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REVIEW

Strategies to enhance efficacy of SPION-labeled stem cell homing by magnetic attraction: a systemic review with meta-analysis

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Abstract: Stem cells possess a promising potential in the clinical field. The application and effective delivery of stem cells to the desired target organ or site of injury plays an important role. This review describes strategies on understanding the effective delivery of stem cells labeled with superparamagnetic iron oxide nanoparticles (SPION) using an external magnet to enhance stem cell migration in vivo and in vitro. Fourteen total publications among 174 articles were selected. Stem cell type, SPION characteristics, labeling time, and magnetic force in vivo are considered important factors affecting the effective delivery of stem cells to the homing site. Most papers reported that the efficiency was increased when magnet is applied compared to those without. Ten studies analyzed the homing competency of SPION-labeled MSCs in vitro by observing the migration of the cell toward the external magnet. In cell-based experiments, the mechanism of magnetic attraction, the kind of nanoparticles, and various stem cells were studied well. Meta-analysis has shown the mean size of nanoparticles and degree of recovery or regeneration of damaged target organs upon in vivo studies. This strategy may provide a guideline for designing studies involving stem cell homing and further expand stem cell.

Keywords: stem cells, homing, SPION, magnetic attraction, stem cell therapy, systematic review

Introduction

Application of superparamagnetic iron oxide nanoparticles with the cellular therapies is an attractive option for the localization of stem cells to sites of interest to repair tissue damage.[1] Stem cell-based therapies are actively studied and used in all areas of regenerative medicine. However, delivery of an appropriate number of cells to defective tissue remains difficult. In addition to stem cells' important abilities such as self-renewal and tissue differentiation, cell migration to damaged cells, known as the homing phenomenon, is also crucial.[2] Among stem cells types, mesenchymal-derived stem cells (MSCs) have a better homing capacity than induced pluripotent stem (iPS) cells, embryonic stem cells, and others. MSCs are defined to adhere to plastic in culture and differentiate into osteocytes, chondrocytes, and adipocytes.[3] Additionally, they must express CD105, CD90, and CD73 and lack expression of CD45, CD34, CD14 or CD11b, CD79 alpha or CD19, and HLA-DR surface molecules.

Currently, there is substantial variability in the strategies used to improve MSC homing. Several groups have demonstrated the homing and migration of MSCs; however, only a small portion of the systemically administered MSCs remain on

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the target organ.[4] Many groups are investigating methods to enhance membrane expression of CXCR4, a critical receptor for bone marrow homing.[5–7] Wiehe et al[7] demonstrated that the CXCR4-stromal-derived factor-1 (SDF-1) axis is critical for homing to the injured myocardium. Shi et al[8] showed that MSCs cultured with a cocktail of cytokines induced high surface expression of CXCR4, with chemotactic receptors of SDF-1α upregulated in ischemic tissue. Because MSCs are trapped in the lung after intravenous injection, Yukawa et al[9] modified the administration of transplanted MSCs in combination with heparin treatment and found that this strategy also significantly decreased MSCs trapped in the lungs. Recently, a "magnetic attraction" method for stem cells was developed.[10] The concept of magnetic tagging and targeting could play an important role for future advances in delivery and noninvasive monitoring of cell-based therapeutic interventions.

Superparamagnetic iron oxide nanoparticle (SPION) was used for monitoring the migration of injected stem cells by magnetic resonance imaging (MRI).[11] These SPIONs are also well known to be harmless and non-cytotoxic, showing normal MSC viability, proliferation, and differentiation in vivo and in vitro.[12,13] SPION can move magnetized MSCs where needed under the presence of a static magnetic field. According to Yun et al's[14] study, SPION-labeled MSCs migrated to injured olfactory tissue guided by a permanent magnet, resulting in improved MSC homing and migration effects in vivo and in vitro, respectively. Song et al[15] reported that when an external magnet (0.32 T) is attached to the skull in the ischemic brain injury rat model for one week, stem cells labeled with SPION after intravenous injection increased 3-fold in the infarct region under the magnet and the infarct size decreased significantly. These theories have long been introduced and can be used to create experimental methods that greatly impact stem cell studies,[16] but there has been no significant progress over 20 years due to the lack of a standardized protocol for magnetized stem cell homing using SPION for magnetic attraction. The methodology to be established for homing is divided into three categories: first, how SPION labels stem cells and whether it is cytotoxic or affects cell differentiation. Second, how do magnetized stem cells home in on a desired location in vivo, given the specific magnetic forces. It is necessary to consider the route of injection in administration of cell (intravenously or directly), the time taken for cells to move to the desired organs post-injection, and the ideal location of the magnet in vivo, in order to evaluate the therapeutic efficacy of magnetized MSC homing in clinical settings. Finally, in order to better understand homing biology and increase homing efficiency in vivo, it is important to establish standardized protocols for the experiments in vitro such as how to change the cellular homing molecules (Figure 1).

Therefore, studies of stem cells combined with nanobiotechnology can offer many benefits to stem cell research, including cell homing, which is currently the limiting factor for further advances in this field. The aim of this review was to analyze publications on SPION-labeled stem cells and their magnetic attraction in vivo and in vitro and summarize the strategies for improving magnetized MSC homing. By providing information on established experimental methods for many researchers in these fields, we aim to help define the optimal expansion protocols.

