SPECIAL ARTICLE

Advances in using MRI probes and sensors for in vivo cell tracking as applied to regenerative medicine

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ABSTRACT

The field of molecular and cellular imaging allows molecules and cells to be visualized in vivo non-invasively. It has uses not only as a research tool but in clinical settings as well, for example in monitoring cell-based regenerative therapies, in which cells are transplanted to replace degenerating or damaged tissues, or to restore a physiological function. The success of such cell-based therapies depends on several critical issues, including the route and accuracy of cell transplantation, the fate of cells after transplantation, and the interaction of engrafted cells with the host microenvironment. To assess these issues, it is necessary to monitor transplanted cells non-invasively in real-time. Magnetic resonance imaging (MRI) is a tool uniquely suited to this task, given its ability to image deep inside tissue with high temporal resolution and sensitivity. Extraordinary efforts have recently been made to improve cellular MRI as applied to regenerative medicine, by developing more advanced contrast agents for use as probes and sensors. These advances enable the non-invasive monitoring of cell fate and, more recently, that of the different cellular functions of living cells, such as their enzymatic activity and gene expression, as well as their time point of cell death. We present here a review of recent advancements in the development of these probes and sensors, and of their functioning, applications and limitations.

KEY WORDS: Regenerative medicine, Stem cells, Magnetic resonance imaging, Paramagnetic contrast agents, CEST, Perfluorocarbon particles, Biosensor, Cell labeling, Cellular function

Introduction

Magnetic resonance imaging (MRI; see Box 1 for a brief history on its development) is a non-invasive imaging technique that allows the visualization of the internal structures of the body in health and disease; it has thus been used as a diagnostic tool, with a wide range of medical applications, for more than 30 years. The principle of MRI is based on manipulating the magnetic properties of the protons and neutrons contained in atomic nuclei present in the body (most commonly, those found in the atoms of hydrogen). The motion of these nuclei produces a small magnetic moment (see Box 2 for a glossary of terms). When a body is placed in the magnetic field of the MRI scanner, the magnetic moment of these nuclei aligns with the direction of the magnetic field. A radiofrequency (RF) pulse is then applied to the body in the scanner, which excites the nuclei such that there are transitions between lower and higher energy spin states. Once the RF pulse is given, the nuclei return to their equilibrium state (a process called relaxation), releasing their absorbed extra energy and emitting an RF signal. This signal is detected by the scanner’s RF coils and is then used to generate a detailed image of the body’s tissues. By using MRI contrast agents (see the following sections, Boxes 2 and 3, and Table 1 for more details), the contrast of this image, and so the visibility of specific body structures, can be improved. Subsequent advancements in this field made it possible to use these contrast agents to label specific cell types and thus to monitor cells at the molecular level.

The application of MRI to cellular imaging in vivo has proved particularly useful in the field of regenerative medicine research, where it allows the tracking of engrafted cells and the monitoring of their physiological responses in a non-invasive manner. Over the past two decades, stem cells have been increasingly used as potential therapies for different disease conditions, particularly those in which cell replacement can restore the normal function of tissue or organs subsequent to their damage or degeneration. For example, as reported in the NIH public clinical trials database (http://www.clinicaltrials.gov; accessed 26 January, 2015; only open studies included, unknown status excluded), 1502 clinical trials at different phases are currently using stem-cell-based therapies to treat various disease conditions, e.g. myocardial infarct, neurodegenerative diseases and autoimmune diseases.

Based on the increasing numbers of cell-replacement therapies, it has become imperative to monitor non-invasively the engraftment of cells in vivo to determine the overall safety and efficacy of these approaches. For example, two FDA-approved cord blood products, ‘Hemacord’ (manufactured by New York Blood Center, Inc.; www.fda.gov; Submission Tracking Number: BL 125397/0) and ‘HPC-Cord Blood’ (manufactured by Clinimmune Labs, University of Colorado Cord Blood Bank; www.fda.gov; Submission Tracking Number: BL 125391/0) are being used for hematopoietic stem cell replacement therapies. Both cell therapies are systemically delivered, non-specific, and rely on the engraftment of an extremely large number of cells (recommended minimum dose: \(2.5\times10^7\) nucleated cells/kg body weight), with the assumption that enough cells will find their way to the target sites. Only non-invasive imaging renders it possible to evaluate the homing of such cells in vivo, thereby confirming the efficacy of such a therapeutic approach.
In certain conditions, such as in neurodegenerative disorders and in type 1 diabetes, poor engrafted cell survival, due to a hostile host tissue microenvironment or to inadequate nutrient support (Robberecht and Philips, 2013; Thomas et al., 2013), is a major barrier to the success of stem-cell-based therapies. Thus, the capacity to determine, in real time, the physiological state of engrafted cells, in terms of their gene expression and enzymatic activity, and to monitor their cell survival, will enable clinicians to better assess the therapeutic outcome of such treatments. This might, in turn, lead to the development of more-effective therapies.

