

## MRI Techniques for Stem Cell Trafficking

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### *Introduction*

Transplanted stem and post-mitotic cells show great promise as therapeutic agents in a number of diseases, including Alzheimer's disease, Parkinson's disease and myocardial infarction, among many others. The curative use of transplanted cells would be aided greatly by non-invasive techniques for visualizing the distribution and location of cells following transplantation or infusion. Particularly in cases where widespread migration of delivered cells is necessary, longitudinal monitoring of cellular migration non-invasively could be crucial. Coupled with functional and metabolic assessments, non-invasive imaging of stem cell grafting would have a profound effect on physicians' ability to serially monitor and adjust treatment.

Magnetic resonance imaging is a powerful tool for visualizing cellular trafficking, homing and migration in intact animals. Centrally important and advantageous to cellular imaging in intact animals, is the ability of MRI examinations to be performed longitudinally and non-invasively, all within appropriately rapid scan times. Cellular detection is most often accomplished via  $T_2$  or  $T_2^*$  based mechanisms following incorporation of contrast agent either within the cell or attached to the cell. In general, superparamagnetic iron oxide based contrast agents are used to generate contrast, and when sufficient metal is accumulated within a cell, and at high enough image resolution, single cells can be detected, even in vivo. Herein are described common methods for labeling cells for cellular imaging and imaging techniques for their visualization.

### *Cell tracking contrast agents*

#### *$T_2$ and $T_2^*$ weighted contrast*

Superparamagnetic iron oxide particles are the most common form of contrast agent for cell tracking by MRI. Extensive reviews of these types of contrast agents can be found here <sup>1,2</sup>. Differing from paramagnetism, where a Boltzman distribution term qualifies the degree of alignment of the magnetic dipoles in a magnetic field, the magnetic dipoles of superparamagnetic materials add in a magnetic field, making them stronger contrast agents. The properties of common particle based cell labeling contrast agents are shown here:

	<b>Coating</b>	<b>Iron oxide content</b>	<b>Overall size</b>
<b>USPIO</b>	Dextran	Single ~ 4-6 nm crystal	10 – 20 nm
<b>SPIO</b>	Dextran	Multiple ~ 4-6 nm crystals	50-100 nm
<b>MPIO</b>	Polystyrene	As high as 40% iron by weight 0.1-10 pg iron per MPIO	0.96 – 5.8 micron

Commercially available USPIOs and SPIOs are most often biodegradable dextran coated and present the opportunity for surface modification to allow the attachment of antibodies or peptides to aid in cellular uptake. Commercially available MPIOs are most often polystyrene-based inert polymer coated, and can be purchased with chemical functionality and fluorescence built directly into the coating. MPIOs package from 0.1 pg iron in a 0.96 micron diameter particle to more than 10 pg iron in a 4.5 micron diameter particle. In fact, cells harboring just single MPIOs can be detected by MRI <sup>3</sup>. USPIO particles have been successfully synthesized to have many different coatings, such as starch <sup>4</sup> or citrate <sup>5</sup>, for example. Polymeric macromolecular iron dendrimers (40-50 nanometers) have also been used to successfully label cells for cell tracking <sup>6</sup>, as have macromolecular complexes of gadolinium, one example being GRID <sup>7</sup>.

Incorporation of superparamagnetic contrast agents in a way that clusters them results in a magnification of their relaxation properties <sup>8</sup>. This is largely the product of several different physical phenomena. One important process, particularly with USPIOs and SPIOs is that clustering of these small particles results in the magnetic properties of this cluster being more like the overall size of the cluster, rather than the individual small particles themselves. This places the relaxation regime of neighboring water molecules in the static dephasing regime <sup>9</sup> and enhances  $T_2^*$  relaxivity <sup>8</sup>. A second potential enhancement mechanism may be magnetic co-operativity between the magnetic cores of the particles, resulting in superferromagnetism within the magnetic field of the MRI magnet.

#### *T<sub>1</sub> weighted contrast*

Whereas most cell tracking studies using MRI have employed  $T_2$  or  $T_2^*$  weighted contrast,  $T_1$  agents have been successfully used to label cells for MRI as well. An early study demonstrated red blood cells labeled with chromium could produce  $T_1$  effects <sup>10</sup>. Continuing along these lines, red blood cells labeled with gadolinium were also shown to produce detectable  $T_1$  contrast <sup>11</sup>. Recently, labeling of lymphocytes with manganese has been demonstrated, with estimates of detectability of 25 cells per 100 micron<sup>3</sup> voxel <sup>12</sup>.