Materials and methods

Search strategy

This review included original English reports that used SPION-labeled stem cells and a magnetic field to promote

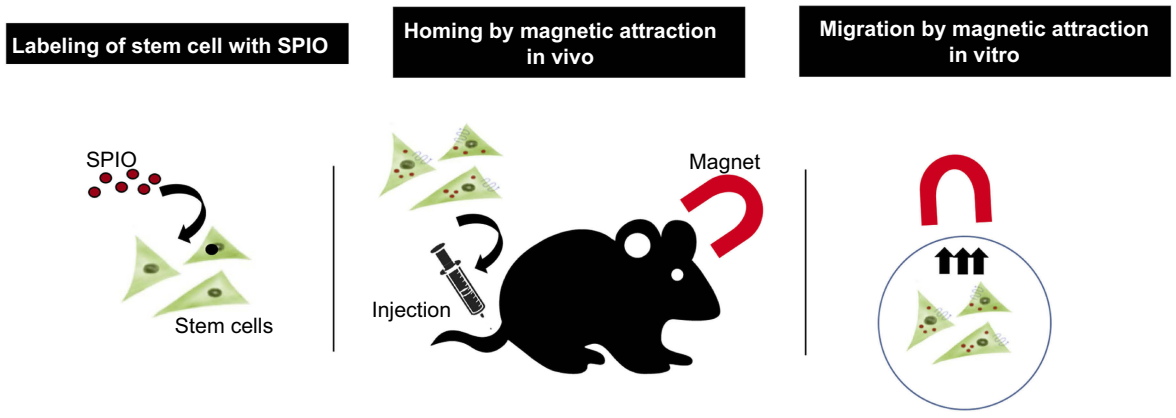

Figure 1 Schematic representation of enhancement of SPION-labeled stem cell homing with magnetic attraction.

homing. Studies published between January 2000 and July 2018 were searched in the following databases: EMBASE, PubMed, and Web of Science. The following selected criteria of interests, Boolean operators (DecS/MeSH), and keyword sequence were used:

(i) EMBASE: "stem cell"/exp OR "stem cell" AND ("iron oxide"/exp OR "iron oxide" OR nanoparticle) AND ("homing") AND "magnetics;"
(ii) PubMed: (((stem cell [MeSH terms]) AND (iron oxide OR SPION OR nanoparticle)) AND "homing") OR (((stem cell [MeSH terms]) AND (iron oxide OR SPION OR nanoparticle)) AND magnetics);
(iii) Web of Science: TS = (stem cell) AND TS = (nanoparticle) AND TS = (homing) OR TS = (stem cell) AND TS = (nanoparticle) AND TS = (magnetics) OR TS = (stem cell) AND TS = (iron oxide) AND TS = (targeting).

Studies which were indexed in more than one database (duplicates); incomplete articles; studies from conferences, book chapters, and non-English papers; and those not related to "nanoparticle" or "stem cells" and "magnetic field" were excluded from this review (Figure 2).

Data compilation and review (data extraction and quality assessment)

In this review, 2 review authors (Y.J.A. and Y.J.S) have independently extracted and randomly selected data using the search strategies cited earlier and verified the eligibility of the references. Discrepancies in study selection and data extraction that appeared between the two reviewers were discussed with a third reviewer and resolved. The reviewed papers were divided into three categories. The characteristics of the nanoparticles used in the experiment, the results of the in vivo experiments, and the results of the experiments in vitro were analyzed separately in Tables 1–3 respectively. We undertook a quantitative evaluation of data by using random-effect meta-analyses.

Outcome measures

We focused on data presented in each study as follows: 1) the hydrodynamic size of nanoparticles, 2) results of in vivo experiments such as the number of observed cells in the target organ of each study, and 3) results in vitro. However, the result of in vitro study cannot be analyzed because of the lack of results.

Statistical analysis

The mean hydrodynamic size of SPIONs was analyzed. The cell number observed experimentally in vivo was compared in "cells/field" units. The raw mean data and standardized mean difference (SMD) were used to pool studies. Between-study heterogeneity was evaluated using the p-value and I^2 statistics. If I^2 was <50%, the fixed-heterogeneity effect model was used, otherwise the random-effect model was performed. All statistical analysis was performed using R software (v. 3.5.0) for Windows with the meta-package.The p-values <0.05 were considered statistically significant.

Results

Overview of the reviewed literature

A total of 174 articles were identified by applying these search strategies. After applying inclusion criteria and removing duplicated articles, 14 total publications were selected (Figure 2). Although stem cell homing studies were used, a majority of the nanoparticle papers used for tracking MRIs were excluded. In this review article, we wanted to analyze only the use of magnetic force to attract stem cells labeled with nanoparticles. Although 19 years of research were analyzed, active research (6 papers, 43%) has been conducted over the past seven years. Asia, especially China, Japan, and South Korea, had the greatest number (43%) of studies conducted, recently followed by European countries and the United States. The reviewed papers were analyzed according to the characteristics of stem cells and SPIONs used experimentally and according to the experimental designs either in vivo or in vitro.

SPION and stem cell characteristics used in homing experiments

There were 9 mesenchymal, 1 bone marrow stromal, 1 endothelial progenitor, 1 neural, and 1 cardiosphere-derived cell lineages used (Table 1). Nine studies sourced their stem cells from humans, 5 from rats, 1 from rabbits, and 1 from mice. Concerning nanoparticle structural characteristics, the absolute amount of Fe used in each experiment is important for determining the magnetic force; however, this could be described through the concentration of the SPION labeled. The cell surface charge (zeta potential), nanoparticle size, and coating agent are important factors for enhancing cell labeling. Although the rate at which cells were labeled by nanoparticles by different factors may vary, most studies have reported over 80% labeling rates in cells were noted. The iron oxide

