Stem Cell Imaging

Monitoring the distribution and growth of transplanted cells

By Tom Schaffner

Stem cell imaging, considered a decade ago as a medical wonder of the future, has emerged from being a water cooler debate to a topic of international focus. But researchers are hoping to unlock the mysteries of these tiny wonders to battle disorders like Alzheimer's, diabetes and even cancer.

In the human body, stem cells are unprogrammed cells, capable of changing into other types of cells. Because stem cells can become bone, muscle, cartilage and other specialized types of cells, they could eventually be used to regenerate organs, reducing the need for organ transplants and related surgeries.

Marc H. Hedrick, MD, an assistant professor at the University of California, Los Angeles School of Medicine, compares stem cells to little children "who, when they grow up, can enter a variety of professions. A child might become a fireman, a doctor or a plumber, depending on the influences in their life – or environment. In the same way, these stem cells can become many tissues by making certain changes in their environment."

Stem cells are essentially divided into two types: embryonic and adult. Embryonic stem cells have the potential to morph into a greater variety of cells than adult stem cells.

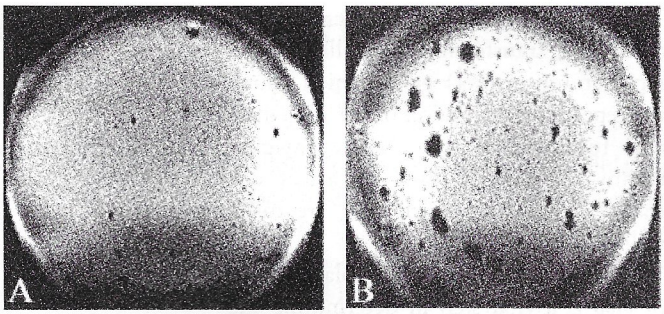
Prior to transplantation into the human tissue for regeneration purposes, stem cells have to go through a differentiation process, a sort of preprogramming of the stem cells to become specific cells. The cells are then injected into the area of the body being targeted for tissue regeneration. When stem cells come into contact with growth chemicals in the body, the chemicals program the stem cells to grow into the tissue surrounding it.

The hurdle for researchers is to find out what causes stem cells to change into particular types of cells in order to grow cells that perfectly match those of the patients.

PET and SPECT Imaging

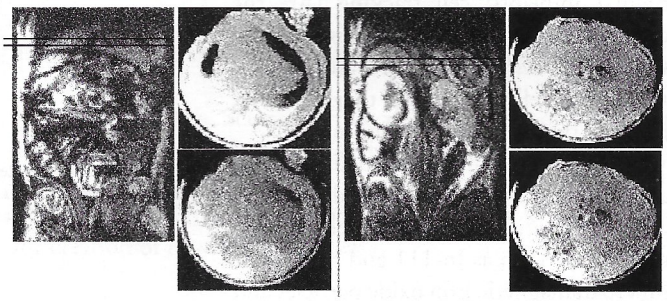
As researchers strive to determine a way to manipulate stem cells to their fullest potential when dealing with disorders, such as Parkinson's disease and myocardial infarction, PET and single photon emission computed tomography (SPECT) have proven to be reliable imaging technologies in tracking and monitoring stem cells.

Recent research by Paul D. Acton, PhD, associate professor and director of molecular physics at Thomas Jefferson University, and Rong Zhou, PhD, assistant professor of molecular imaging at the University of Pennsylvania, both in Philadelphia, has tracked cardiomyoblasts injected into infracted myocardium. "We have used direct cell labeling with In-111 and Tc-99m-MIBI SPECT to monitor both cell trafficking and myocardial viability simultaneously," Acton says. "We are now investigating the use of reporter genes to track the cells to get more information than In-111 labeling can provide."

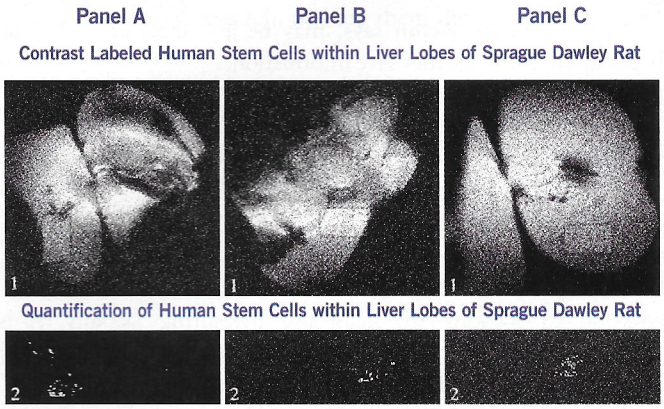


Randall E. McClelland, PhD

In vitro MRI of embedded liver stem cells in agarose gels: A) unlabeled cells, B) labeled cells