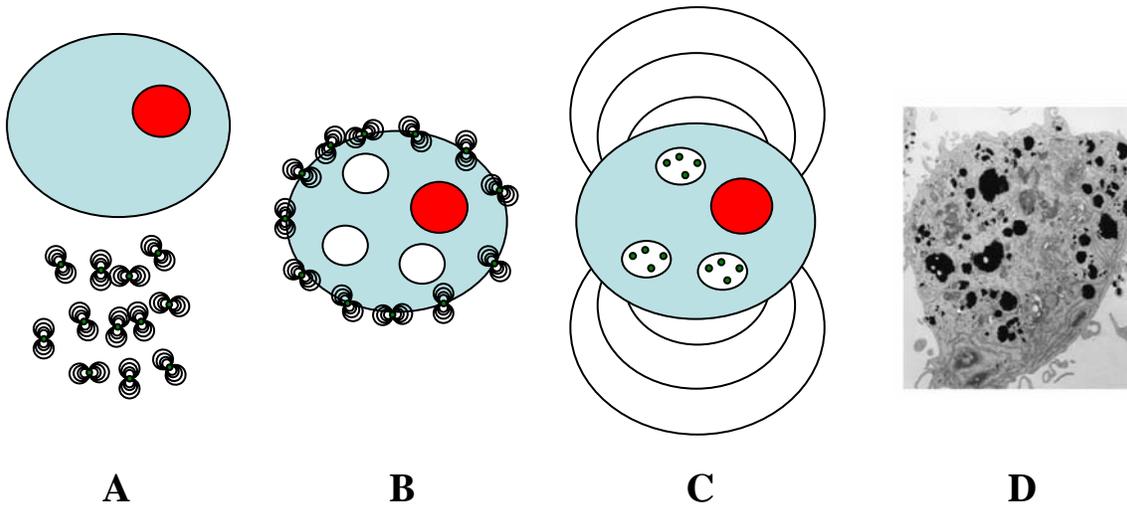
#### *In vitro cell labeling for cell tracking*

The main objective of magnetic cell labeling is to maximize incorporated contrast agent without interfering with cellular functions or adding new ones. To this end, there are different methods for cell labeling depending on which contrast agent is used. In early experiments <sup>13</sup>, cells in culture were labeled with native USPIOs by simple incubation, relying on fluid phase pinocytosis for intracellular uptake. Importantly, cellular function did not seem to be altered by the incorporation of the particles. However, simple incubation usually proves to be an inefficient method for cell labeling with USPIOs. Other early experiments used a biotinylated antibody to target cells in culture, followed by streptavidin, and subsequently followed by biotinylated particles to effect cell labeling <sup>14</sup>. In an effort to amplify intracellular labeling with USPIOs, Josephson et al <sup>15</sup> attached the HIV-1 TAT protein, a peptide which conveys membrane translocation, to a chemically cross-linked version of their USPIO and realized a gain of 100X in intracellular iron, yielding a high of ~ 2.5 pg iron/cell. Similar multiplied gains in

labeling efficiency were realized in mouse neural progenitor cells, human CD34+ lymphocytes and hematopoietic stem cells, and mouse splenocytes, achieving 10-30 pg iron/cell<sup>16</sup>. This was achieved by using 40 ug of contrast agent per 10<sup>5</sup> cells.

For cell labeling with SPIO particles, simple incubation of cells in culture with native particles also provides for cell labeling. However, significant work has been undertaken in complexing SPIOs with transfection agents, such as poly-lysine<sup>17</sup>, protamine sulfate<sup>18</sup>, or other commercially available transfection agents<sup>19,20</sup>. While careful titration of contrast agent and transfection agent are necessary to prevent precipitation of the contrast agent, in general a concentration of SPIO at 25 to 50 ug/ml with an SPIO:poly-lysine ratio of 15-30:1, incubated overnight, achieves cell labeling as high as 15-20 pg iron/cell<sup>21</sup>. Recently, electroporation has been successfully used to label cells with SPIO particles<sup>22</sup>. Cellular iron content following electroporation was equivalent to that achieved with transfection agent assistance.

An alternative to magnetic cell labeling with nanoparticles is to use micron sized iron oxide particles, MPIOs, in essence a pre-clustered version of USPIOs<sup>23,24</sup>. For cells that grow adherent to culture dishes, magnetic cell labeling is accomplished by simple overnight incubation of particles<sup>24</sup>. In this manner, iron contents as high as hundreds of pg iron can be achieved with minimal cell death. As particles are heavy enough to sink, cells which grow in suspension can be labeled by first pre-targeting with biotinylated antibodies, then labeled with streptavidin coated MPIOs.



