ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

A review on fundamental principles of cellular and molecular imaging in practice with magnetic resonance imaging using magnetic nano particles; Stem cells tracking oriented

Daryoush Fatehi[1], Hossien Masoumi[2], Ayoob Rostamzadeh[3]*

[1]Department of Medical Physics, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.

[2]Department of Radiology, Faculty of Paramedicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.

[3*]Department of Anatomy and Neuroscience, Shahrekord University of Medical Sciences, Shahrekord, Iran.

***Corresponding author: E-Mail: ayoobrostamzade@gmail.com, Tel: +989187225635, Fax: +983813334911**

Abstract

Nowadays, application of stem cells in treatment is increased incredibly. The stem cells are capable to self-reproduce. Molecular imaging is a novel procedure that is capable to evaluate cell function on molecular level directly with no interference and disorder for the cells. In other word, molecular imaging is able to trace specific molecules on the individual anatomical locations in the body. Feasibility of MRI cell tracking with SPION based agents as labels has been well demonstrated in animal experiments. Clinical trials of MRI cell tracking are also ongoing. Different to animal studies, some general requirements for clinical cell tracking should be met for the labeling agents. The labeling agents must be shown to be non-toxic to cells in culture and to animals. The labeled cells should be more widely categorized to control any effects of the labeling procedure on cell functionality.

KEY WORDS: molecular imaging, cellular imaging, magnetic resonance imaging, magnetic nano particles, Stem cells tracking.

1. INTRODUCTION

Nowadays, application of stem cells in treatment is increased incredibly. The stem cells are capable to self reproduce (Mohammadi, 2015; Rostamzadeh, 2014; Belegu, 2007). They also are able to differentiate to new cells. Scientists have used many of different stem cells to treat different diseases such as ischemic, degenerative, immune, and genetic diseases. Stem cells are applied for repairing of damaged tissues from heart, cartilage, bone, and so on. However, graft of the stem cells introduces the following questions (Pluripotent).

- Whether local injection of stem cells causes tissue carcinogenesis of the region?
- Where the stem cells go after intra venous injection?
- Do the grafted stem cells that reached to their target immigrate?
- What are mechanisms between the reached stem cells to the target and microenvironment around them?
- Are the grafted stem cells that reached to their target able to differentiate target tissue?
- Are the grafted stem cells that reached to their target coordinate with target tissue? For instance, do the grafted stem cells to an ischemic brain differentiate to neurons or they differentiate to neuroglia?
- How much stem cells should reach to the target?
- How many of the stem cells that are in the target are alive? What is their half-life?

Based upon the above questions, one can conclude that in the graft of stem cells the main issue is deducing of molecular mechanism and behavior of the cells in the tissue or organ that is under treatment (Rostamzadeh, 2014; Saberi, 2016). We believe that stem cell tracking in the live patient (or animal) is important, without biopsy or killing the animal. Therefore, scientists improved methods for tracking and imaging of the stem cells. In the present article we introduce the main molecular imaging procedures for stem cell tracking (Smith, 2010). Additionally, we will explain tracking methods for intra-cell media and molecules. Nowadays, molecular imaging and nano particles evolutionary improved in medical diagnostic imaging. We know that these novel procedures do not replace the conventional medical imaging, but they have increased accuracy of them. Nano-biotechnology is defined as research and improvement of technology on molecular and atomic level (1-100 nm) in order to deducing phenomena and materials in a nano-metric level of structures and systems of the human (or animal) body (Renn and Roco, 2006). Nano is a basic technology which came after micro technology. There are coming reasons which show materials have very different properties when they have nanometer size, comparing with the same in a normal size. Nano technology (nTech) lets to produce materials that are thinner and tighter, while they have grate magnetic properties, more thermal conductivity, better light emission control, and lesser destruction (Gerardin and Paccagnella, 2010). Thus, nTech has prepared an atmosphere, in which scientists have access to new methods and materials that capable they improve their specific science. In the near future nTech will have a major role in imaging procedures, producing biosensors, bio-recognition, self-equipment implants, and drug delivery (Leeuw, 2007). Molecular imaging is a novel procedure that is capable to evaluate cell function on molecular level directly with no interference and disorder for

the cells. In other word, molecular imaging is able to trace specific molecules on the individual anatomical locations in the body. Historically radioactive tracking was developed by George Hevesy for studying chemical processes in the metabolism of animals. His experiments are a base for nuclear medicine and after that molecular imaging. Noninvasive tracking for cell moving inside the body is necessary to develop therapeutic strategies (Kang, 2012). Since, tracking of the treating cells is accompanied with histological analysis and needs animal scarifying or biopsy; therefore, scientists improved noninvasive tracking of the grafted stem cells with labeling applying nTech. Up to now, many procedures are improved for stem cell tracking, e.g. Micro computed tomography (micro-CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), Optical imaging (OI); however, in this review we focus on MRI (Janib, 2010).