MRI is an ideal tool for the in vivo tracking and ‘sensing’ of engrafted cells because of its ability to image deep inside tissue and to gather accurate anatomical and physiological information with high temporal resolution and sensitivity (Srivastava and Butle, 2014). MRI could also be used to monitor alterations in cell function, tissue damage and changes in the dynamics of the biological processes that are associated with certain diseases (Haris et al., 2014; Yoo and Pagel, 2006). This use of MRI for non-invasive cell tracking first emerged from the use of MRI to label immune cells (Bulte et al., 1992; Bulte et al., 1993), and was followed by the first clinical application of MRI cell tracking to label and follow the fate of anti-tumor dendritic cells, used as cancer vaccines (de Vries et al., 2005).

In recent years, great progress has been made in the development of novel MRI sensors to monitor the different cellular functions of engrafted cells. In this Special Article, we describe recent advances in the development of MRI probes and sensors that are used for cell tracking and for detecting cellular functions in vivo. We also discuss the limitations of these approaches, their associated safety issues and the opportunities for their further improvement.

**Following the cell: MR contrast agents**

Contrast agents (see Table 1) are exogenous compounds that are administered intravenously or orally prior to MRI in order to improve the visibility of body structures for clinical diagnostic purposes. These agents change the relaxation rate of protons in water, creating a change in signal on MRI (see Box 3). However, contrast agents can also be used to pre-label cells ex vivo before transplantation, which is the most commonly used approach in MRI-based cell tracking.

There are different ways to incorporate contrast agents into living cells, such as by, for example, the use of transfection agents (Frank et al., 2002) and the use of translocation peptides. In this section, we discuss the main types of magnetic resonance (MR) contrast agents, how they function and their applications in clinical settings, as well as in experimental cell-tracking and regenerative approaches.
MRI. Gadolinium (III) (Gd\(^{3+}\)) chelates (see Box 2) are the most effective contrast agents, owing to their seven unpaired electrons. The unpaired electrons of Gd\(^{3+}\) create a magnetic moment that increases the T1 of the surrounding water proton spins, creating a ‘positive’ contrast on a T1-weighted scan (see Box 3). As a research tool, Gd\(^{3+}\) has been used to label and track different types of stem cells, such as hematopoietic progenitor cells, monocyctic cells, endothelial progenitor cells and mesenchymal stem cells in cell transplantation studies in small animals (Agudelo et al., 2012; Guenoun et al., 2012; Hedlund et al., 2011). Because they are not nanoparticles, the cellular uptake of Gd\(^{3+}\) chelates occurs by pinocytosis (a non-specific form of endocytosis in which small particles present in the extracellular fluid are internalized into cells) or via electroporation (see Box 2). However, overall, the low sensitivity of these contrast agents and their low uptake by cells is one of the main barriers to cell labeling with Gd\(^{3+}\). Different methods, e.g. transfection using transfection agents (lipofectin and lipofectamine) (Rudelius et al., 2003) or coupling of the contrast agent to a membrane-translocation peptide (13-mer HIV-tat peptide) (Bhorade et al., 2000), can be used to increase the uptake of paramagnetic chelates. In the last few years, several advanced paramagnetic contrast agents with improved uptake efficiency, e.g. Gd hexanedione, liposomal-based Gd nanoparticles, and gadofluorine (Nejadnik et al., 2012b), have been developed and successfully used for cellular imaging (Ghaghada et al., 2009; Tseng et al., 2010).

However, the major limitation to the use of Gd\(^{3+}\) contrast agents is the long-term presence of Gd\(^{3+}\) in cells, which might lead to toxicity, either to the cells themselves or to the surrounding host tissue. This has been reported in the case of Gd\(^{3+}\) chelates that are not rapidly cleared in patients with impaired kidney function, causing severe fibrosis and even death (Thomsen, 2006). In addition, in stroke models, as for example in a rat model of middle cerebral artery occlusion (MCAO), transplantation of neural stem cells labeled with gadolinium-rhodamine dextran (GRID) did not significantly improve the therapeutic outcome. To the contrary, T2-weighted MRI of MCAO rats monitored for 1 year revealed that the presence of GRID-labeled cells slightly increased the infarct size in the treated rats when compared with MCAO rats that were not treated with cells, whereas the lesion size was decreased by 35% in animals that were treated with cells that did not have gadolinium labeling (Modo et al., 2009). The exact mechanism of gadolinium toxicity is not known, but dechelated (free) gadolinium is believed to be taken up in the bone marrow, where it might interfere with metal ion hemostasis of bone marrow stem cells.

A different class of paramagnetic contrast agents is represented by the so-called PARACEST (paramagnetic chemical exchange saturation transfer) agents (see Boxes 2, 3; Table 1) (Zhang et al., 2001). Because different metals induce different chemical shifts (see Box 2 for a glossary of terms) in resonance frequencies, PARACEST MRI can be used to track two different types of cell populations simultaneously by labeling them with two different PARACEST agents (Aime et al., 2005). As further proof-of-principle, the two PARACEST agents Yb- and Eu-HPPO3A have been successfully used in vivo to track two cell populations (macrophages and melanoma cells) (Ferrauto et al., 2013). One of the limitations of PARACEST agents, however, is their intrinsically low contrast sensitivity; to detect sufficient contrast typically requires concentrations ranging from 1 to 10 mM. At such concentrations, these paramagnetic metal agents might become toxic when present in the body for prolonged periods, as in the case for Gd\(^{3+}\) described above.

