REVIEW ARTICLE

Magnetic resonance imaging of transplanted stem cell fate in stroke

Hamid Reza Aghayan[1,2], Masoud Soleimani[1,3], Parisa Goodarzi[1,4], Abbas Norouzi-Javidan[1,4], Seyed Hasan Emami-Razavi[1], Bagher Larijani[2,5], Babak Arjmand[2,6]

[1]cGMP-compliant stem cell facility, Brain and Spinal Cord Injury Research Center, [2]cGMP-compliant stem cell facility, Endocrinology and Metabolism Research Center, [3]Department of Hematology, Faculty of Medicine, Tarbiat Modares University, [4]Cellul Fanavaran Knowledge-Based Organization, [5]Medical Ethics and History of Medicine Research Center, [6]cGMP-compliant stem cell facility, Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran

Nowadays, scientific findings in the field of regeneration of nervous system have revealed the possibility of stem cell based therapies for damaged brain tissue related disorders like stroke. Furthermore, to achieve desirable outcomes from cellular therapies, one needs to monitor the migration, engraftment, viability, and also functional fate of transplanted stem cells. Magnetic resonance imaging is an extremely versatile technique for this purpose, which has been broadly used to study stroke and assessment of therapeutic role of stem cells. In this review we searched in PubMed search engine by using following keywords; *"Stem Cells", "Cell Tracking", "Stroke", "Stem Cell Transplantation", "Nanoparticles", and "Magnetic Resonance Imaging"* as entry terms and based on the mentioned key words, the search period was set from 1976 to 2012. The main purpose of this article is describing various advantages of molecular and magnetic resonance imaging of stem cells, with focus on translation of stem cell research to clinical research.

Key words: Cell fate, cell tracking, magnetic resonance imaging, nanoparticle, stem cells, stroke, tracking

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INTRODUCTION

Recent scientific findings about the possibility of regeneration of the nervous system have revealed the differentiation of stem cells into neural cells and stem cell-based brain tissue regeneration.[1,2] Although, several basic mechanisms were defined for stem cell journey and functions in the body and their therapeutic roles, it needs to be fully clarified.[3] This clarification can be performed by experimental and clinical research projects to promote stem cell transplantation.[4] Therefore, the migration, engraftment, long term viability, and also functional fate of transplanted stem cells should be assessed by noninvasive imaging modalities particularly, in clinical cell transplantation trials.[5-7] Magnetic resonance imaging (MRI) is an extremely versatile technique for this purpose,[1] which has been successfully contributed to study stroke and evaluation of therapeutic role of stem cells. Therefore, MRI as a "Magnetic Imaging" technique has taken its place among the other imaging modalities.[8] In this review, we will describe MRI for evaluating the migration, engraftment, and fate of transplanted stem cells in stroke by using nanotechnologies, with focus on translation of stem cell research to clinical research.

MATERIALS AND METHODS

In this review to introduce MRI as a clinical compatible imaging technique for evaluating the fate, engraftment and migration of transplanted stem cells in Stroke, *"Stem Cells", "Cell Tracking", "Stroke", "Stem Cell Transplantation", "Nanoparticles", and "Magnetic Resonance Imaging"* were used as search terms by using PubMed search engine. Subsequently, the search period was set from 1976 to 2012.

Stroke

Stroke is defined by the World Health Organization (WHO), as "rapidly developing signs of focal or global disturbance of cerebral function lasting more than 24 hours (unless interrupted by surgery or death) with no apparent cause other than a vascular origin".[9] There are two types; ischemic stroke (caused by an occlusion) and hemorrhagic stroke (initiated by a rupture) of a blood vessel in the brain. Among them, ischemic stroke is the most common type.[10] Stroke is the main cause of adult disability and one of the leading causes of irreversible neurological damages and death worldwide.[11-16] It is also the 6th leading cause of diseases burden, while around one third of deaths from stroke occur

Address for correspondence: Dr. Babak Arjmand, Endocrinology and Metabolism Research Center and Brain and Spinal Cord Injury Research Center, Tehran University of Medical sciences, Shariati Hospital, North Kargar, Tehran - 1411413137, Iran.
E-mail: b_arjmand@farabi.tums.ac.ir
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in developing countries.[17] According to the estimation of the WHO, in 2004 the stroke mortality rate was reported around 5.7 million, that was equal to 9.7% of the deaths worldwide. Of them, more than 85%, happened in low and middle-income countries.[18] There are some considerable geographic and regional variations and differences in stroke types, incidence, mortality, and distribution around the world.[19]

Incidence pattern of stroke in Iran

The epidemiologic data of stroke in the Middle East regions are limited and unreliable.[19] Stroke is becoming a serious health problem in this region, with the mortality rate expected to double by 2030. There is a lack of data on the burden of stroke in Iran.[17] Tran *et al*., reported stroke incidence rate as 10.4 per 1,00,000 in Iran, with a higher rate in women.[18] Azarpazhooh *et al*., demonstrated that, the incidence of stroke in Iran is significantly greater than most of the western countries, with an occurrence in younger ages. They also indicated the history of hypertension as a main risk factor[16] followed by smoking and diabetes.[17] Ischemic stroke is the most common type in Iran, similar to previous reports from other regions of the world.[17] As stroke is an important health problem, with a huge burden on health economy, it specifically requires effective and novel treatments to regenerate damaged brain tissue.