One of the new emerging applications of magnetic cell labeling concerns magnetic resonance cell tracking. One of the new coils, such as the cryogenic probe, allows sub-milimetric resolution and gives the means to perform cellular MRI in vivo. The advantage of the cryogenic probe to improve the signal-to-noise (SNR) ratio and concomitantly improve the image resolution has been demonstrated throughout the last decade in several studies. MRI and spectroscopy of human subjects in vivo have enjoyed over 30 years of steady progress (Gore, 2011; Salehi, 2016). Today, MRI is well recognized as the single most valuable imaging modality accessible in radiological practice, especially for the detection and characterization of soft tissue pathologies such as solid tumors in cancer (Wu, 2006; Srinivas, 2010). MRI is proficient of high resolution imaging with outstanding inherent soft tissue contrast, and is well-suited for longitudinal, noninvasive imaging because it does not rely on probes with short half-lives. Moreover, the applications of imaging know-hows have also changed from conventional radiological diagnosis to addressing other types of problem in biomedical research and patient management (Mohammadi, 2015).

Anatomic images have always been the center of gravity in the daily work of radiologists. They provide the basis of many diagnoses supplemented by physiologic MRI data or metabolic profiling if necessary (De Backer, 2010). Despite the sophistication of these techniques and the wealth of information that can be obtained, the diagnostic information often remains nonspecific, and evidence regarding the nature of the underlying disease commonly remains circumstantial. In contrast to generic contrast agents used in the clinic, the molecular imaging (MI) field uses reporter molecules tailored for in vivo detection of specific molecular or cellular events (De Backer, 2010). Formally, MI encompasses techniques that directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications (De Backer, 2010). Following an early diagnosis, MI could closely monitor the effectiveness of therapeutic interventions. Imaging is progressively used to assess function rather than just anatomy (e.g, for studies of the brain) or to characterize individual phenotypes for targeted drug therapies. Much greater use is made of quantitative measurements from images rather than subjective judgments, and preclinical imaging of animal models of disease has increased dramatically with the development of particular equipment (Gore, 2011). The Society of Nuclear Medicine has defined molecular imaging as follows: Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems. Molecular imaging agents are probes used to visualize, characterize and measure biological processes in living systems. Both endogenous molecules and exogenous probes can be molecular imaging agents (Sekhon, 2008).

How to monitor the migration and homing of transplanted cells as well as their engraftment efficiency and functional capability remains a critical issue to be solved in the field of cellular therapy. Because MRI offers a good depth penetration and high spatial resolution, and exhibits a superior ability to extract molecular and anatomic information concurrently, it has been vigorously examined in the previous years and so far the first choice for tracking implanted cells. Fundamentally, MRI cell tracking includes three components: labeling agents, labeling of cells of interest, and MRI tracking (Wang and Shan, 2012).

The labeling agents are synthesized with procedures similar to those developed for organ imaging, with more attention in their cellular internalization, intracellular retention, and cytotoxicity, ex vivo labeling by incorporation of a contrast agent into a population of purified cells *in vitro* (Bulte and Kraitchman, 2004). With ex vivo labeling, excess contrast agents and dead cells can be detached purely; the labeled cells can be carefully characterized before transplantation; and non-specific labeling of irrelevant cells can be well controlled by purifying the relevant cell population before labeling (Wang and Shan, 2012). MRI cell tracking studies in animals first started in the early 1990s; however, the first study in humans was performed delayed to 2005. A variety of conventional MRI techniques is used to determine the presence of iron in various tissues over a wide range of magnetic field strengths. In general, T2- and T2*-weighted imaging techniques employing spin echo orgradient echo pulse sequences are available on all clinical and experimental MR scanners, and are commonly used to detect the presence of iron in the brain in the form of ferritin, liver, heart and other organs as a result of hemorrhage or iron storage diseases (i.e, hemosiderosis). Using these sequences, the signal intensity of these tissues containing the various forms of iron is decreased or becomes hypo intense compared with healthy control tissues. The extent of the decrease in signal intensity or image contrast