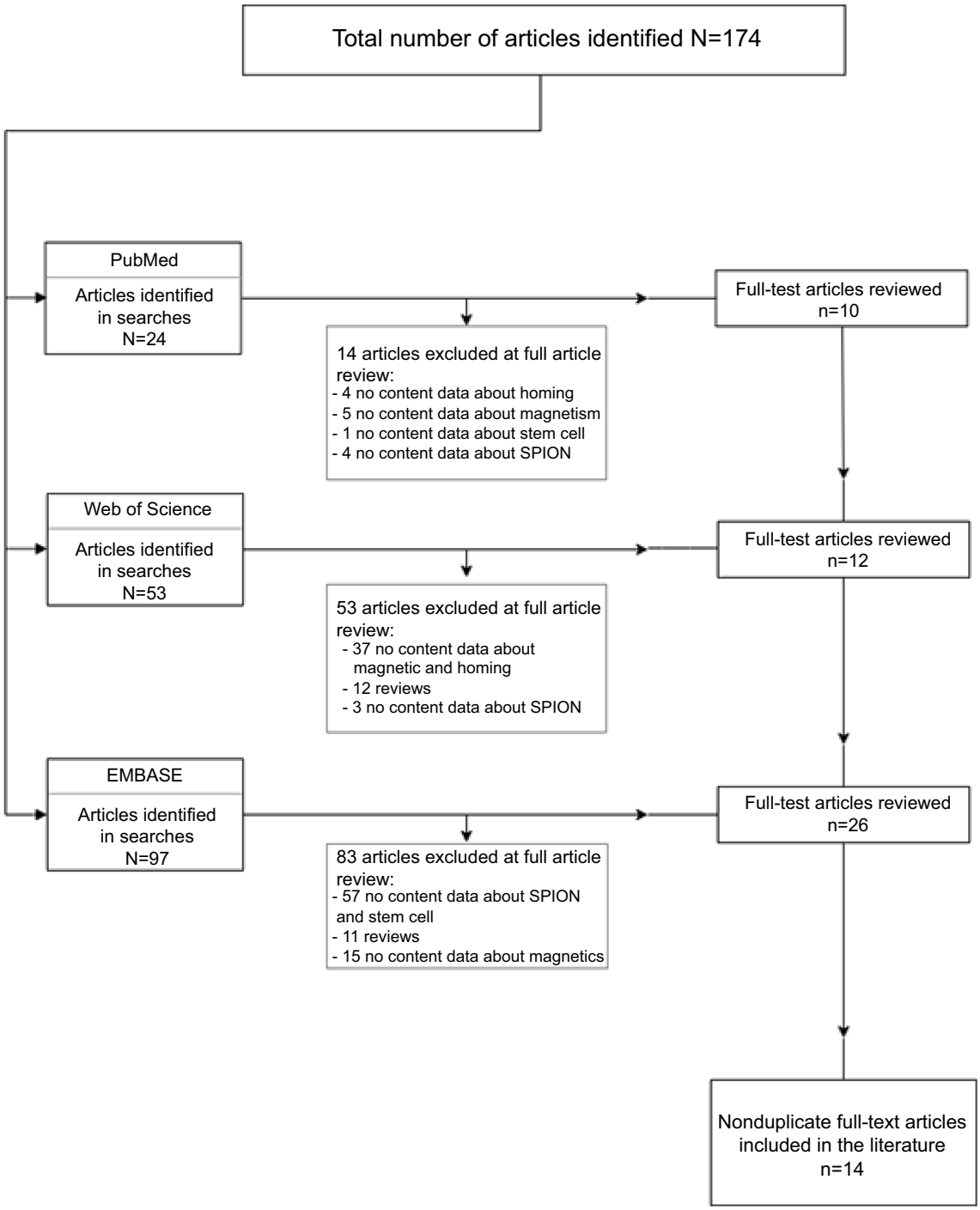

Figure 2 Flowchart of the article screening process for inclusion in this review.

nanoparticles used in eight studies among fourteen selected publications were commercial nanoparticles; the remaining six were laboratory-synthesized. Among studies that had used commercially synthesized iron oxide nanoparticles, four studies used Feridex® or Endorem™ (Advanced Magnetic, Cambridge, MA, USA), one used Resovist® (Bayer Schering Pharma AG, Berlin, Germany), two used FluidMag (Chemicell, Berlin, Germany), and one used Molday ION Rhodamine B (BioPAL, Inc., Waltham, MA, USA). These findings suggest that the higher prevalence in utilization of commercially synthesized iron oxide nanoparticles may be due to immediate availability and proven safeness of the material. The diameter of the nanoparticle used ranged from 4 nm to 1200 nm. In meta-analysis of hydrodynamic size of

Table 1 Characteristics of stem cells and iron oxide nanoparticles labeled in homing experiments

Reference	Stem cell			Superparamagnetic iron oxide nanoparticle (SPION)								Cell viability	
	Type	Source	Concentration	Type	Species of metal	[Fe] (μg/mL)	hydrodynamic size (nm)	Coating agent	Charge (zeta potential, mV)	Incubation Time	Efficiency (%)	Methods	Results
Arbab et al (2004)[54]	MSC	Human	$2x10^4$	Feridex	Fe	50	120–180	PLL	n/a	Overnight	n/a	n/a	n/a
Nishida et al (2006)[30]	bmSC	Rat	$1x10^5$	Feridex	Fe	25	120–180	PLL	n/a	24 h	n/a	n/a	n/a
Wilhelm et al (2007)[20]	EPC	Human	$2x10^4$	AMNP	Fe	2 mM, 5 mM, 10 mM	7.5	n/a	negative	15 min to 2 h	40%	Trypan blue	No significant difference
Kyrtatos et al (2009)[23]	MSC	Human	$1x10^6$	Endorem	Fe	500	120–180	n/a	n/a	Overnight	n/a	MTS	No significant difference
Song et al (2010)[15]	NSC	Human	$4x10^6$	Feridex	Fe	25	120–180	PLL	n/a	60 min	n/a	n/a	n/a
Cheng et al (2012)[24]	CDC	Rat	$5x10^5$	SPM	Fe	500:1 SPM/cell ratio	n/a	n/a	n/a	24 h	n/a	CCK8	No significant difference
Yanai et al (2012)[44]	MSC	Rat	$1x10^6$	FluidMag	Fe	50, 250, 500	200	Starch	n/a	2 h	n/a	MTT	No significant difference
Vaněček et al (2012)[21]	MSC	Rat	$1x10^6$	SPION	Fe	15.4	n/a	PLL	−46	72 h	84%	n/a	n/a
Riegler et al (2013)[26]	MSC	Rabbit	$1x10^6$	FluidMAG-D	Fe	620	182±18	Dextran	1±6	24 h	80%	MTS	No significant difference
Landázuri et al (2013)[17]	MSC	Human	$2x10^6$	SPION	Fe	2,10 μg/cm^2	30.8±2.4	n/a	n/a	24 h	100%	MTS	No toxic effect noted
Li et al (2013)[25]	MSC	Human	$1x10^6$	SIO_4@SPIONS	Fe	10	124	Silica	−37.5	2 h	n/a	CCK8	No significant difference
Oshima et al (2014)[27]	MSC	Human	$1x10^5$	Resovist	Fe	3.52 *0.5 mol/L	4.2/62	carboxydextran	n/a	24 h	n/a	n/a	n/a
Meng et al (2017)[28]	MSC	Human	$1x10^3$	Molday ION Rhodamine-B	Fe	25	n/a	n/a	n/a	12 h	>80%	MTT	increased
Yun et al (2018)[14]	MSC	Mouse	$1x10^5$	IRB	Fe	15	5.22±0.9	Oleic Acid	+15±0.3	24 h	n/a	CCK8	No significant difference