Stem cell transplantation in stroke

In spite of recent considerable advances in stroke management, current acute treatment methods, have only mild effects and moderately restore its lost function.[11,15] Therefore stroke remains a major cause of disability and needs much more efficient management methods, such as cell replacement in the ischemic region to prevent further disability.[11,20] Cell transplantation is a novel treatment method in many fields of medicine, including stroke as a nervous system disorder.[21] In the last decade, evidence of neurogenesis probability in the human adult brain has provided the basic scientific hypothesis of (stem) cell transplantation therapy in various neurological disorders including; Parkinson's disease, multiple sclerosis, and stroke, to improve neurological defects and relieve disability.[11,22-24] Initial animal and experimental studies explored the significant benefit of stem cell transplantation in neuroregeneration and improvement in neural functioning.[11,13,25,26] Stroke is one of the neurological disorders, which has been selected as a pioneering trial in the clinical application of stem cell.[27] Furthermore several studies demonstrated the feasibility of stem cell-based therapy for the restoration of lost brain function[13,14,20] and improvement of the clinical outcome in stroke patients.[10,28-30] Several experimental and clinical researches have introduced different types of stem cell for transplantation in stroke.[11,14-16,20,22,27-37] In recent years, different experimental and clinical cell transplantation studies have been started in Iran, as a leading country in the Middle East[38] for central nervous system (CNS) disorders including; spinal cord injury (SCI)[21,39-41] and stroke.[42] Also, based on our suggestion, the Iranian Food and Drug Organization (FDO) started working on a policy to regulate and harmonize human cell and tissue manufacturing activities specially for improving clinical (stem) cell transplantation safety and efficacy as a national standard. This national standard can be a basic reference for cell therapy facilities, to address minimal safety considerations.[43] According to such scientific and organizational advances, in the field of stem cell therapy in the country, it sounds crucially important to clarify the shady corners of cell transplantation therapy.[34] Moreover clarification of optimal cell dosing, route of transplantation, cell delivery methods, and *in vivo* cell imaging techniques is needed to ensure safety, efficacy, expected outcome, and more success of potential stem cell transplantation trials.[30]

Stem cell imaging and tracking modalities in stroke

Stem cell's therapeutic effects in the treatment of various neurological disorders have been experimentally demonstrated. Well-guided stem cell migration, differentiation, survival, and engraftment to the site of injury, may reinforce these beneficial effects on the treatment of human damaged brain.[44,45] Therefore, stem cell therapy needs to be assessed and monitored by imaging and tracking the transplanted cells,[46] for developing the brain restorative treatment strategies.[4,47-49] Hence, to achieve this aim, stem cell imaging techniques are performed as pioneering investigation to monitor, control, and treat biological systems, in particular, the brain.[50] Moreover, stem cell imaging by non-invasive modalities allows their monitoring overtime. Therefore, finding a non-invasive method to track stem cells in the human body is an essential step before translating stem cell research into clinical research. Several studies in animal models and humans have demonstrated that, clinical translation of imaging modalities from the basic to the clinical research is feasible.[51] During the last few years, various imaging techniques, particularly magnetic resonance imaging, equipped with different contrast agents, have been applied for this purpose.[52-55] It is elucidated that, noninvasive imaging modalities facilitates accomplishing suitable therapeutic effects in clinical stem cell trials [47,56-58] while understanding stem cell migration and differentiation mechanisms.[59] Ideally, *in vivo* stem cell tracking and imaging depict stem cell migration, viability, and also their functions,[3,60] considering the preferential engraftment of stem cells on to the site of the injured brain.[61] These techniques are crucial to guide and promote significant advances in stem cell transplantation research and its clinical application for neurological diseases in the future.[62] There are various cell imaging modalities