MRI of total body perfusion and using severe combined immune deficiency (SCID)/nod mouse: (Left panel) Control mouse containing unlabeled stem cells; (Right panel) Treated mouse with labeled stem cells



MRI Signal Radius (um)			avg. radius (um)	# cells/signal (um²)	# cells/signal (um³)
A	B	C		actual	spheroid extrapolations
107.87+22.2	174.88+101	117.5+19.5	133.4+47	220+28	3260+149

To establish parameters of "human stem cell (HSC) colonies," this figure illustrates a comparative analysis of "actual" cell spheroid sizes versus "MRI signal" responses of the same cells. In this figure, microscopy images of small aggregates parameters (11.2µm radius) are also confirmed as numerous MRI contrast voids (labeled stem cells) as shown in A2. Furthermore, medium and large HSC aggregates with microscopy parameters of 44.1 and 73.1µm, respectively, are also MR detectable as larger contrast voids, as shown in B2 and C2.

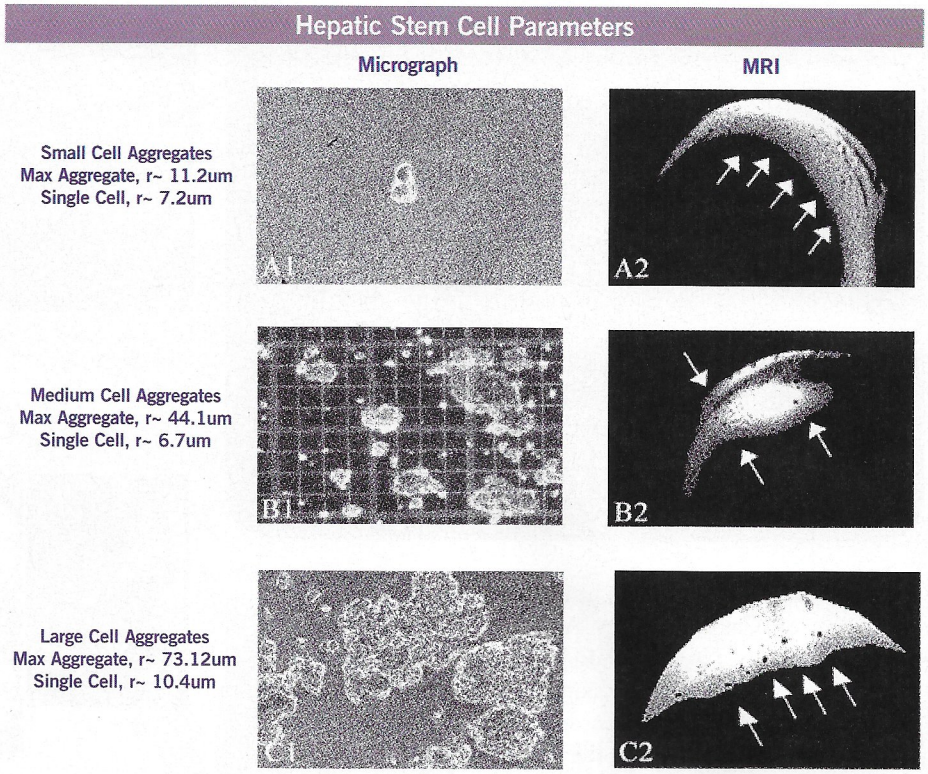
Using PET or SPECT, researchers can track the transfection of cells with a reporter (or marker) gene, which allows the repeated visualization of the migration and function of cells. These imaging techniques can be used to assess cell trafficking with methods that are easily translatable to humans.

"Following the fate of transplanted cells *in vivo* is a vital step in determining the efficacy of the implant," Acton says. Non-invasive imaging techniques have been used to monitor cells and can provide information on three important features of cellular implants: cell tracking (Are the cells reaching the target tissue?), cell viability (Once they get there, are they still alive?) and cell function (If they are alive, then are they actually functioning?).

Acton says it is important to differentiate among cell tracking, viability and function. Tracking studies with direct cell labeling techniques, such as In-111 and SPECT or superparamagnetic iron oxide particles and MRI, give no information on cell viability. "If the cell dies or the label diffuses out of the cell, the signal could remain. Thus, the imaging signal may have little or no relationship to the number of surviving cells," he says.