in areas containing iron-labeled cells, for example, will depend on the iron concentration, T2 and T2* relaxation times of the surrounding microenvironment, the MRI magnetic field strength along with the imaging sequence used (Arbab, 2006).Cells labeled with Gd-based agents can be detected on T1- or T2*-weighted images depending on the concentration of the paramagnetic agent in the cell. T1-weighted images can be used to detect USPIO nano particles following an intravenous injection of the agent and uptake into the extracellular space or by resident macrophages. Cells labeled with USPIO contrast agents are usually detected by T2- and T2*-weighted imaging owing to the intracellular compartmentalization in endosomes. It may be problematic to differentiate between signal loss caused by the SPIONs in the cells and native low signal in tissue due to their inherent short T2 and T2* relaxation times (Garwood, 2013). To improve the sensitivity to track magnetically labeled stem cells or other cells, several positive contrast or white marker pulse sequences have been developed. The signal intensities on MRI surrounding the SPIONs have a characteristic barbell pattern and have been theoretically derived for a single dipole in a homogeneous magnetic field. The off-resonance-based positive contrast pulse sequences allow for visualization of high signal intensities against a suppressed background in regions containing SPION-labeled cells. These pulse sequences employ spectrally selective RF pulses to excite and refocus off-resonant water in regions near the labeled cells, thereby producing high signal intensities in contrast with the surrounding environment (Arbab, 2006). Positive-contrast or white-marker techniques are being developed on clinical MRI scanners in order to improve the conspicuity of the SPION-labeled cells in tissues; however, it is not clear if these approaches can differentiate the presence of magnetically labeled cells from hemorrhage or SPIONs in the extracellular space. Quantifying the number of labeled stem cells in target tissues is of great importance to optimize the dose and timing of cellular therapy. T2-weighted spin echo imaging has been used to quantify the amount of iron within tissue. By determining the T2 relaxation times at 0.5 and 1.5 Tesla using a multiple spin echo technique, some researchers demonstrated a linear relationship between the T2 relaxation rates and iron content obtained from marmosets with hemosiderosis – an iron storage disease. However, T2 is sensitive to both the concentration and distribution of SPIONs, thereby greatly complicates its use for quantifying iron oxide (IO) concentration. To achieve high sensitivity for the detection of intracellular SPIONs, the T2* relaxation rate of the tissue should be measured. Using a multi gradient echo pulse sequence, T2* relaxation times can be determined for the labeled cells in tissues and can be used to approximate the number of labeled cells within a voxel (Liu and Frank, 2009). Unfortunately, the T2* relaxation rate is not only influenced by SPIONs in labeled cells, but also by macroscopic susceptibilities that arise from air–tissue interfaces. These susceptibility artifacts lead to overestimated relaxation rates or obscure low concentrations or numbers of SPIO-labeled cells. Several other methods have been proposed to correct for the macroscopic magnetic susceptibility influence such as increasing the spatial resolution, altering the slice selection gradient, utilizing the tailored RF pulse, performing improved shimming of the magnetic field and correction of the main field in homogeneities to compensate for magnetic field susceptibilities from tissues that do not contain magnetically labeled cells (Dahnke and Schaeffter, 2005). It is possible that the use of ultra-short echo time pulse sequences samples the water protons magnetization at less than 100 ms prior to the dephasing of the water proton spins, due to the presence of directly implanted SPIO-labeled cells in tissues, and should allow for the ability to produce more accurate T2* maps for quantifying the number of iron containing cells in tissues. The development of new MRI scanner hardware and pulse sequences, along with analysis and segmentation software, may improve the sensitivity and delineate various iron-containing pathologies from magnetically labeled cells in tissues (Arbab, 2006).

How magnetic nano particles (MnP) transfer to a tissue?

Passive transition: In this fashion the following parameters of the MnP are involved: blood circulation, tissue accessibility, SPION decay and bio-distribution, cellular uptake. These all affected from hydrodynamic ammeter and surface potential of the SPION. Here we explain certain cases of the passive transition of magnetic probes in MRI. Different tracks for MnP uptake determine MRI contrast in different tissues. Small particles collect by reticoluendothelial system. In cancer cells due to absence of the reticoluendothelial system, relaxation time has no change however, that there are available MnP as a contrast media. Hence, detection of lymphatic nodules, liver and brain tumor, is possible if one access to MRI images obtained through MnP. SPION probes do not have capability for molecular imaging in biological environments. Therefore, they normally will guided under the physiological processes including non-individual cell uptake, capturing by phagocytes is macrophages of the tumor and inflamed tissues, and finally gathering in the spleen, liver, and lymphatic nodules (Levine, 1939).

Liver imaging: Researches reveals that MnP bigger than 150 nm covered by dextran could non individually uptake with liver Kupffer cells. Since in a cancerous liver there are no Kupffer cells, one can easily distinguish between normal and tumor liver cells (Anthony, 1973).

Lymphatic nodules imaging: Particles with 30 nm diameter could collect in the lymphatic nodules by lymphatic vessels. SPIONs collection in the lymphatic nodules causes reduction of T2 (Branca, 2010). Contrarily, absence of the SPIONs in the lymphatic nodules demonstrates disorders in the lymphatic current and or metastasis in the

lymphatic nodules. Thereby, with no application of a specific marker one can differentiate between normal and abnormal structures. Thus, a macrophage which is including MnP produces a black image while tumor appears white (Cole, 2011).

Solid tumors imaging: SPIONs can detect solid tumors directly. SPIONs can passively be available in the region of interest through leakage from vessels and or macrophage uptake. The leakage is depending to porosity of the tumoral vessels. In bone marrow research scientists use AMI-25 (a MnP which is applicable in MRI); where, the damages are diagnosed with a high sensitivity (Therasse, 2000).