Abbreviations: MSC, mesenchymal stem cells; bmSC, bone marrow stromal cells; EPC, endothelial progenitor cells; NSC, neural stem cells; CDC, cardiosphere-derived cells; n/a, not indicated; AMNP, anionic magnetic nanoparticles; SPM, superparamagnetic microsphere; SPION, superparamagnetic iron oxide nanoparticles; SIO, silica-coated iron oxide.

Table 2 Experimental designs of homing - in vivo

Reference	Magnetics				Experimental model					In vivo homing			
	Type	Force (T)	location	Exposure time	Animal	Animal model	Groups	Route	Dose (cell count)	Duration of experiment	Histological image	Magnetic resonance image	Efficiency (%)*
Arbab et al (2004)[54]	NdFeB	0.34	Over the target organ (liver)	1, 8, 15, 22, and 29 days	Rat	n/a	2 groups (4 per magnet exposure time) control: 5	IV (tail vein)	$8x10^5$–$1x10^6$	29 days	DAB-enhanced Prussian blue and corresponding HLA-I staining of serial consecutive sections proved the presence of iron-containing human cells.	Hypointense liver signal after intravenous administration of FE-PLL–labeled MSCs	1-fold increase
Nishida et al (2006)[30]	NdFeB	0.38	para-vertebral muscles at T7 level	1 day	Rat/SD	Spinal cord injury	2 groups (magnet group: 5, non-magnet group: 5)	SI (subarachnoid injection)	$1x10^5$	1 day	Aggregations of GFP-positive cells were observed mainly on the dorsal surface of the spinal cords of rats in the magnet group.	n/a	3-fold increase
Wilhelm et al (2007)[20]	NdFeB	0.3	Above the matrigel	18 hrs	Mouse	n/a	n/a	SC (matrigel)	$5x10^4$	n/a	n/a	18 hrs post injection EPC with magnetic field moved towards the magnet	Movement of EPC toward magnet 18 hrs post injection

(*Continued*)

Table 2 (Continued)

Reference	Magnetics				Experimental model					In vivo homing			
	Type	Force (T)	location	Exposure time	Animal	Animal model	Groups	Route	Dose (cell count)	Duration of experiment	Histological image	Magnetic resonance image	Efficiency (%)*
Kyrtatos et al (2009)[23]	NdFeB	1.195	Adjacent to the ventral aspect of the neck	2 mins	Rat/SD/M 380–420 g	Vascular injury (common carotid artery)	N/A	IA (common carotid artery)	$5x10^6$	24 h	Application of an external magnetic force using the actuator during and after cell delivery increased CD133 cell engraftment to the injured vascular surface by a factor of 5.4 compared with cell delivery without the magnet.	n/a	1.16-fold increase
Song et al (2010)[15]	NdFeB	0.32	External magnet attached with an adhesive in the area above the injury	7 days	Rat/SD/M 250–300 g	Transient focal cerebral ischemia model	3 groups (control, with, and without magnet) Total: N=33	IV (tail vein)	$4x10^6$	7 days	Area of Prussian blue staining was significantly larger in brain slices from ICM rats as compared with IC rats (2.6±1.6 mm^2 vs 0.7±0.2 mm^2).	n/a	3-fold increase
Cheng et al (2012)[24]	NdFeB,	1.3	Above the heart	~3 weeks	Rat/WKY/F	Myocardial infarction model	Total: N=82	Intracoronary injection	$5x10^5$	3 weeks	More GFP+ cells were evident in the Fe-CDC and magnet group than in the Fe-CDC group in both risk and normal regions.	n/a	6.4-fold increase

(*Continued*)

Table 2 (Continued)

Reference	Magnetics				Experimental model					In vivo homing			
	Type	Force (T)	location	Exposure time	Animal	Animal model	Groups	Route	Dose (cell count)	Duration of experiment	Histological image	Magnetic resonance image	Efficiency (%)*
Yanai et al (2012)[44]	NdFeB	0.26	Within the orbit. limbal incision was made in the superior fornix and magnet pushed back into orbital apex	1 month	Rat S334ter-4 heterozygous transgenic rat	Ocular injury	n/a	DI (vitreous cavity) IV (tail vein)	DI-$2x10^5$ IV-$1x10^6$	1 month	Retinal cryosections identified that magnetic MSCs could be identified in both the outer as well as the inner retina if a magnet had been placed in the orbit.	n/a	Accumulation of 20% in magnet group and 0.6% in non-magnet group
Vaněček et al (2012)[21]	NdFeB	0.35	Above the lesion site	At least 1 week to a max of 2 weeks	Rat/Wistar/M 280–300 g	Balloon-induced spinal cord compression lesion model	2 groups Total: N=32 (with magnet: 4, without magnet: 4)	DI (intrathecal injection)	$5x10^6$	2 weeks	Prussian blue staining colocalized with the GFP signal in serial histological sections. In the control groups, fewer cells were identified in the lesion area.	Larger hypointense signal of SPION-labeled cells in the lesion area of the magnet group in contrast to the weak signal observed in the control group	3-fold increase

(*Continued*)

Table 2 (Continued)