including optical imaging, bioluminescence imaging (BLI), ultrasound, computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) that clinical stem cell trials will benefit from[29,49,56,63] real-time depiction of cell migration and journey in the body, and promotes optimizing preclinical and clinical stem cell transplantation studies. Advantages and disadvantages of several imaging modalities are demonstrated in [Table 1].[53,64,65] Nowadays, PET, SPECT, and MRI are suitable candidates for human nervous system cellular imaging. Among them, PET is more sensitive to low concentration of contrast agents. However, it has some limitations as low spatial resolution, radiation exposure, and short-term signal production.[50] Another technique is optical imaging, which is a sensitive method with some distinct advantages in small animal models but, it is not feasible for human whole-body visualization because of the limited penetration depth and low spatial resolution. Although, a high spatial resolution can be provided by other methods like micro-CT, this technique is not always suitable for *in vivo* human studies and it needs to be optimized for a better cell detection in the whole body.[66] With respect to the full commitment of clinical studies and trials to patients' safety, radiation and radioactive exposures are important limitations of CT, PET, SPECT, and Scintigraphy. Therefore, MRI can be preferred as a superior method for cell tracking and imaging particularly, in clinical trials.[3,51] Several stem cell tracking studies have been performed by using MRI. A main clinical field in which, MRI has been used for stem cell tracking is neurological diseases.[56,67]

MR Imaging of transplanted stem cells in stroke

Nowadays, *in vivo* stem cell imaging introduces a novel view on stem cell research in stroke.[68] Although, various clinical trials have depicted the stem cell transplantation safety in stroke, revealing probable mechanisms of cell delivery is crucially important as a main subdivision of stem cell therapy[11,36] in different types of disease such as neurological disorders. Recently, MRI stem cell tracking has become an important method for real-time, noninvasive imaging and following cell migration, engraftment, survival, differentiation, and subsequently the efficacy of clinical cell therapy trials.[25,56,69,70] MRI is a well-defined noninvasive cell imaging technique, which has some valuable advantages,[71] for instance, it is able to provide an excellent image quality, high spatial 3D resolution, superior sensitivity, identifying labeled cells in their anatomical context, additional information about the surrounding milieu, and clinical applicability with no exposure to ionizing radiation.[3,5,26,54,56,63,66,68,72-75] The MR tracking of transplanted progenitor cells in the CNS has been performed by several investigators. The first relevant studies were reported in 1992, in which superparamagnetic contrast agents were used for cell imaging in rat brain.[26] Today, imaging of Superparamagnetic iron oxide (SPIO) labeled (stem) cells is already routinely used in animal models of neurological diseases[52,56] including, SCI and stroke.[26] In summary, MRI provides many requirements of a noninvasive cell imaging and monitoring *in vivo*,[4] while it can be equipped with nanotechnology.[76] It can be also used serially to follow and identify the distribution scenery of transplanted stem cells in stroke.[61] Hence, its application in human trials needs safety controls. Gadolinium chelates and iron oxide particles are currently the best contrast agent candidates to label cells for MRI, because they are well tolerated when directly injected in the blood stream.[50,77,78] Several contrast agents which are used in MR cell imaging are compared in [Table 2].[71] Some of them such as Gadolinium may be coupled with the fluorescent compound allowing their detection by histology in experimental studies.[61,77,79]

Gadolinium as a T1-weighted contrast agent increases T1 relaxation time thus resulting in bright contrast image. On the other hand, SPIO nanoparticles as negative contrast agents reduce T2 relaxation and consequently, produce

Table 1: Several in vivo cell imaging modalities

Modality	SPECT	PET	Ultrasound	MR-based contrast agents	^{19}F MRI	Fluorescence	Bioluminescence
Clinical applicability	Yes	Yes	Possible	Yes	Possible	No	No
Relative sensitivity	++	+++	+	+++	+	+	++
Longitudinal cell tracking	+	+	+	+++	+++	+++	+++
Quantification of cell numbers	+++	+++	+	+	+++	+	++
Assessment of cell viability or function	No	Yes	No	No	No	Yes	Yes
Radiation Radioactive Exposure	Yes	Yes	No	No	No	No	No
Depth	no limit	no limit	1 mm-1 cm	no limit	no limit	<1 cm	1 cm
Acquisition time	minutes	minutes	second to minutes	microsecond to hours	microsecond to hours	minutes	minutes
Resolution	1-2 mm	1-2 mm	50 µm	10-100 µm	10-100 µm	2-3 mm	2-3 mm

hypointense negative (black) signals.[3] Furthermore, in susceptibility weighted image (SWI) and diffusion-weighted image (DWI) of MRI, labeled stem cells with SPIO nanoparticles produce dark spots.[80]