Macrophage imaging: MRI in destructive and inflame diseases, with high macrophage activity, acts based on uptake particles by macrophages and other phagocyte cells. Therefore, MRI provides detection of graft rejection's place and atherosclerosis plaques. It also is capable to detect narrowing of the vessels before it appear. These all certainly have positive effect on the treatment. Macrophages play an effective role in imaging in order to find abnormalities such as stroke, MS, brain tumors, and carotid atherosclerosis. One should notice that image contrast is mainly depended to the time of blood circulation as well as surface electric potential. Experiments demonstrate that MnP with 15-30nm diameter and more circulation time provide a better contrast in the inflamed tissues (Corot, 2004).

Finally, studies reveal that MnP categorized by their diameter size and decreasing in the diameter of the MnP increases the image contrast and particle half time. Production of the MnPs in an environment without water and high temperature provides a better control for the producers on the size and crystallite of the MnP; while it reduce the solvent of the particles. To overcome the later, a surface coater is used for the MnP. There are certain coating materials for different applications. Hence, there are different types for the MnPs. MnP coating is an efficient factor in medical application; since it can reduce toxicity of the particle, and increase mechanism of the particle rejection, functionality of the surface of the particle, easy connection with the anti-bodies, and Physiochemical stability of the MnPs in the body. There are certain MnP for medical application including MION, CLIO, magnetoferritin, magneto dendrimer, magneto liposome. These MnP could passively transit to tissues and provide imaging of the certain tissues e.g. liver, spleen, lymphatic nodules, cancer, and macrophages (Corot, 2004).

Active transition: When a particle is small it could escape from phagocyte systems; thus, in order to purposive transition scientist applied MnP accompanying with the specific markers. Purposeful active transition is superior to the passive transition since it not only increases the image contrast; but also it is able to detect molecules. That means the active transition can provide physiological information as well as intuition from molecular mechanism. These lead to a faster and more accurate diagnosis. Up to now imaging procedures applying active MnP transition are used for imaging of inflamed tissues, infarction, angiogenesis, apoptosis, gene status, and cancer. In order to access purposes of the active transition of SPION, one need to a special biomarker for each individual tissue. In the first step the agent is connected to a hydrophilic coated surface. Polymeric coats have active factor groups such as amino, sulfhydryl, and carboxyl; that accelerate process of conjugation. The next step is enough collection of the MnP in the tissue. Perhaps the most important impediment for active transition is placing a suitable quantity of SPIONs in the damaged location to access sufficient contrast. Thus, certain techniques are available in order to increasing MnP collection in an individual location. In this regard intracellular capturing technique has more application in which receptors are used and it creates a high contrast image for the damaged cell. Other procedures are including two-step enhancement, such as using biotin antibody that collect in the area of the disease as well as SPION connected to stereptovidin. Since avidin and biotin have high intention, they collect MnP all together in the region of interest. Furthermore, MRI probes, that sometimes are accompanied with fluorescent dye; conjugate with the targeted cancer antibody for in-vitro and in-vivo studies (Chevion, 1988).

Cardiovascular imaging: Molecular imaging provides conditions in which we could diagnose cardiovascular diseases, atherosclerosis, thrombosis, and myocardial deficiencies at their early stages. Early diagnosis of atherosclerosis is possible when one apply targeted SPION with anti-VCAM-1 and its specific peptide. E-selection, which is a pre-inflamed marker for endothelial cells, could apply for early diagnosis of atherosclerosis and angiogenesis. Applying conjugated SPION with anti human E-selection MRI can detect quantity of this factor in the cell culture. Furthermore, thrombosis can be detected using αIIb-β3, which is released from activated platelets. The activated platelets are detected by applying conjugated SPION with RGD. Applying SPION along with RGD provides a better contrast in comparison with SPION alone. It could detect thrombus of 2mm diameter. Additionally, applying mono-crystal iron oxide nano particle (MION) combined with anti myosin could use for diagnosis of myocardial infarction. This combination performs based on electrostatic gravity or covalence between lysine antibodies and superficial hydroxyl activated by potassium periodate. The point is that a myocardial infarction cell has a more porosity membrane in comparison with a normal cell. Using conjugated SPION with anti myosin Fab could entered through the cell membrane more effectively in the damaged cells and detect myosin. These infracted cells are black color in the image; while cells that have no marker create a low image contrast (Taylor, 2010).

Angiogenesis imaging: Angiogenesis is a necessary process for growth of the new blood vessel as well as tissue development and tissue repair. Angiogenesis is also a clear symbol for tumor growth. Thus, evaluation of angiogenesis development is obviously an efficient parameter in diagnosis of tumor and during its treatment. In angiogenesis process there are a few molecules which have important roles. These are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), PD-EDGF, Tie2 receptor, integrin, E-selection, VEGF, Tie2, and E-selection were more investigated (Folkman and Beckner, 2000).