Reference	Magnetics				Experimental model					In vivo homing			
	Type	Force (T)	location	Exposure time	Animal	Animal model	Groups	Route	Dose (cell count)	Duration of experiment	Histological image	Magnetic resonance image	Efficiency (%)*
Riegler et al (2013)[26]	12 element k=3 rabbit Halbach cylinder	0.21	External cylindrical magnet placed around the leg with femoral injury	Immediately after the injury and additional 40 mins	Rabbit/ NZW/M 2.0–2.5 kg	femoral artery balloon injury model	n/a	IA (central lumen of femoral artery)	$1x10^6$	1 day	n/a	50% of the injured vessel circumference was attached with magnetically labeled cells. The application of an external magnetic field during cell delivery lead to a 6.2-fold increase.	6.2 fold increase
Landázuri et al (2013)[17]	NdFeB	n/a	Over the proximal portion of the tail vein	n/a	Mouse/ nude 4–5 weeks old	n/a	2 groups (With magnet and without magnet)	IV (tail vein)	$2x10^6$	Mouse euthanized immediately after cell injection	IF: The signal emitted by DiR was 6.4-fold higher in the presence of the magnet.	n/a	6.4-fold increase
Li et al (2013)[25]	n/a	0.3	On the skull of the ischemic hemisphere	120 mins	Mouse/ICR/ M 25–30 g	Transient middle cerebral artery occlusion	2 groups	IV (jugular vein)	$1x10^6$	1 day	Brain atrophy volume was significantly reduced in M-SiO4@SPIONs-EPCs group.	n/a	3-fold increase

(*Continued*)

Table 2 (Continued)

Reference	Magnetics				Experimental model					In vivo homing			
	Type	Force (T)	location	Exposure time	Animal	Animal model	Groups	Route	Dose (cell count)	Duration of experiment	Histological image	Magnetic resonance image	Efficiency (%)*
Oshima et al (2014)[27]	Magnetic device	1.5	n/a	10 mins	Rat/ nude, 9 weeks	Limb muscle injury	9 groups (magnetic force) 3 groups (exposure time)	DI (muscle)	$1x10^5$	7 days	Masson trichrome staining and IF: 3 weeks after cell transplantation remarkably decreased fibrotic area. Control: 18.0 $\pm 1.58x10^5$ um^2; case: $7.51\pm0.72x10^2$ um^2	n/a	Fibrotic area decreased twice compared to the control group.
Meng et al (2017)[28]	NdFeB	0.5	On the surface of the wound	6 hrs/day	Mouse/ BALB/c nude	Skin defect	3 groups (Control, SPIONs-MSCs, SPIONs-MSCs with EMF) total: N=30	DI (wound)	$2x10^6$	1–7 days	IHC and IF: Increased	Increased SNR, CNR, and displacement at 1, 2, and 7 days	More than 80% of cells were seen in the injury site after 24 hrs.
Yun et al (2018)[14]	NdFeB	0.3	Directly on the site of injury	7 days	C57BL mouse	Olfactory injured model	2 groups	DI (nostrils)	n/a	7 days	IF: MSC injection with IRBs under a magnetic field showed significantly higher values compared to both MSC injection without IRBs and MSC injection with IRBs	n/a	3-fold increase

Note: *Versus the control group.

Abbreviations: NdFeB, neodymium iron boron; SD, Sprague-Dawley; M, male; F, Female; WKY, Wistar-Kyoto; NZW, New Zealand White; n/a, not indicated; IV, intravenous, SI, subarachnoid injection, SC, subcutaneous, IA, intra-atrial, DI, direct injection, IHC, immunohistochemistry, IF, immunofluorescence.

Table 3 Experimental designs of migration - in vitro

Reference	Cell			Magnetics				In vitro Migration			
	Type	Concentration (cell count)	Labeling Time	Type	Force (T)	Location	Exposure time	Time of test	Histological image	Magnetic resonance image	Efficiency
Arbab et al (2004)[54]	HeLa	40,000/cm2	Overnight	NdFeB	0.34	Under the culture dish	48 hrs	48 hrs	High concentration of labeled cells in the area near the edge of magnet.	Hypointense ring above the area magnet was placed	n/a
Wilhelm et al (2007)[20]	EPC	$5x10^4$	15 mins to 2 hrs	Magnetic tip	0.33	Directly on the matrigel test area	18 hrs	18 hrs	Approaching the magnetic tip, vasculogenesis was observed by videomicroscopy in the matrigel in medium containing endothelial growth factors.	Localized EPCs on the site with magnet	n/a
Kyrtatos et al (2009)[23]	hMNC	$1x10^6$	Overnight	NdFeB	1.195	1 mm from the cell	5, 10, 15 s	15 mins	Capture of SPIO-labeled cells in in-vitro flow system in area with magnet	n/a	252-fold increase in the capture of cell 1 mm distance from the magnetic actuator
Song et al (2010)[15]	NSC	$1x10^5$	1 hr	NdFeB	0.32	Below the culture dish	24 hrs	24 hrs	Aggregation of labeled cells in area surrounding the magnet observed.	n/a	n/a
Yanai et al (2012)[44]	MSC	n/a	2 hrs	NdFeB	0.26	Below the culture flask	12 hrs	12 hrs	Aggregation of labeled cells in area surrounding the magnet observed.	n/a	n/a
Vaněček et al (2012)[21]	MSC	$1x10^6$	72 hrs	Permanent magnet	0.35	Below the culture dish	48 hrs	48 hrs	Cell evenly distributed in the culture plate in non-magnet group and cells attracted to region above the magnet in the magnet group	n/a	n/a

(*Continued*)

Table 3 (Continued)

Reference	Cell			Magnetics				In vitro Migration			
	Type	Concentration (cell count)	Labeling Time	Type	Force (T)	Location	Exposure time	Time of test	Histological image	Magnetic resonance image	Efficiency
Riegler et al (2013)[26]	MSC	$3x10^4$	24 hrs	NdFeB	0.44	Below the culture dish	10–120 mins	120 mins	Twice the number of cells attached to the test site were observed	n/a	Doubled cell attachment rate
Landázuri et al (2013)[17]	MSC	n/a	24 hrs	NdFeB	1.2	Below the culture dish	4 hrs	4 hrs to 3 days	Cells accumulated on the sites with highest magnetic field gradient	n/a	n/a
Li et al (2013)[25]	MSC	$1x10^4$	2 hrs	No Information	0.3	Below the culture dish	24 hrs	24 hrs	Cells accumulated on the sites with highest magnetic field gradient	n/a	n/a
Yun et al (2018)[14]	MSC	$1x10^6$	24 hrs	Permanent magnet	0.3	Beside the culture plate	24 hrs	24 hrs	Attraction of the cells toward the magnetic field gradient was noted upon fluorescent microscopy	n/a	42.92% of magnetized MSCs adhered to the front