### Paramagnetic manganese agents

In addition to Gd\(^{3+}\), manganese (Mn II) is another potentially useful positive contrast agent for T1-weighted MRI (see Box 3 and Table 1). The kinetics and behavior of Mn\(^{2+}\) ions in the cell mimic those of calcium (Ca\(^{2+}\)) ions, because Mn\(^{2+}\) ions enter cells through ligand- or voltage-gated Ca\(^{2+}\) ion channels (Narita et al., 1990). Therefore, Mn\(^{2+}\)-enhanced MRI (MEMRI) has been used to study neuronal activity and to visualize neuronal connectivity in different animal models (Silva and Bock, 2008). Few reports exist concerning the use of manganese-based contrast agents in cell tracking. One study used MnCl\(_2\) as a contrast agent for the labeling of lymphocytes as proof-of-principle (Aoki et al., 2006), but no in vivo studies using this approach have been performed. By contrast, manganese oxide (MnO) nanoparticles have been used as an MR contrast agent to

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**Table 1. Overview of exogeneous contrast agents for cell labeling**

<table>
<thead>
<tr>
<th>Contrast agent</th>
<th>Contrast</th>
<th>Sensitivity</th>
<th>Potential toxicity</th>
<th>Clinical use(^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gadolinium</td>
<td>Positive</td>
<td>Low</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Manganese</td>
<td>Positive</td>
<td>Low</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Manganese oxide (MnO)</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dysprosium</td>
<td>Negative</td>
<td>Low</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>(U)SPIO</td>
<td>Negative</td>
<td>High</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PARA(CEST)(^{1})</td>
<td>Color-encoded</td>
<td>Low</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>DIA(CEST)</td>
<td>Color-encoded</td>
<td>Low</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PFCs</td>
<td>Positive (tracer)</td>
<td>Low</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^{1}\)These paramagnetic metals are bound to chelates to prevent cytotoxicity.

\(^{2}\)As cell-labeling agent.
detect cells in vivo. One of the advantages of using these nanoparticles in vivo is that they can be strong enough to visualize a small number of cells (Fig. 1). The presence of thousands to millions of magnetically aligned iron atoms in the SPIO core enables sensitive detection after the cells internalize these nanoparticles. Iron particle size varies from 10-50 nm [very small or ultra-small SPIO (VSOP and USPIO, respectively)] to >1 μm [micrometer-sized iron oxide (MPIO)] (Bulte and Kraitchman, 2004). SPIOs were first utilized to label and track transplanted cells in the rat brain (Hawrylak et al., 1993; Norman et al., 1992). Over the years, iron oxide particles have been developed that have higher relaxivities and more efficient intracellular labeling (Nkansah et al., 2011; Tang and Shapiro, 2011). Non-phagocytic cells cannot internalize SPIO nanoparticles; the most commonly used approach to label such cells is through the use of transfection agents, as originally demonstrated for magnetodendrimers (see Box 2 for more details) (Bulte et al., 2001).

As an example of their clinical use, SPIO nanoparticles have been used to track, using MRI, the homing of injected mesenchymal stem cells to the central nervous system (CNS) of individuals with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) (Karussis et al., 2010). This approach is particularly valuable because SPIO nanoparticle labeling does not affect the differentiation capacity (Nejadnik et al., 2012a; Wang et al., 2011), viability or function (Richards et al., 2012) of transplanted stem cells, except for chondrogenesis by mesenchymal stem cells, where it interferes with forming the extracellular matrix of cartilage (Bulte et al., 2004; Kostura et al., 2004).

In the past, only two clinical-grade, iron-oxide-based agents have been developed and approved for MRI of the liver: (1) SPIO nanoparticles coated with dextran as a polysaccharide (i.e. ferumoxides, also known as Endorem® in Europe and Feridex® in the USA) and (2) SPIO nanoparticles coated with low molecular weight carboxydextran (i.e. ferucarbotran, also known as Resovist®). Although these SPIO formulations have been used for several clinical studies in the past, they are no longer manufactured because of economic considerations (Bulte, 2009). The agents never sold well for their FDA-approved application (i.e. detection of liver tumors) and, over time, abdominal MRI techniques have become more advanced to detect those masses without the need of administering contrast.

A limitation to the use of SPIO nanoparticles is their occasional extracellular deposition in tissues, either by active exocytosis (Cromer Berman et al., 2013) or passive release through the death of transplanted cells (Terrovitis et al., 2008). Deposited iron particles are scavenged by macrophages, which can then generate a false signal on MRI. As a result, the clinical application of these nanoparticles is limited to their short-term use and includes the MRI-guided delivery of cells in real time (Barnett et al., 2007; Kraitchman et al., 2003) and the subsequent immediate monitoring of their homing and engraftment. As such, the use of these iron nanoparticles is restricted to assessing the acute retention of labeled cells and their short-term distribution in the body.