Apoptosis imaging: Targeting SPION is an issue in apoptosis imaging. Apoptosis is a controlled cell death process which is seen in cancer pathogenicity, destruction of the neural tissues, acute and chronic myocardial infarction. Apoptosis also applies for illustration of drugs' efficiency. The first step for detecting apoptosis is destruction of phosphatidylserine in the cell membrane. In order to detect this step one uses synaptotagmin and annexin. Applying SPIONs connected to C2 region of synaptotagmin could detect apoptotic cells. Resolution of this type of imaging is 0.1 nm which is three times greater than other conventional procedures. The other method for apoptosis imaging is use of phosphatidylserine and Annexin V. Applying conjugated MnP with concentration of 0.1 µg Annexin V can detect the apoptotic cells in in-vitro (Belhocine, 2002).

Gene status imaging: Gene status is one of the branches of biological sciences. There are certain methods for gene status; however, they have certain limitations such as low penetration depth in optical imaging and small resolution in imaging using radioisotopes (Scintigraphy). Gene status imaging evaluates transferrin receptor in tumor cells. Therefore, in this procedure one applies MIONs coated with transferrin. If there is a Transferrin receptor in the region, it connects with the MIONs. In MRI when gene status is more, reduction in T2 gradient will be more (Attard, 2009).

Cancer imaging: Noninvasive diagnoses of cancer are very important issue not only for patient but also for physician. While conventional MRI could detect masses with a diameter of 1 cm; applying MnP in MRI will increased the power of resolution to a molecular scale. It also can evaluate molecular reactions and detects cancerous masses with a very small size. Many of cancer markers are known as targets for SPION direct ligands. Markers that are selected for targeting transition not only must have a higher level of grade in cancer cells, but also they should provide possibility for more collection in the cell through endocytosis via receptor. Transferrin receptor is one of the markers which have high grade for many cancerous cells specially breast cancer cells. This marker provides a high grade and adjustment of the receptor for SPIONs connected to transferrin receptors in in-vitro as well as in-vivo conditions. Moreover, coating SPION with folic acid (Folate) provides faster and more effective uptake by cancerous cells that have more folic acid. They also illustrate 38% decreasing of signals in T2 intensity. Another marker that could be detected with SPIONs is MUC-1, a tumor anti-gene; which is a usual factor in many of epithelial cells adenocarcinoma. MUC-1 is available in tissue cancers such as breast, pancreas, lung, colorectal, prostate, and stomach; and could trace with peptide of EPPT1. MMP2 (matrix metalloproteinase 2) neutral endopeptidases connected to the membrane is another marker that use in cancer imaging and has a high grade in glioma. Particle targeting with specific receptors on the surface of the normal cells, which is not stated in the cancerous cells, is another procedure to detect cancer cells. For instance, CCK receptor is connected to the SPION and will detect ACCK receptors in surface of the normal pancreas cell. Therefore, one could find tumor region since there is a reduction in T2 of the normal cells. Liver carcinoma is mostly appears following a metastasis from breast, lung, rectum, and colon cancers. Researchers were followed to find a marker in order to use it as a contrast agent in MRI specifically for detection liver carcinoma. They found that asialoglycoprotein (ASG) which is available in normal hepatocytes, in cancer cells will change its usual form. Hence, they applied SPIONs connected to arabinogalactan (AG), which is a polysaccharides ligand, for targeting imaging. They believe these MR images demonstrate that applying AG-SPION will create a high quality contrast between the two normal and cancer tissues (Charles-Edwards, 2006).

Cell tracking and cell imaging: One of the applications of SPION is evaluation of cell distribution in in-vivo condition after graft and cell inject therapy following the diseases such as multiple sclerosis (MS), Parkinson, trauma, stroke, infertility, and so forth. Nowadays, SPIONs are vastly use for stem cells (e.g. embryonic stem cells (ESCs) and neural stem cells (NSCs)) tracking around the world. Today, researchers could identify and explained cell individually in its internal dimensions. Following procedures are used for mentioned propose:

- Replacing SPIONs accompanying of the cells in a live person and tracking them in order to find the exact location of the SPIONs.
- Combining SPIONs with T-cells.
- Particle entering via endocytosis related to cell receptor and evaluation of the cell immigration. For instance, they conjugated a transferrin anti receptor antibody to MnP, and then the MnP will entered to a cell via an endocytosis depended to receptor, which provides evaluation of movement of the oligodendrocytes cells.

- Application of changes in magnetic relaxation of SPIONs collection. This change creates a phenomenon called magnetic relaxation susceptibility (MRES), in which collection of SPIONs is effective in de-phasing of the spins of the region and terminates to increase T2 spin-spin relaxation time. Recently researchers apply this technique in order to detect bio-molecules. Thus having a high sensitivity imaging modality they are capable to diagnose oligonucleotide protein, enzyme and enantiomers. Notice that precision of this method is 500 atoms. MRES can detect magnetic changes in situations that cells are muddy and or lyses without need to protein purity. Moreover, MRES provides evaluation of SPIONs in the depth of the tissues; hence it is a nice option for in-vivo imaging (Goldman, 2005).