Abbreviations: EPC, endothelial progenitor cell; hMNC, human mononuclear cell; MSC, mesenchymal stem cell; NdFeB, neodymium iron boron; SPION, superparamagnetic iron oxide, n/a, not indicated.

nanoparticles, studies which presented the hydrodynamic size mean and standard deviation and the nanoparticle size were included. Finally, 3 studies were pooled, and the pooled hydrodynamic size was 72.67 nm (95% CI: 25.90–119.44) with high heterogeneity (I^2=100%) (Figure 3). Riegler et al[11] used especially large commercial nanoparticles while Yun et al[14] and Landázuri et al[17] used relatively small laboratory-synthesized nanoparticles. Thus, high heterogeneity was shown. For commercialized nanoparticles used for homing, sizes up to 120–200 nm were used to label cells, which is the size of clathrin-mediated endocytosis. Smaller nanoparticles were also used in some laboratories, but there was no significant difference in labeling efficiency. SPION coating with poly-L-lysine (PLL) as the transfection agent was demonstrated in most of the reviewed studies. In addition to PLL, starch, dextran, silica, and oleic acid were used; these are commonly known as harmless to the human body. To insert nanoparticles into cells for labeling, the zeta potential in contact with the negatively charged cell surface is important. While most selected studies did not report the material's charge, many were negative. Negatively charged nanoparticles have a lower capacity for cell entry than positively charged ones because the cell surface is also negatively charged.[18,19] However, when internalized by the cell, negatively charged nanoparticles show less cytotoxicity and are thus preferred. The nanoparticle concentration used for labeling ranged from 2 to 500 μg/mL. The stem cell concentration used for labeling ranged from 2×10^4 in Wilhelm et al[20] to 4×10^6 in Song et al.[15] The labeling time for nanoparticles to enter stem cells ranged from 15 min in Wilhelm et al[20] to 72 hrs in Vaněček et al;[21] a labeling time of 24 hrs was most commonly used. Stem cells were commonly incubated with nanoparticles for 24 hrs, but the shortest time ranged from 15 mins to 2 hrs. Therefore, by the mean rate of labeling time applied in the different kinds of literature, 24 hrs are recommended to maintain high-efficiency labeling. When cell viability was observed at these incubation times, none of 11 papers reported cytotoxicity. To determine cell viability, 4 studies used cell counting kit (CCK), 3 used 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), 2 used Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1 used trypan blue and 1 used CASY2 analyzer.

Migration of SPION-labeled stem cells by magnetic attraction in vitro

Ten studies analyzed the homing competency of the iron oxide nanoparticle-labeled MSCs by observing the migration of the cell toward the external magnet (Table 3). Wilhelm et al[22] used video microscopy to observe migration of the labeled cell. After endothelial precursor cells (EPCs) were magnetically labeled via 2 hr incubation, the cells were seeded in Matrigel applied by a thin magnetic tip of 0.33 T. Cells massively migrate toward this tip (over 2–4 hrs) to form dense tissue surrounding the tip (14 hrs). Kyrtatos et al[23] reported the highest migration efficacy in vitro among selected studies, showing a rapid movement toward the magnet of 19 pN at 5–15 s in real-time video. They developed an "in vitro flow system" with a 5 bar magnet array in aluminum casing for evaluating intraluminal migration and suggested the possibility for the magnetic control of cells inside the vasculature using a circumferential external magnetic force. They found that capturing cells in vitro for 15 mins at 1 mm resulted in 252-fold increase in capture rate (mean=$41\text{x}10^3\pm6\text{x}10^3$ cells) versus control specimens (mean=163±41 cells). Yun et al[14] showed the quantitative result of magnetically dragging MSCs with a 0.3 T permanent magnet affected the cells at a distance of 15 mm, increasing cell density by 43% (at 0–5 mm). Results analyzed in vitro could not be quantitatively compared or meta-analyzed due to a lack of data.

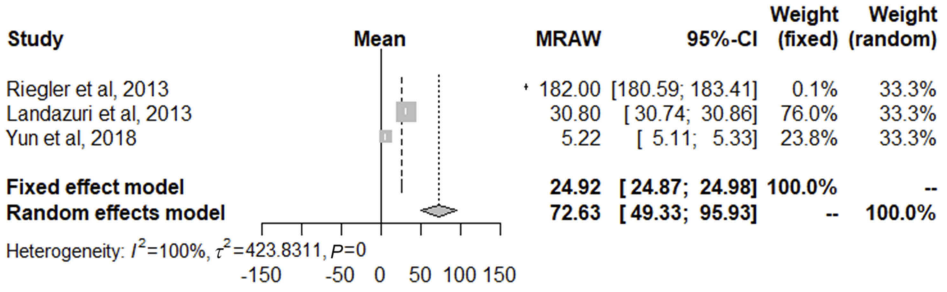

Figure 3 Forest Plot showing hydrodynamic size of nanoparticles and meta-analysis results of mean hydrodynamic size of nanoparticles.

Homing of SPION-labeled stem cells by magnetic attraction in vivo

For in vivo experiments, the magnet's force and location of placement were considered as an important factor for attracting magnetized cells. The homing of iron oxide nanoparticle-labeled MSCs was enhanced through the application of a magnet to a desired site (Table 2). Nine studies used a neodymium iron boron magnet, 2 used a permanent magnet, 2 used a magnetic device, 1 used a magnetic tip, and 1 study did not mention the magnet type. The corresponding magnetic forces ranged from 0.21 T to 1.5 T.