Reporter genes, Acton says, may be a better approach, since they give information, not just on cell tracking, but also viability. If the cell dies, the gene switches off, and the signal disappears.

"Most cell tracking experiments stop at this point," he says. "However, just because the cells are viable does not necessarily mean they are functioning. Therefore, in addition to tracking the cells and measuring viability, it is important to study the function of the cells [to find out if they are] leading to functional recovery of the organ or tissue under study."



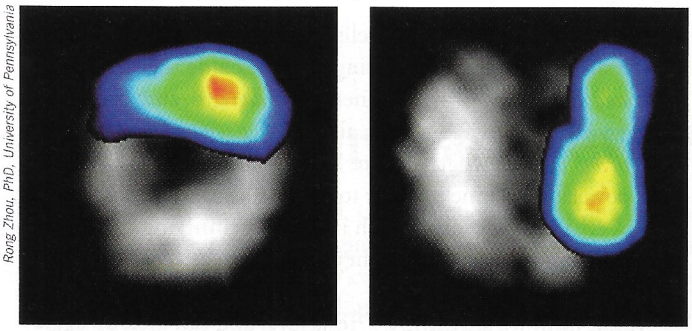
MR signal responses of contrast-labeled HSC aggregates are confirmed with the three-panel *ex vivo* study. This figure illustrates image effects for day-0 transplanted cells of three animal models. These animals are seeded with layers of 2-D contrast labeled cell aggregates recently removed from culture wells and loaded through liver portal veins. After seeding, the animals are immediately exsanguished with tissue perfusion and tissue extraction for MR investigation. Panel A1 illustrates two liver lobes with many contrast signals throughout the tissues. Particularly, an outlined five-sided pentagram surrounds a dense concentration of HSC signals visualized in the left liver lobe. Furthermore, Panel A2 depicts these contrast signals as thresholded "white specs" and are used for cell aggregate dimensioning. Comparatively, animal models shown in panels B1 and C1 illustrate dense signal concentrations within the square and oval partitions, respectively. Again, thresholded "white spec" signals in panels B2 and C2 and are also used for dimensioning. Tabularized MRI data of cell aggregates are displayed under the image panels. As shown, liver lobes A, B and C exhibit average signal radii of 107.87, 174.88 and 117.5ìm, with a combined signal average of 133.4µm. Furthermore, the quantity of HSC cells within these MR signals are normalized against an actual HSC cell radius of 8ìm. In this way, the average total cells found in an area (μm^2) of one MR signal is 222 HSCs, without correcting for the MRI/microscope ratio deviation. By 3-D spheroid extrapolation into volumetric units (μm^3), the theorectical number of cells per signal is 3,260.

As an example, Acton uses the monitoring of dopaminergic function in Alzheimer patients with the tracer FDOPA and PET after stem cell implantation. The implant is not useful unless it leads to the production of dopamine (a chemical naturally produced in the body). "Simply tracking the implant is not giving the whole story," he says.

At this year's annual meeting of the Society of Nuclear Medicine in Toronto, researchers from Seoul National University in South Korea announced that SPECT can be used to evaluate the effectiveness of stem cell therapy with ischemic or coronary heart disease. Using electrocardiographically (ECG)-gated myocardial single SPECT, researchers determined that stem cell therapy improved the regional function of the damaged heart more than blood flow to the damaged heart muscle.

"Our findings indicate that stem cells helped improve the damage caused by heart attack beyond the formation of new vessels into the damaged myocardium," says Dong Soo Lee, MD, professor in the department of nuclear medicine at Seoul National University. "Due to its non-invasiveness and convenience, peripheral stem cell therapy will be widely used in patients with ischemic heart disease," says Lee. "Gated myocardial SPECT will help evaluate treatment effect and suggest the underlying mechanism whereby the damaged heart muscles improve after stem cell infusion. With SPECT, information can be obtained on global function, heart volume and regional blood flow and function. Gated myocardial SPECT can compare function before and after a successful treatment," he says.

Korean researchers have also labeled stem cells with F-18 FDG and successfully assessed tissue distribution. "The use of PET to evaluate stem cell treatment of heart disease adds specificity and feedback to stem cell treatment," says Josef Machac, MD, director of the clinical PET center and nuclear medicine at Mount Sinai School of Medicine in New York City. "Rather than



Rong Zhou, PhD, University of Pennsylvania

A horizontal short-axis slice (left) and a vertical long-axis slice of ^{111} In-oxine-labeled cardiomyoblasts overlaid on a grayscale image of Tc-99m-MIBI in a rat myocardium, obtained using ultra-high resolution pinhole SPECT

just infusing the stem cells, closing one's eyes and hoping for the best, the imaging of F-18 FDG-labeled stem cells provides valuable information about the location and number of cells successfully implanted. One can then link this with eventual improvement in function, investigated by the same group with gated myocardial SPECT," he says.