Fluorinated agents
Fluorine (19F) is the naturally abundant isotope of fluorine, with a nuclear magnetic resonance (NMR; see Boxes 1, 2) sensitivity of 83% compared with hydrogen (1H). It is also not radioactive, in
contrast to the $^{19}$F isotope, which is used in positron emission tomography (PET; see Box 2) imaging. $^{19}$F MRI has emerged as a novel technology for not only tracking transplanted immune cells, but also endogenous macrophage-type cells that are present in several inflammatory disorders, such as autoimmune myocarditis and inflammatory bowel disease (Ahrens and Bulte, 2013; Ahrens and Zhong, 2013; Temme et al., 2012). Although SPION nanoparticles are currently the most commonly used agent for MRI cell tracking (Cromer Berman et al., 2011), the relationship between the contrast, free iron and the concentration of SPION is non-linear, and, therefore, it is difficult to quantify (Lebel et al., 2006; Rad et al., 2007). In addition, the SPION-induced negative contrast is not truly specific and could be difficult to interpret at times, particularly when other sources of low signal contrast are present, as occurs for example in blood vessels when a patient is bleeding – following illness or injury – where blood iron accumulates and is converted to a negative iron oxide contrast agent. By contrast, $^{19}$F MRI provides a more accurate, unambiguous detection of labeled cells (given the lack of background signal). Moreover, the relationship between the concentration of the $^{19}$F and signal intensity is directly proportional and linear over a wide range of concentrations, and the signal can be quantified directly from the acquired images (Srinivas et al., 2007). The lack of a detectable background in the $^{19}$F signal in biological tissues leads to higher visibility of the target cells, much like ‘hot spots’ emerging from an empty background (Bulte, 2005).

Perfluorocarbons (PFCs), which have many fluorine atoms with identical chemical shifts, are most commonly used for $^{19}$F MRI cell tracking applications, and they include perfluoro-15-crown-5-ether, linear perfluoropolyethers and perfluorooctyl bromide (Kadayakkara et al., 2014; Ruiz-Cabello et al., 2011). PFCs, being lipophobic and hydrophobic, are formulated into stable nanoemulsions by high-energy sonication for cell labeling (Janjic and Ahrens, 2009). Similar to SPION-labeled cells, cells are labeled with PFC nanoemulsions ex vivo and then injected into the body (Ahrens and Zhong, 2013). Of note is that PFCs with different chemical shifts can be used to visualize two distinct populations of cells simultaneously in vivo (Partlow et al., 2007). As for regenerative medicine, neural stem cells have been successfully labeled with PFCs, and then visualized following their implantation into the brain (Fig. 2) (Bible et al., 2012; Boehm-Sturm et al., 2011; Ruiz-Cabello et al., 2008). Previous studies have shown that PFC labeling has a minimal effect on cell viability and cellular functions, such as differentiation and proliferation, both in vitro and in vivo (Boehm-Sturm et al., 2011; Ruiz-Cabello et al., 2008; Barnett et al., 2011; Keupp et al., 2011; Partlow et al., 2007).

In addition to tracking cells, PFCs can be used to probe cell function by sensing intracellular oxygen tension. For example, one study combined cell-labeling technology with the oxygen-sensing ability of PFCs to measure the intracellular oxygen tension in brain tumor models (Kadayakkara et al., 2010; Zhong et al., 2013), based...
on the principle that O₂ molecules change the ¹⁹F T₁ relaxation time. This study reported that changes in oxygenation following tumor treatment with chemotherapy and immunotherapy provided a reliable imaging biomarker for evaluating the efficacy of these anti-tumor treatments. This approach could potentially be used to assess possible hypoxia at the site of stem cell transplantation, and to determine its effect on stem cell survival. The promising preclinical data and safety profile of PFCs make ¹⁹F MRI ready for clinical translation; recently, the feasibility of using ¹⁹F imaging of cells in a clinical setting has been demonstrated (Ahrens et al., 2014).

There are, however, limitations to the amount of PFCs that can be incorporated into cells: their size, similar to MPIOs, means that they take up a significant amount of cytoplasmic volume. Thus, the population number of cells to monitor should be above a certain threshold for detection, and that might vary depending on cell type. The minimum amount of cells that can be detected using ¹⁹F-based imaging ranges from 10⁵ to 10⁶ cells (Ahrens and Bulte, 2013). ¹⁹F MRI also requires the use of separate coils for image acquisition, or use of a multinuclear detector coil, neither of which is readily available in many MRI centers. However, the resonance frequency of ¹⁹F is close to that of ¹H; therefore, existing ¹H coils could be adapted for use as dual-tuned coils, capable of imaging both ¹H and ¹⁹F nuclei.