*Figure 1: A) Contrast agent (green dipoles) is added to growth medium containing cells (blue circle). B) Contrast agent attaches to edge of the cell. This can be helped by transfection agents or by charging the particles. C) Contrast agent is taken up by a variety of methods, from pinocytosis for small particles to phagocytosis for larger particles. Cross-membrane translocation can be aided by the use of the TAT peptide. Particles almost always are shuttled to endosomes and/or lysosomes (white circles). At this point, the cell becomes the contrast agent, more so than the individual particles themselves. Particles almost never enter the nucleus (red circle). D) Electron micrograph of a mesenchymal stem cell loaded with 1.63 micron MPIOs (scattered black contrast).*

### ***In vivo cell labeling for cell tracking***

Injection of USPIOs intravenously results in specific labeling of resident phagocytotic cells of the reticuloendothelial and mononuclear phagocyte systems, as well as peripheral cells such as macrophages. Peripheral macrophages home to sites of various diseases, and labeled macrophages will report on conditions such as rheumatoid arthritis<sup>25</sup>, organ rejection following transplantation<sup>26</sup>, atherosclerosis<sup>27</sup>, mouse models of multiple sclerosis<sup>28</sup> and ischemic brain damage<sup>29</sup>. These topics have recently been reviewed<sup>30</sup>. In general, USPIO doses range from 10's to 100's  $\mu\text{mol iron/kg}$  and imaging takes place 24 hours after injection. USPIO administration is preferred over larger SPIOs for in vivo macrophage labeling largely due to the much longer blood half-life versus SPIO, enabling longer contact time with cells. However, a measurement of iron loading per cell has not yet been fully investigated.

Recently, it has been demonstrated that MPIOs can label neural stem cells in vivo<sup>31</sup>. Since individual MPIOs pack as much as 10 pg iron within a single particle, only one or a few particles are necessary for robust MRI detection. This allows inefficient labeling schemes in areas not accessible by blood injections. Neural stem cells lining the lateral ventricles in adult rats were labeled with MPIOs by directly injecting MPIOs into the ventricle. The MPIOs were first incorporated into the stem cells, and then passed on to daughter cells. When these daughter cells migrated from the ventricle to the OBs, this migration could be detected by MRI.

### ***MRI methods for detecting magnetically labeled cells***

With most cell tracking experiments being performed in animals, there is an emphasis on both high resolution and rapid imaging time. Generally speaking, rodents can be anesthetized and successfully maintained for a period of several hours. However, during this time, metabolic fluctuations, as well as physical motions can and do occur, even when the rodents are mounted in appropriate imaging frames. Furthermore, the cellular migration that we are interested in is occurring during the procedure and rapid migration can actually blur the location of the contrast in the image. Therefore, it is usually advisable to keep the imaging scans as fast as possible, to within an hour or two.

Because of the heavy iron load in cells labeled with iron oxide particle based contrast agents, diffusion sensitized  $T_2$  and  $T_2^*$  weighted contrast work best. The thick coating of the particles restricts the closest distance water molecules can approach the particles, rendering  $T_1$  and real  $T_2$  relaxation processes relatively inefficient. To this end, sequences which are sensitive to magnetic field gradients are most often used to detect labeled cells. Extended echo spin echo or gradient echo pulse sequences can provide for both contrast and high resolution, especially when performed as 3D sequences. At clinical fields, FISP based sequences are efficient and well suited for imaging<sup>32</sup>. However, as the field moves towards single cell detection, a premium will be put on imaging resolution. For example, a recent demonstration of in vivo single cell detection in mouse liver was accomplished using voxel sizes of 100 x 100 x 300 microns, employing  $T_2^*$  contrast<sup>33</sup>.

### *What's on the horizon?*

The future of MR based cell tracking is bright. Already, a first study tracking USPIO labeled dendritic cells in humans has been accomplished<sup>34</sup>. Major steps moving forward will likely involve a few key areas. The first will be continued improvements in contrast agents. A possible problem with T<sub>2</sub> and T<sub>2</sub>\* based contrast is that there is a loss of signal, and spurious dark contrast is present from blood and tissue/air interfaces. Stronger contrast agents would allow shorter echo times while achieving the same contrast, which, especially for T<sub>2</sub>\* contrast, will make less spurious dark contrast in the images.

Another area where major advances would be important is in reporting MRI agents. The dark MRI contrast from a labeled cell is only indicative of the presence of the contrast agent, and it is difficult to determine whether the contrast is from the originally transplanted cell, or from free particles, or from a macrophage which endocytosed released material, simply from the MR image. Contrast agents which are sensitive to biological events inside the cell, or which can report on altered gene expression, i.e. when a stem cell turns into a neuron, will aid in removing this uncertainty. These contrast mechanisms may involve aggregation events which alter the relaxivity of the contrast agent<sup>35</sup> or ferritin expression<sup>36</sup>.

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