Of these selected publications, 8 studies used rats, 5 used mice, and 1 used rabbits. Among the experimental animal models, 5 used a vascular injury model, 2 used a spinal cord injury model, 1 used muscular injury model, 1 used skin injury model, 1 used cardiac injury model, 1 used ocular injury model, and 1 used olfactory injury models. The most common type was the vascular injury model; three used thread occlusion[15,24,25] and two performed balloon inflation to cause injury.[23,26]

Iron oxide nanoparticle-labeled MSC dosage ranged from $5x10^4$ to 5×10^6. The routes of stem cell administration in the selected studies were 5 studies by direct injection to the injury site, 4 studies by intravenous injection, 2 studies by intra-arterial injection, and 1 study by subcutaneous injection. The in vivo homing experiment was observed from immediately to 1 month. Four studies observed the experiment until day 7,[14,15,27,28] while 2 studies,[29,30] observed for about 1 month in order to determine the duration of MSC retention in the injury site. The researchers were able to observe MSC in the site of injury until 1-month post-administration.

The reviewed studies observed an enhanced migration capacity of SPION-labeled MSCs to the injury site with or without application of an external magnetic field to promote effective delivery of MSCs to the desired site. Although there have been few papers reported specific values for homing efficiency in vivo, most papers reported that the efficiency was increased when magnet is applied compared to those without. Cheng et al[24] reported that the efficiency in a myocardial infarction rat model can be increased about 6.4-fold with a 1.3 T NdFeB magnet above the heart. Yun et al[14] used a 0.3 T permanent magnet directly on the site of injury in the olfactory-injured mouse model, showing a high efficiency of a 3-fold increase after 7 days. Six studies have used MRIs to assess the therapeutic efficacy of stem cell homing with the application of an external magnet, and 13 of 14 selected publications had histological images taken either in immunohistochemistry (IHC) or immunofluorescence (IF).

In meta-analysis, quantitative comparisons of observed stem cells at the target organ were performed with and without magnets. Studies were included that reported the mean and standard deviation of observed stem cells in the target organ and the number of subjective animals. Finally, 5 studies were pooled. The SMD of the pooled magnet was 2.23 (95% CI: 0.43–4.03) with moderate heterogeneity (p-value=0.05, I^2=58%, Figure 4).

Discussion

Stem cell-based therapies are rapidly evolving for several diseases such as strokes, myocardial infarctions, liver diseases, and demyelinating/dysmyelinating disorders of the central nervous system.[31–35] Karp and Leng Teo[36] suggested defining cellular homing as an arrest of MSCs within the vasculature of the respective tissue followed by a transmigration process across the endothelium. Several stem cells including MSCs and EPCs, which are multipotent regarding regeneration, are currently used due to their ability to migrate toward damaged tissue. They also exhibit an intrinsic homing property enabling them to direct migration to sites of injury, inflammation, and tumors.[37–39] However, approaches which rely on cell delivery suffer

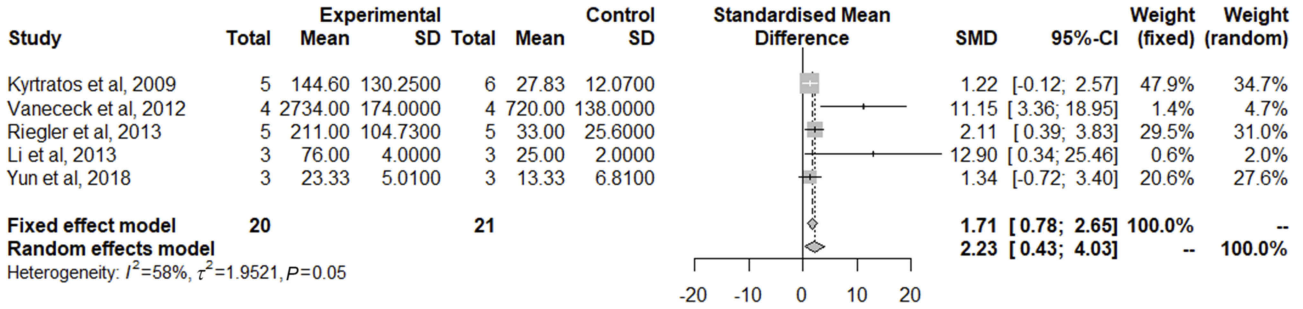

Figure 4 Forest plot showing stem cell observed in the target organ and meta-analysis results for quantitative comparison.

from poor cell retention at the target site and has potential for adverse effects due to generalized distribution.[40] Devine et al[4] demonstrated a low engraftment efficacy, estimated to range from 0.1% to 2.7% and observed in the kidney, lung, liver, thymus, and skin. Lee et al[41] recently quantified MSC accumulation in the lungs after systemic administration. They found the presence of nearly all (80%) infused MSC in the lungs 15 mins after infusion; after 4 days, the human-specific signal decreased exponentially to 0.01%. Thus, several methodologies have been applied to increase the efficacy of stem cell homing.

The measurement of the velocity of magnetically labeled cells submitted to a magnetic field gradient was referred to as magnetophoresis,[42] attracting interest as a new approach.[22] However, the in vivo mechanisms involved in MSC homing and the conditions which govern their homing behavior are still poorly understood and require extensive in vitro and in vivo testing. Several factors are important, including the cellular iron concentration, effective cellular SPION saturation, cellular nanoparticle distribution, external magnetic field strength, magnetic field gradient, injection methodology, and especially the design of animal experiments regarding clinical translation. This review focuses on the current knowledge of magnetically labeled stem cell homing toward an external magnetic field. It is important for the future use of MSCs as a cellular therapeutic agent. Song et al[15] demonstrated that rats wearing an external magnet (0.32 T) on their skull for 1 week exhibited an increased number of SPION-labeled stem cells after intravenous injection, resulting in a 3-fold or greater increase in the infarct area below the magnet as well as a significant decrease in infarct size. Shen et al[10] introduced another approach for the magnetic attraction of stem cells to injury sites after traumatic brain injury via intra-carotid delivery. Hsiao et al[43] reported that using 1.5T MRI at single-level 3d gradient echo sequence for 4 repetitions have detected about 45.2% of the labeled MSCs. Yun et al's study[14] suggested the possibility that magnetic retention of SPION-labeled MSCs can increase the homing efficiency of MSCs clinically. MSCs labeled with nanoparticles under a magnetic field also showed a significant difference in the olfactory-injured mice model compared to MSCs injected without nanoparticles ($p \leq 0.05$).