Highly-shifted proton MRI
In 2014, a novel ‘hot spot’ highly shifted proton (HSP) MRI technique for cell tracking was described that directly detects dysprosium (Dy)- or thulium (Tm)- 1,4,7,10-tetramethyl-1,4,7,10-tetraacetic acid (DOTMA)-labeled protons inside cells (Bulte, 2014; Schmidt et al., 2014). The principle behind HSP MRI is that certain lanthanides (see Box 2 for a glossary of terms), in particular Tm and Dy, cause a chemical shift of proton resonance frequency, one that is far away (100 ppm) from the water peak (from which the MRI signal is normally collected). When the MRI excitation pulse is applied at that chemical shift, only cells that contain the Dy- or Tm-bound protons will resonate and generate a signal. A customized pulse sequence, termed ultra-short echo time (UTE) MRI, was used to take advantage of the dramatically shortened T₁ value of the protons bound to the chelate (Schmidt et al., 2014). When human fibrosarcoma tumor cells were labeled with Tm-DOTMA by electroporation and implanted into the flank of nude mice, the tumor could clearly be detected as a ‘hot spot’ on HSP MRI, and the overlay with conventional T₂-weighted MRI allowed its anatomical localization (Fig. 3). HSP MRI is thus somewhat comparable to ¹⁹F MRI, and has a lower approximate cell detection number of 1×10⁴ cells.

Beyond cell tracking: determining cellular function with MRI
Beyond cell tracking, MRI now has the added potential of being used to monitor enzymatic activity, gene expression, metal ion homeostasis and cell death in vivo. In this section, we discuss the most recent advances in the development of MRI probes and sensors to monitor cellular function, and also discuss the potential applications of these approaches to regenerative medicine.

Monitoring enzymatic activity: use of sensors
In several preclinical studies, stem cells have been genetically modified to overexpress growth factors and enzymes in order to enhance their therapeutic properties in different disease conditions (Li et al., 2014; Krakora et al., 2013; Liang et al., 2013a; Suzuki et al., 2008; Xie et al., 2007). However, changes in the expression or level of activity of endogenous enzymes can also be associated with different diseases, requiring imaging methods to be developed that can distinguish between the two conditions.

In recent years, several MRI-based sensors have been designed to measure the expression and activity of both endogenous and recombinant enzymes. Most of these studies have been performed in vitro as proof-of-principle, with few in vivo examples as of yet. These sensors can either be substrates of the enzyme of interest conjugated to a contrast agent, or a substrate that changes its

Fig. 3. MRI of highly shifted proton (HSP)-labeled cells in mice. (A) Ultra-short echo time (UTE) image of a mouse subcutaneously implanted with Tm-DOTMA-labeled fibrosarcoma shows that these cells are detectable in vivo immediately (left) and 8 days after (right) injection. The ‘hot spot’ HSP signal is displayed superimposed over the anatomic ¹H MRI T₂-weighted images. In addition to the tumor (red arrow), a reference tube containing 0.25 mmol/l of aqueous Tm-DOTMA solution (white arrow) is shown. (B) The Tm-DOTMA signal in the tumor decreases with time, due to cell division. au, arbitrary units. (C) Corresponding mass spectrometric Tm image (right; color scale in arbitrary units) corresponds to tumor location on histology (left, red arrow), whereas surrounding fatty tissue (black arrow) showed no Tm signal. Reproduced with permission (Schmidt et al., 2014).
Fig. 4. Schematic depicting the different mechanisms of enzyme-induced changes in contrast. (A) T1 (positive) contrast can be increased by enzymatic removal of a blocking group (Louie et al., 2000). (B) T1 (positive) contrast can be increased by enzymatic polymerization, where the polymerized contrast agent has a higher relaxivity than the single molecules (Chen et al., 2004). (C) T2 (negative) contrast can be increased by enzymatic cleavage/conversion of superparamagnetic iron oxide (SPIO) agent surface groups, leading to particle aggregation (Schellenberger et al., 2008). (D) Chemical exchange saturation transfer (CEST) contrast can be reduced (blue) by enzymatic cleavage/removal of the exchangeable proton (Liu et al., 2011) or increased (red) by accumulation of the probe in the cell following enzymatic phosphorylation (Bar-Shir et al., 2013c). Vertical color bar represents CEST contrast in color-coded hot scale.

decting gene expression: use of reporters

With recent advances in the field of molecular imaging, it is now possible to non-invasively monitor transgene expression in cells in vivo using genetically encoded reporters. The principle behind this is that a new, foreign gene encoding an MRI reporter protein is introduced into the cell’s genome (either stably or transiently), and is then transcribed and translated to produce a new protein, peptide or enzyme that can affect the MRI contrast in a manner that will enable its detection with MRI. Several landmark studies have utilized such genetically encoded proteins as MRI reporters, including the human transferrin receptor (Koretsky et al., 1996), β-gal (Louie et al., 2000), and proteins that are involved in iron metabolism (Alfke et al., 2003) and storage (Cohen et al., 2005; Cohen et al., 2007; Genove et al., 2005; Zurkiya et al., 2008) (Table 2). An overview of these gene reporter systems has been described in detail in several previous reviews and so is not discussed further here (Airan et al., 2013; Gilad et al., 2007b; Gilad et al., 2008b; Vandsburger et al., 2013).