Impact of MnP on cell viability: Indeed, one of the significant aspects in cell labeling is also the assessment of cell functions after SPION internalization. Cell proliferation should be monitored over a period of at least 5 days [9]. The preservation of cell functions and differentiation capacities might differ among distinct cell types therefore should be determined specifically. The expression of specific genes of interest can also be quantified to assess subtle phenotypically alterations following SPIO labeling. To date different cell types have been labeled with SPIONs (immune cells, endothelial cells, cancer cells, primary culture or established cell lines and progenitors cells, to mention just a few) and detrimental effects on cell proliferation and cell functions at short and long terms, *in vitro* or in vivo, were not observed. The labeling of stem cells is trickier as these cells should conserve their self-renewal and property of lineage multi potency after internalization of SPIONs. Controversial effects were observed on the multi-lineage differentiation capacity of mesenchymal stem cells. The chondrogenesis (i.e. the capacity to differentiate in cells of cartilage) was partially inhibited in one study, but not in others, whereas adipogenesis and osteogenesis were not impaired. On the contrary, while labeling cells with citrate-coated SPION, we could modulate the amount and the physical state of nano particles interacting with cells and could conclude that only high dose of SPIONs or an aggregated state could have adverse effects on cell differentiation (chondrogenesis). Labeling conditions with perfectly stable SPION is thus recommended for use in cell therapy assays (Hoskins, 2012).

A global signal loss, which is less dependent on MRI parameters In a pioneering study using cellular MRI in vivo, MRI could be used to monitor the migration of lymphocytes injected intravenously to tumor bearing mice. Lymphocytes were targeted to tumor cells through immune recognition, where the MRI showed a complex cell migration pathway. This study was important from a methodological point of view, showing, for the first time, that single cells could be detected by MRI directly in vivo in a tumor. Together with numerous studies by dissimilar groups, we can realize that MRI offers a great potential for cell tracking, which is also progressively being integrated in clinical assays. Be that as it may, despite the fact that MRI might provide us with real-time insight in cell distribution in vivo, we should corroborate its results with other, even post-mortem methods of cell detection, such as histology (Hoskins, 2012).

Limitations of MRI cell tracking: There are several limitations for MRI cell tracking, especially when long-term tracking of the cells is necessary. These limitations are either technical or physiology-pathology-related. These limitations can be summarized into four major aspects, which should be considered in designing studies of MRI cell tracking (Bulte, 2009).

Live vs. dead cells: The signal intensity in MRI depends primarily on the local values of longitudinal and transverse relaxation rates of water protons. SPION agents are not detectable themselves, but are detected by their effects on surrounding water protons. When the transplanted cells of interest die, the SPION agents may remain in or around dead cells until the agents are cleared away.

False positivity: These host cells can be mistaken to be the transplanted cells. Tissues with high iron content such as bone marrow and hemorrhage can lead to misinterpretation of MRI signal.

Dilution of the labeling agents among daughter cells: This issue is more prominent for rapidly dividing cells and stem cells. Stem cells may divide asymmetrically, leading to an unequal distribution of the labeled agents among daughter cells. The unequal distribution not only leaves some cells having less contrast agents and undetectable quickly, but also makes the quantification of cell number less precisely.

Quantification of cell numbers: Although MRI visualizes cells in vivo, cell number quantification is challenging. Between the SPION signal and the absolute number of live cells (Bulte, 2009).

2. CONCLUSION

In conclusion, the feasibility of MRI cell tracking with IO based agents as labels has been well demonstrated in animal experiments. Clinical trials of MRI cell tracking are also ongoing. Different to animal studies, some general requirements for clinical cell tracking should be met for the labeling agents. The labeling agents must be shown to be non-toxic to cells in culture and to animals. The labeled cells should be more extensively characterized to determine any effects of the labeling procedure on cell functionality. Any design strategies for agent synthesis, cell labeling and in vivo MRI tracking would necessarily need to take the approval of regulatory agencies into consideration. Iron oxide nano particles can be used for magnetic labeling of different types of cells. The labeling of