The application of SPION in the nanomedical field has been increasing; with the recent advances in tissue engineering technologies, various experimental trials have been undergone using SPION.[11,15,44,55] Despite of debates on SPION toxicity caused by concentration, size, zeta-potential, and oxidative stress effect, the SPION is also well known to be widely used in biomedical applications, for example, magnetic resonance imaging, targeted delivery of drugs or genes, and in hyperthermia. Some kind of SPIONs were approved by FDA and commercially available, and other synthesized SPION which have undergone cell viability tests to verify the cytotoxic effect were used. In the studies we have reviewed, many researchers suggest that SPION is safe to use in in vitro and in vivo examinations. Most of these studies used commercially available SPIONs as they are FDA-approved materials; However Resovist® is now available only in few countries and Feridex® has been stopped development and have withdrawn from the market due to economic reasons.[45] Jo et al[46] indicated that it is practically necessary for efficient cell labeling to accurately control the size and surface state of the iron oxide nanoparticles. SPION labeling has several limitations. First, as the labeling of these particles is time-consuming, transfection agents are added to enhance cellular uptake of the particles. Most of the reviewed studies used PLL as this transfection agent, functioning as a coating on SPIONs; PLL is commonly used to enhance cell adhesion to the culture dish in vitro and is a prospective vehicle for transporting nanoparticles into cells. Increasing the particle coating or reducing its breakdown when internalized within a cell can greatly reduce toxicity.[47] Thus, these findings suggest that coating nanoparticles with a transfection agent enhances the efficacy of cellular SPION-labeling. Second, the SPION surface potential also contributes to cellular internalization. Jo et al[46] suggests that since the cell surface is negatively charged, nanoparticles with positive surface potentials would ionically interact with the cell surface and result in the enhanced cell internalization of nanoparticles. Third, SPION size is considered as a strong factor of efficient cellular SPION labeling since different particle sizes are correlated with different endocytosis pathways. Arbab et al,[29] Nishida et al,[30] Kyrtatos et al,[23] Song et al,[15] Riegler et al,[11] and Li et al[25] used SPION sizes greater than 120 nm.

A nanoparticle placed in the external milieu of a cell can interact with the exterior of the plasma membrane, which can lead to nanoparticle entry through a process termed endocytosis. The pathway through which the nanoparticles are internalized is yet to be known; however, due to their shape and size, it is assumed that they are internalized through caveolae-mediated or clathrin-mediated endocytosis.[13,48] Huang et al[17] proposed that

the internalization of nanoparticles had a stimulatory effect leading to an increase in reactive oxygen species (ROS) due to hypoxic conditions. Excess ROS are generated during a variety of cell stresses and may contribute to inflammation and cell or tissue damage.[49] Yun et al[14] also demonstrated that the increase in CXCR4 by MSC internalization of iron oxide nanoparticles improved magnetized MSC homing to the injury site by SDF-1/CXCR4 chemotaxis. When cells are labeled with SPION, they exhibit issues concerning cytotoxicity or differentiation, and this is of great clinical concern. In the reviewed 14 studies, no cytotoxicity was noted in the SPION-labeled stem cells. This suggests the safety of iron oxide nanoparticles as a labeling agent to enhance homing rates.

Most of the reviewed studies applied the external magnet directly to the site of interest to enhance the homing effect of the MSC. The World Health Organization (WHO) suggests occupational limits for the general public of 5,000 mG (milligauss) and 1,000 mG for electric and magnetic fields, respectively. Schäfer et al[50] proposed that the paramagnetic ions NA^+ and K^+ are crucial for maintaining membrane potential, and their distribution may be influenced by magnetic fields; thus, further investigation is needed to determine the molecular mechanisms of interactions between stem cell biology and magnetic fields. According to Marycz et al,[51] the static magnetic field is classified as a weak (<1 mT), moderate (1 mT to 1 T), strong (1 T to 5 T), or ultrastrong (>5 T) field. Most reviewed papers used magnets with a moderate magnetic field, and no changes in cell viability were shown. Lew et al[52] suggested that the proliferation of dental pulp stem cells enhanced by a 0.4 T static magnetic field (SMF) is considered to be a model of the p38 MAPK signaling pathway as well as intracellular calcium ion activation. Most reviewed papers applied a magnet to the external surface of the injury site; this may be due to easy application and to avoid the risk of infection during surgical insertion of the magnet. The magnet application site and type may vary according to the target organ or area.[53]

In meta-analysis of our study, there are different types of nanoparticles, stem cells, animal models, and target organs used in each study, resulting in a bias in statistical results. Our meta-analysis shows just basic information of previous studies. However, in most of the studies we reviewed, the detailed quantitative results were not disclosed even in supplements, which become a major limitation of our study. The future studies will be suggested that it is desirable to be able to present quantitative results together. Regarding SPIONs, many reports did not show the number, size, or zeta potential of the SPIONs used. Some studies did not report the number of stem cells observed in each target organ, but they showed the degree of recovery or regeneration of damaged target organs in vivo. In in vitro studies, quantifiable results were rarely reported. Additional studies should be reported in the future and accompanied by quantitative reports.

Conclusion

This review has provided information on overall experimental methods. However, further studies should be conducted. First, the mechanism of endocytosis during SPION labeling should be clarified. We must reveal the mechanisms used, from endocytosis to exocytosis, and develop various types of SPIO for reliable labeling. If we can control this mechanism, safer magnetized MSCs can be produced clinically. Using magnetic attraction, location tracking via MRI would be able to accurately evaluate the efficiency of magnetized MSCs. It will be necessary to develop a SPION that is safe, noncytotoxic to stem cells, and able to maximize magnetic attraction. This is also important to ensure the localization of the circulating cells to the desired location in the vascular system without being washed out in the lungs. Although there are many important issues to address, cell therapies using the attraction of magnetized MSCs are suggested as potential treatment, repair, or replacement strategies for stem cells.

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Disclosure

The authors report no conflicts of interest in this work.

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