We have previously demonstrated that the artificial CEST-based lysine-rich protein (LRP) reporter gene can be used to distinguish transplanted rat glioma cells that overexpress the reporter transgene from control cells in vivo (Gilad et al., 2007a). Since then, CEST MRI has been used to detect expression of the herpes simplex virus (HSV) type-I thymidine kinase (tk) (Bar-Shir et al., 2013c), and to sense cellular signaling using a genetically encoded biosensor (Airan et al., 2012). The key advantage here is that the gene is replicated with each cell division, enabling longitudinal MRI tracking of the dynamics of biological processes. At this early stage of
However, the assessment of cell death after access to oxygen and nutrients during the post-transplantation period is challenging. One way to ‘sense’ cell death is to monitor changes in intra- and extracellular pH, e.g. extracellular pH (pHe) is reduced in tumors (Gillies et al., 1994). Because of this control, abnormal pH values can indicate cell death or pathological changes in a tissue.

Table 2. Overview of current approaches for developing MRI reporters and sensors

<table>
<thead>
<tr>
<th>Contrast mechanism</th>
<th>Gene product</th>
<th>Reporter/sensor</th>
<th>Substrate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 or T2*</td>
<td>Transferrin</td>
<td>Reporter</td>
<td>No</td>
<td>Deans et al., 2006; Koretsky et al., 1996; Moore et al., 2001; Weissleder et al., 2000</td>
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<tr>
<td></td>
<td>Ferritin</td>
<td>Reporter</td>
<td>No</td>
<td>Cohen et al., 2005; Cohen et al., 2007; Deans et al., 2006; Genove et al., 2005; Gottesfeld and Neeman, 1996; Iordanova and Ahrens, 2012; Iordanova et al., 2010; Ziv et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Tyrosinase</td>
<td>Reporter</td>
<td>No</td>
<td>Alfke et al., 2003; Weissleder et al., 1997</td>
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<tr>
<td></td>
<td>MagA</td>
<td>Reporter</td>
<td>No</td>
<td>Zurkiya et al., 2008; Rohani et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Protein kinase A</td>
<td>Sensor</td>
<td>No</td>
<td>Shapiro et al., 2009; Lee et al., 2014</td>
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<tr>
<td>T1</td>
<td>lacZ (β-galactosidase)</td>
<td>Reporter</td>
<td>Yes¹</td>
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<tr>
<td></td>
<td>Biotag/Blr/A</td>
<td>Reporter</td>
<td>Yes¹</td>
<td>Bartelle et al., 2012</td>
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<td></td>
<td>Bacterial cytochrome P450-BM3</td>
<td>Sensor</td>
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<td>Shapiro et al., 2010</td>
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<tr>
<td></td>
<td>(BM3h)</td>
<td>Reporter</td>
<td>Yes²</td>
<td>Bartelle et al., 2013</td>
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<td></td>
<td>Divalent metal transporter</td>
<td>Reporter</td>
<td>Yes</td>
<td>Westmeyer et al., 2014</td>
</tr>
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<td></td>
<td>Secreted alkaline phosphatase</td>
<td>Reporter</td>
<td>Yes</td>
<td>Westmeyer et al., 2014</td>
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<td>Chemical exchange</td>
<td>Lysine-rich protein³</td>
<td>Reporter</td>
<td>No</td>
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<tr>
<td>saturation transfer</td>
<td>Cytosine deaminase</td>
<td>Reporter</td>
<td>Yes</td>
<td>Liu et al., 2011</td>
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<td>(CEST)</td>
<td>Herpes simplex virus thymidine kinase</td>
<td>Reporter</td>
<td>Yes</td>
<td>Bar-Shir et al., 2013b; Bar-Shir et al., 2013d</td>
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<td>PKA sensor³</td>
<td></td>
<td>Sensor</td>
<td>No</td>
<td>Airan et al., 2012</td>
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<td>Human protamine-1</td>
<td>Reporter</td>
<td>No</td>
<td>Bar-Shir et al., 2014</td>
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<td></td>
<td>Bacterial carboxypeptidase G2</td>
<td>Reporter</td>
<td>Yes</td>
<td>Jamin et al., 2013</td>
</tr>
<tr>
<td>³¹P</td>
<td>Creatine kinase</td>
<td>Reporter</td>
<td>No</td>
<td>Koretsky et al., 1990; Koretsky and Traxler, 1989</td>
</tr>
<tr>
<td></td>
<td>Arginine kinase</td>
<td>Reporter</td>
<td>No</td>
<td>Walter et al., 2000</td>
</tr>
<tr>
<td>¹⁹F</td>
<td>lacZ (β-galactosidase)</td>
<td>Reporter</td>
<td>Yes⁴</td>
<td>Kodibagkar et al., 2006; Liu et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Bacterial carboxypeptidase G2</td>
<td>Reporter</td>
<td>Yes⁴</td>
<td>Jamin et al., 2009</td>
</tr>
<tr>
<td>Hyperpolarization</td>
<td>Aminoaclylase-1</td>
<td>Reporter</td>
<td>Yes</td>
<td>Chen et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Bacterial carboxypeptidase G2</td>
<td>Reporter</td>
<td>Yes</td>
<td>Jamin et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Urea transporter</td>
<td>Reporter</td>
<td>Yes</td>
<td>Patrick et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Gas vesicles</td>
<td>Reporter</td>
<td>Yes⁴</td>
<td>Shapiro et al., 2014</td>
</tr>
</tbody>
</table>

A summary of the most commonly studied genes that have been used as MRI reporters or sensors. There are two categories of reporter genes: those that can be visualized without the need to administer a substrate in the form of a contrast agent, and those that rely on substrate injection to provide contrast.