living cells allows a variety of biomedical applications ranging from cell manipulation to diagnostics and regenerative medicine. This tutorial provides the basic requirements for efficient cell labeling with anionic (citrate coated) iron oxide nano particles and includes sections on troubleshooting to prevent the occurrence of potential cell damage during the labeling procedure. Magnetically labeling cells with existing MRI contrast agents cannot be used to interrogate the transplanted labeled cells' viability, function or ability to differentiate towards a desired lineage. Multimodal imaging approaches combining MRI with nuclear medicine approaches (e.g, positron emission tomography or single photon emission tomography) or optical and bioluminescent imaging in experimental models may be useful to determine the functional status of the repaired tissue, although the resolution of the images will not be comparable with that of MRI. Cellular MRI could be used to determine the migration and homing of administered cells at the site of interest for the first few days to weeks and then nuclear medicine or other imaging modalities could be used to determine the functional improvement of the tissues or organs. Finally, we also evaluate the potential of cell manipulation that can be exploited both *in vitro* for tissue engineering and in vivo in cell therapies. Moreover, the feasibility of MRI cell tracking with SPION based agents as labels has been well demonstrated in animal experiments. Clinical trials of MRI cell tracking are also ongoing. Different to animal studies, some general requirements for clinical cell tracking should be met for the labeling agents. The labeling agents must be shown to be non-toxic to cells in culture and to animals. The labeled cells should be more extensively characterized to determine any effects of the labeling procedure on cell functionality.

REFERENCES

Anthony P, Vogel C & Barker L, Liver cell dysplasia, a premalignant condition, Journal of clinical pathology, 26, 1973, 217-223.

Arbab A.S, Liu W & Frank J.A, Cellular magnetic resonance imaging, current status and future prospects, Expert review of medical devices, 3, 2006, 427-439.

Attard G, Swennenhuis J.F, Olmos D, Reid A.H, VickersE, A'hern R, Levink R, Coumans F, Moreira J & Riisnaes R, Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer, Cancer Research, 69, 2009, 2912-2918.

Belegu V, Oudega M, Gary D.S & Mcdonald J.W, Restoring function after spinal cord injury, promoting spontaneous regeneration with stem cells and activity-based therapies, Neurosurgery clinics of North America, 18, 2007, 143-168.

Belhocine T, Steinmetz N, Hustinx R, Bartsch P, Jerusalem G, Seidel L, Rigo P & Green A, Increased uptake of the apoptosis-imaging agent 99mTc recombinant human annexin V in human tumors after one course of chemotherapy as a predictor of tumor response and patient prognosis, Clinical Cancer Research, 8, 2002, 2766-2774.

Branca RT, Cleveland ZI, Fubara B, Kumar CS, Maronpot RR, Leuschner C, Warren WS & Driehuys B, Molecular MRI for sensitive and specific detection of lung metastases, Proceedings of the National Academy of Sciences, 107, 2010, 3693-3697.

Bulte JW & Kraitchman D.L, Iron oxide MR contrast agents for molecular and cellular imaging, NMR in Biomedicine, 17, 2004, 484-499.

Bulte JW, *In vivo* MRI cell tracking, clinical studies, AJR, American journal of roentgenology, 193, 2009, 314.

Charles-Edwards EM, Diffusion-weighted magnetic resonance imaging and its application to cancer, Cancer imaging, 6, 2006, 135.

Chevion M, A site-specific mechanism for free radical induced biological damage, the essential role of redox-active transition metals, Free Radical Biology and Medicine, 5, 1988, 27-37.

Cole AJ, David AE, Wang J, Galban CJ, Hill HL & Yang VC, Polyethylene glycol modified, cross-linked starch-coated iron oxide nano particles for enhanced magnetic tumor targeting, Biomaterials, 32, 2011, 2183-2193.

Corot C, Petry K.G, Trivedi R, Saleh A, Jonkmanns C, Le Bas J.F, Blezer E, Rausch M, Brochet B, & Foster-Gareau P, Macrophage imaging in central nervous system and in carotid atherosclerotic plaque using ultrasmall superparamagnetic iron oxide in magnetic resonance imaging, Investigative radiology, 39, 2004, 619-625.

Dahnke H, & Schaeffter T, Limits of detection of SPIO at 3,0 T using T2* relaxometry, Magnetic resonance in medicine, 53, 2005, 1202-1206.

De Backer M, Nabuurs R, Van Buchem M & Van Der Weerd L, Mr− Based Molecular Imaging of the Brain, The Next Frontier, American Journal of Neuroradiology, 31, 2010, 1577-1583.

Fatehi D, Naleini F, Salehi M.G, Afshari D, Mirfendereski S & Farzizadeh M, Traumatic Spinal Cord Injury, Theranostic Applications of Advanced MRI Techniques, Biomedical and Pharmacology Journal, 8, 2015, 891-903.

Folkman J & Beckner K, Angiogenesis imaging, Academic radiology, 7, 2000, 783-785.

Garwood M, MRI of fast-relaxing spins, Journal of Magnetic Resonance, 229, 2013, 49-54.

Gerardin S & Paccagnella A, Present and future non-volatile memories for space, Nuclear Science, IEEE Transactions on, 57, 2010, 3016-3039.

Goldman RD, Spector DL & Swedlund AC, Live cell imaging, a laboratory manual, Cold Spring Harbor Laboratory Press Cold Spring Harbor NY, 2005.

