result will be an MRI theranostic, combining therapy and diagnosis in a single modality, creating a completely new class of imaging/therapy.

Financial & competing interests disclosure

JJ Connell and PS Patrick are funded by the UK Regenerative Medicine Platform. TL Kalber is funded by an EPSRC Early Career Fellowship (EP/L006472/1). MF Lythgoe receives funding from the Medical Research Council (MR/J013110/1); King's College London and the UCL Comprehensive Cancer Imaging Centre CR-UK & EPSRC, in association with the MRC and DoH (England); the National Centre for the Replacement, Reduction and Refinement of Animal in Research (NC3Rs); UK Regenerative Medicine Platform Safety Hub (MRC: MR/K026739/1); Eli Lilly and Company. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Executive summary

Magnetic targeting

- Magnetic targeting enables therapeutic cells tagged with superparamagnetic iron oxide nanoparticles (SPIONs) to be delivered and retained at the target organ by application of a magnetic field.

Magnetic particles

- A large variety of SPIONs are available for use in regenerative medicine-based studies.
- Particle choice is influenced by magnetic moment, particle coating, cellular uptake, toxicity and application.

Cell tracking with MRI

- MRI has proven to be a sensitive and reliable way to confirm the location of therapeutic cells loaded with SPIONs.
- Injection accuracy can be confirmed and the migration of transplanted cells can be assessed.

Magnetic actuation

- Therapeutic cells can be manipulated to control activation, differentiation, proliferation and migration through use of magnetic nanoparticles.
- Magnetic nanoparticles can enhance cell gene transfection.

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