¹Gadolinium-based substrate required; ²manganese-based substrate required; ³artificial gene, synthesized de novo; ⁴hyperpolarized xenon required.

development, nearly all of these studies have been merely proof-of-principle and not yet applied in specific disease settings.

Although there is some overlap between reporter genes and sensors in their function to serve as a beacon, reporters are in general genetically encoded and synthesized by cells, whereas sensors are most often man-made and added to the cells. Importantly, sensors have the capability to sense the extracellular environment and are not limited to act as a beacon for events occurring within or on the surface of the cell. Examples of other recent developments in the field include approaches to express reporters in a specific tissue or cell type [as demonstrated by Bartelle and colleagues, who have taken a creative approach to expressing a multicomponent reporter specifically in endothelial cells (Fig. 5) (Bartelle et al., 2012)]; reporter genes that report on cellular metabolic changes and on the conversion of a pro-drug to an active drug [such as the carboxypeptidase G2 enzyme, a bacterial enzyme with putative therapeutic potential (Jamin et al., 2009)]; and biosensors that detect the activity of proteins, such as PKA (Airan et al., 2012).

Monitoring cell viability using pH nanosensors

The death of therapeutic cells from immunorejection and/or poor access to oxygen and nutrients during the post-transplantation period is an important factor that reduces the efficacy of stem cell therapies. However, the assessment of cell death after in vivo transplantation is challenging. One way to ‘sense’ cell death is to monitor changes in intra- and extracellular pH, a measure of acid-base balance that assesses the effective concentration of hydrogen ions (H⁺). In vivo, this acid-base balance is regulated through various pathways that enable the production and transportation of hydrogen ions so that intra- and extracellular pH are physiologically controlled (Gillies et al., 2004). Because of this control, abnormal pH values can indicate cell death or pathological changes in a tissue.

Conventional methods that enable the measurement of intra- or extracellular pH in vitro include the use of pH electrodes (Roos and Boron, 1981) and the use of optical dyes (Li et al., 2006; Thomas et al., 1979). However, several MRI-based methods that are non-invasive, such as MRS, T1 contrast MRI and CEST contrast MRI, allow pH to be measured in live animals. Our group has recently developed a new technique using pH-sensitive CEST agents to monitor the viability of hepatocytes non-invasively in vivo using MRI (Chan et al., 2013). CEST contrast provides an alternative MRI method with which to measure pH (Aime et al., 2002; Liu et al., 2013; Sherry and Woods, 2008; Ward and Balaban, 2000) through changes in the water proton (MRI) signal. This technique offers several advantages; for example, CEST contrast is not constantly ‘turned on’ as T1 relaxation agents are, but is instead switched on through the addition of an appropriate saturation pulse(s) at the start of the imaging sequence, which allows for more-specific detection of contrast resulting from pH changes.

Imaging of tissue-mimicking scaffolds

As mentioned above, one of the persisting limitations of stem cell transplantation is their poor survival immediately post-
Fig. 5. Schematic of the Biotag reporter system. The Biotag reporter system, as reported by Bartelle and colleagues (Bartelle et al., 2012), was used here to label endothelial cells in a mouse model of angiogenesis. This system is based on the coexpression and interaction of a Biotag and BirA, and in this study the authors relied on the strong affinity between avidin and biotin. (A) The authors generated transgenic mice that express multiple (nx) copies of a ‘Biotag’ (shown in red). The Biotag was fused to a signal sequence (SS; targeting to the secretory pathway) and the Myc-tagged transmembrane (TM) domain of the platelet-derived growth factor receptor (PDGFR-TM), followed by an internal ribosome entry site (IRES) sequence (allowing translation initiation in the middle of the mRNA sequence) to co-express the hemagglutinin (HA)-tagged BirA enzyme (which is a biotin ligase), modified with SS and KDEL (Lys-Asp-Glu-Leu amino acid) sequences. The expression of this bicistronic construct can be driven by different vascular endothelial cell promoters to restrict its expression to specific cell subtypes. (B) On translation, the ribosome recognizes the N-terminal SS of each protein to insert them into the lumen of the endoplasmic reticulum (ER). In the ER, the BirA enzyme (green) ligates free biotins (black rectangles) onto the Biotag protein (red), and, by continuing through the secretory pathway, the resulting biotinylated Biotag protein will be expressed on the surface of endothelial cells, whereas the BirA enzyme is retained in the ER by the KDEL sequence. (C) Thanks to the strong affinity between biotin and avidin, once on the cell surface, the biotins bound to the Biotag protein will be accessible for binding to the avidinated probes, which can be labeled with fluorescent or paramagnetic agents. (D) Based on the labeling, avidinated probes can be imaged via fluorescence or MR imaging, allowing for the selective detection of the vasculature endothelial cells (red) that express the transgene. (E) To image vascular endothelial cells in vivo, transgenic Ts-Biotag mice were generated by using a minimal promoter for tyrosine-protein kinase receptor 2 (T-shrot; Ts). After injection of DTPA-gadolinium-labeled avidinated probes, Ts-Biotag plugs could be visualized via MRI. Here, in vivo MR images of angiogenic endothelial cells within a Matrigel pellet doped with vascular endothelial growth factor, acquired before and 1 hour after injection, are shown (n=4). After injection, the immediate vascular tissue close to the plugs is clearly enhanced (arrow) compared with wild type (WT; n=4). Figure and data reproduced and modified with permission (Bartelle et al., 2012). Scale bar: 1 mm.
and free 19F iCEST probe, we exploited the dynamic exchange in vivo to be used based approaches. Although the iCEST technique has the potential to a detectable level) – a feature that is not available for 1H MRI- 