Limitation of MR imaging of stem cells

MRI has some limitations which restrict its unique advantages in some cases particularly, long-term cell imaging. For instance, contrast agent may be diluted due to cell division, especially when the cells are rapidly dividing.[45,56,81-83] Sometimes, certain endogenous conditions can introduce hypointense MR signals, which can be confused with the MRI contrast agents. Another limitation can be induced by macrophages, while are loaded with hemosiderin from hemorrhage or contrast agents, and shown as hypointense signals similar to the labeled cells.[56,84] Also, discrimination between live and dead cells is not possible by MRI, because magnetic contrast agents could remain in the site of injured or ischemic brain tissue and produce detectable signals.[45,56,72,81] Furthermore, clinical imaging has more limitation compared with experimental animal studies, for instance, animal MRI scanners can reach16T or higher, whereas high field in human studies is around 7T, as most clinical MRI scanners being less than 3T in the country.[2,8] Another concern is the probable negative effect of MRI contrast agents such as SPIO or USPIO, on the differentiation and metabolism of labeled cells, which were reported in a few studies, while several researches stated no negative or harmful effect.[77,85,86] In spite of MRI single cell detection in some experimental studies, in most cases it requires clusters of labeled cells to detect.[74] Of course, some novel techniques of transfecting agents and new methods for producing contrast labels try to overcome the limitations of MRI stem cell tracking in neurological diseases, which introduce promising results for future clinical stem cell tracking trials using these novel methods[5] in different disorders including neurological diseases.

CONCLUSION AND FUTURE PERSPECTIVES

Neuro-transplantation by using (stem) cells has introduced some promising aspects for the treatment of several CNS disorders such as stroke.[34] Monitoring of transplanted cells is an interesting field to achieve desirable therapeutic effect after transplantation. Although this modality is still in its infancy of development, several experimental and further clinical studies have demonstrated that molecular imaging methods as *in vivo* monitoring modalities can potentially depict the manner of cell migration and journey in the body.[72] *In vivo* cell imaging and tracking can provide special purposes, for instance, cell engraftment, migration, and survival.[3,58] It has also been performed based on extensive longitudinal and histopathological studies, which are conducted by sacrificing animal subjects in different intervals after transplantation, whereas molecular imaging modalities can track transplanted (stem) cells in real time.[58,64,87] Various studies have revealed that tracking transplanted (stem) cells in SCI and stroke is perfectly feasible. Such modalities can be linked to stem cell transplantation methods for facilitating translation of stem cell transplantation from the basic research into the clinic.[58,88] As it has been described previously, MRI cell imaging has several advantages over other techniques such as PET, SPECT, CT, and ultrasound that make it more compatible to clinical grade cell monitoring purpose. On the other hand, MRI has an established role in different preclinical and clinical studies on stroke.[6] Therefore, it is becoming a fundamental part of clinical cell transplantation trials to periodically monitor the distribution of transplanted (stem) cells[25,89] in various diseases including, ischemic brain disorders.[68] For instance, successful cell tracking in 19 human trials, have been reported by McColgan *et al*., which revealed increasing progress in imaging techniques as a crucial part of various medical disciplines.[47] Stem cell imaging can also guide cell delivery, optimize transplantation protocols, and subsequently increase desirable therapeutic effects of (stem) cell therapy trials. Ideally, various imaging technologies should be combined to make it a noninvasive, safe and highly efficient multimodal manner which will be able to perform as a qualitative and also quantitative technique[48,53] to play its cardinal role in the field of advanced stem cell therapy[54] and transfer stem cell-based therapies from the bench to the bedside.[71] Thus, application of these methods to neurological diseases can increase (stem) cell therapeutic

Table 2: MRI contrast agents for cell imaging

MRI contrast gents	SPIO & MPIO	Gadolinium	Reporter gene
Labeling method	Direct *Non specific* (magnetofection and magnetoelectroporation) Indirect *Specific* (receptor mediated)	Direct *Non specific* (incubation with contrasts and transfection) Indirect *Specific* (receptor mediated)	Gene transfection (viral vectors, electroporation)
Relative sensitivity	High and extremely high (Single cell)	Low (> 10^5 cells)	Extremely low (Tissue)
Advantages	High sensitivity Minimal biological effects SPIOs are FDA approved	Positive contrast detection Less ambiguous qualification	No dilution with cell division No exogenous contrast required
Disadvantages	Negative contrast detection Endogenous sources of negative contrast confound quantification Dilution with cell division MPIOs are not FDA approved	Low sensitivity Probable toxicity Dilution with cell division	Complicated labeling procedure Extremely low sensitivity

effects and improve the patient's outcome.[81] Furthermore, more clinical grade studies are needed to overcome some limitations of existing MR cell imaging methods.[79,90] For instance, recently, transgenic cell lines with inbuilt contrast agents are proposed for transplantation.[8,55,91] Also MR reporter genes were introduced for reporting the survival of implanted (stem) cells and overcoming agent dilution following cell division that were two major limitations of the present MR imaging techniques by using routine contrast agents.[56,57] In spite of performing such advanced instances of researches, for contributing novel cell imaging techniques,[3] further investigations are needed to elucidate different points of view towards clinical stem cell imaging in the human body.[66] This review suggests that equipping various (stem) cell therapy modalities with noninvasive MR imaging techniques particularly, in neurological disorders such as stroke, will strongly improve cellular therapy protocols, subsequent therapeutic effects, and patients' outcome.

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