Gore JC, Manning HC, Quarles CC, Waddell KW & Yankeelov TE, Magnetic resonance in the era of molecular imaging of cancer, Magnetic resonance imaging, 29, 2011, 587-600.

Hoskins C, Wang L, Cheng W.P & Cuschieri A, Dilemmas in the reliable estimation of the *in-vitro* cell viability in magnetic nano particle engineering, which tests and what protocols? Nanoscale research letters, 7, 2012, 1-12.

Janib SM, Moses AS & Mackay J.A, Imaging and drug delivery using theranostic nano particles, Advanced drug delivery reviews, 62, 2010, 1052-1063.

Kang SK, Shin IS, Ko M.S, Jo J.Y & Ra J.C, Journey of mesenchymal stem cells for homing, strategies to enhance efficacy and safety of stem cell therapy, Stem cells international, 2012.

Leeuw TK, Reith R.M, Simonette R.A, Harden M.E, Cherukuri P, Tsyboulski D.A, Beckingham K.M & Weisman R.B, Single-walled carbon nanotubes in the intact organism, near-IR imaging and biocompatibility studies in Drosophila, Nano Letters, 7, 2007, 2650-2654.

Levine M, The cytology of the tumor cell in the Rous chicken sarcoma, The American Journal of Cancer, 36, 1939, 276-302.

Liu W & Frank JA, Detection and quantification of magnetically labeled cells by cellular MRI, European journal of radiology, 70, 2009, 258-264.

Mohammadi R, Faeghi F, Rostamzadeh A & Shokouhi J, Applications of diffusion weighted imaging in detection of radiating pain to lower extremities, Journal of Basic Research in Medical Sciences, 2, 2015, 1-9.

Pluripotent U.H, A Review of the National Institute of Health's Guidelines for Research Using Human Pluripotent Stem Cells, Renn O & Roco M.C, Nanotechnology and the need for risk governance, Journal of Nano particle Research, 8, 2006, 153-191.

Rostamzadeh A, Rezaie M.J, Ahadi R, Farzizadeh M, Rahmani Tanha R & Shabani A, Tracking Bone Marrow Mesenchymal Stem Cells Transplanted to Experimental Rabbit Model with Brain Trauma by MRI, International Journal of Pediatrics, 2, 2014, 34-34.

Rostamzadeh A, Shabani A, Ahadi R, Farzizadeh M, Gharib A & Miraki S, Noninvasive Stem Cell Labeling Using USPIO Technique and their Detection with MRI, International Journal of Pediatrics, 2, 2014.

Saberi M, Grading of Glioma Tumors by Analysis of Minimum Apparent Diffusion Coefficient and Maximum Relative Cerebral Blood Volume, Caspian. J. Neurol. Sci, 2, 2016, 42.

Salehi M & Al E, Role of diffusion tensor imaging as an imaging biomarker and theranostic tool in structural imaging of traumatic brain injury, Journal of Chemical and Pharmaceutical Sciences, 2016.

Sekhon BS, Chemical biology, past, present and future, Current Chemical Biology, 2, 2008, 278-311.

Smith MH, Stem Cell Definition, Classification and Research History, Srinivas M, Heerschap A, Ahrens E.T, Figdor C.G & De Vries I.J.M, 19 F MRI For quantitative in vivo cell tracking, Trends in biotechnology, 28, 2010, 363-370.

Taylor AJ, Cerqueira M, Hodgson J.M, Mark D, Min J, O'gara P & Rubin G.D, Accf/Scct/Acr/Aha/Ase/Asnc/Nasci/Scai/Scmr 2010 appropriate use criteria for cardiac computed tomography, a report of the American college of cardiology foundation appropriate use criteria task force, the society of cardiovascular computed tomography, the American college of radiology, the American heart association, the

American society of echocardiography, the American society of nuclear cardiology, the north American society for cardiovascular imaging, the society for cardiovascular angiography and interventions, and the society for cardiovascular magnetic resonance, Journal of the American College of Cardiology, 56, 2010, 1864-1894.

Therasse P, Arbuck S.G, Eisenhauer E.A, Wanders J, Kaplan R.S, Rubinstein L, Verweij J, Van Glabbeke M, Van Oosterom A.T & Christian M.C, New guidelines to evaluate the response to treatment in solid tumors, Journal of the National Cancer Institute, 92, 2000, 205-216.

Wang P.C & Shan L, Essential elements to consider for MRI cell tracking studies with iron oxide-based labeling agents, Journal of basic and clinical medicine, 1(1), 2012.

Wu DH, Archer AG, Hast LJ, Elledge B, Beall D, Walter M, Edwards S.M, Webb H.R, Greer B.J & Rubesova, E, Fundamentals of achieving functional and anatomical imaging with breast MRI, Physical and clinical concepts, Ed, Suri, JS and Rangayyan, RM, Recent Advances in Breast Imaging, mammography, and Computer-Aided Diagnosis of Breast Cancer, Bellingham, Wash, 2006, 51-109.