production, the paramagnetic Zn\(^{2+}\)-responsive T1 agent was administered, resulting in an increase in T1 MRI contrast in healthy mouse pancreas. Because Zn\(^{2+}\) release is tightly correlated with insulin release from healthy pancreatic cells, the same experiment performed in diabetic mice (who do not produce sufficient insulin) did not produce any change in MRI contrast. This experiment demonstrates how MRI can reveal cell functionality in vivo by detecting changes in metal ion levels, a method that could, in the future, be used to detect changes in insulin release in islet cell transplantation for the treatment of diabetes.

It remains a challenge, however, to image metal ions that are present at low levels with high specificity. To overcome this, we have demonstrated a novel, metal-ion-imaging approach that combines 19F MRI with CEST MRI (Bar-Shir et al., 2013a; Bar-Shir et al., 2015) in an approach called (multi)-ion-CEST [(m)iCEST] (see Box 2). Taking advantage of the difference in the ion-specific 19F NMR chemical shift offset (\(\Delta \omega\)) values between the ion-bound and free iCEST probe, we exploited the dynamic exchange between ion-bound and free iCEST probe to obtain MRI contrast. It was demonstrated that a prototype single 19F iCEST probe could separately visualize mixed Zn\(^{2+}\) and Fe\(^{3+}\) ions in a specific and simultaneous fashion. A key advantage of this technique is that it allows the detection of low (biologically relevant) concentrations of metal ions by simply reducing the concentration of the 19F probe (up to a detectable level) – a feature that is not available for 1H MRI-based approaches. Although the iCEST technique has the potential to be used in vivo, further studies are required to prove the feasibility of this approach.

**Clinical translation and current limitations**

As pointed out, owing to their metal toxicity once de-chelated, it is highly unlikely that manganese, gadolinium and other lanthanides will have opportunities for clinical translation. However, these contrast agents will continue to aid in the assessment of cell transplantation strategies in experimental settings, which are pivotal as proof-of-principle studies. Tracking cells in patients has only been performed so far with SPIO and perfluorocarbons, and this is likely to remain for some time. Both agents have proven to be safe, with the iron in SPIO being metabolized and reused in the normal body iron pool, whereas fluorine is incompatible with any known biochemical reaction in humans, eventually leaving the body as an inert gas compound. Clinical reporter-gene-based cell imaging using the HSV-tk reporter gene has been implemented using PET and an \(^{18}\)F-labeled thymidine kinase substrate (Yaghoubi et al., 2009), raising the possibility that similar studies could be done with a CEST MRI substrate (Bar-Shir et al., 2013b; Bar-Shir et al., 2013c). However, there are different regulatory guidelines for using highly sensitive PET probes at a micro-dosing range, as compared to CEST substrates that need to be given at higher doses. Although the PET reporter gene was developed some 20 years ago (Tjuvajev et al., 1995), the CEST reporter gene/sensor field has just begun. CEST agents are based on natural products and do not contain metals; however, they could be involved in immunological or other biological reactions that are at present unknown. Future bio-clearance and dose-toxicity studies will be needed for the further consideration of CEST agents as a clinically viable approach, but so far there have been no indications that clinical translation is prohibited.

**Conclusions and future prospects**

MRI-based cell imaging is a versatile technique because it can employ several different types of contrast. It has evolved, in a short period of time, from being a simple cell-tracking approach to an applied method for sensing the physiological state of cells. Except for SPIO- and 19F-labeled cell tracking performed clinically, the newer functional sensing and reporter gene approaches are still in their infancy, just entering from the test tube phase into preclinical testing. We anticipate that at least a few of these newer probes will be further developed and become commercially available, increasing
their potential user group dramatically and allowing the field to grow. Then, a forward path towards clinical translation could become a bright new path ahead.

Competing interests
A.A.G., M.T.M. and J.W.M.B. are founders and co-owners of SenCEST, LLC.

References