MR Imaging

While radioisotopes like In-111 oxine SPECT have been the preferred choice for tracking stem cells for limited periods of time – its lifespan is usually only a few days after administration – researchers still hope to find a radioisotope with a longer life that is safe enough for use in humans.

The importance of being able to track a stem cell's progress for longer periods of time has prompted some researchers to look into contrast-enhanced MR as an additional strategy.

Albert Lardo, PhD, assistant director of cardiovascular imaging and director of the image-guided cardiotherapy laboratory and his fellow researchers at Johns Hopkins in Baltimore focus on myocardial regeneration therapy. Where X-ray fluoroscopy is usually used to guide the delivery of cells into the heart, the Hopkins team is developing methods of MRI guidance to accurately target the cells' final destination. The cells can be tagged with markers, such as the superparamagnetic iron oxide Feredix, in order to examine the spatial distribution and function of the cells over time.

"But the downside for cardiac applications is whenever you inject cells into a region of a previous myocardial infarction, there's the possibility of setting up the substrate for arrythmias (abnormal heart rhythms) and, specifically, ventricular tachycardia (abnormal rapidity of heart action), which commonly plagues patients who have had a previous myocardial infarction," Lardo says.

For the Hopkins team, the dilemma they face is whether an implantible cardioverter defibrillator – more commonly known as an ICD – needs to be implanted concurrently at the time the stem cells are delivered. Lardo says there have been several studies on animals that have shown a restoration of function in the region of the heart where stem cells have been implanted. However, the problem with these studies is that researchers have not completed any further tests to determine whether the animals are susceptible to arrhythmias.

Lardo also notes the ongoing controversy of Feridex-labeled cells. "There are some investigators who believe that Feridex labeling actually degrades the ability of the cells to be regenerative and lessens the yield of the cells that survive the injection. So when you tag a cell and it goes away, you're not really sure why it went away. 'Did it die apotopically? Did it migrate out of the imaging field? Are the cells that are attached to the Feridex alive?' There's really no way to know," he says.

Researchers are really interested in knowing if the cells survived and will eventually differentiate, Lardo says. "If we can actually image that then I think we've got a much better quantitative assessment of whether or not the cells are going to work."

Problems with imaging stem cells are not just related to tagging; the imaging power behind the scanners is also being blamed for misinformation in research.

"It all boils down to 'Can we see [the stem cells] when we are using any type of imaging equipment?'" says Randall E. McClelland, PhD, a mechanical and biomedical engineer in the cell and molecular physiology department at the University of North Carolina – Chapel Hill. "When you are using particular machines, whether it's MRI or PET, you are looking for some sort of contrast – black and white or red and orange, for example

The Evolution of Stem Cell Research

1858 – Pathologist Rudolf Virchow publishes his trailblazing work, *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebenlehre* (*Cellular Pathology as Based upon Physiological and Pathological Histology*) and promotes his theory that disease starts at the cellular level in his Berlin laboratory.

1968 – First sibling bone marrow transplant successfully used in treatment of severe combined immune deficiency (SCID)

1981 – A derivation of mouse embryonic stem cells are produced.

1994 – Human embryonic stem-like cells are generated.

November 1998 – Scientists from the University of Wisconsin and Johns Hopkins University derive first human stem cells.

August 2001 – President George W. Bush blocks federal funding for creation of new stem cell lines.

November 2001 – Scientist in Massachusetts performs the first cloning of human embryos. Through therapeutic cloning, or somatic cell nuclear transfer, cloned embryonic stem cells generate replacement tissues that patients' bodies will not reject.

November 2004 – Californians vote to spend $3 billion over 10 years on stem cell research, making them the first state to do so.

May 2005 – The U.S. House of Representatives approves a bill to loosen President Bush's restrictions on federal funding for stem cell research. Bush vows to veto any legislation that would ease restrictions he imposed in 2001.

– to pinpoint where the stem cells are," he says.

McClelland explains that using an iron oxide in an MRI, for instance, can modify the magnetic wavelength and produce a black spot on an image just as air in the tissue could do. "You need to be careful what you are looking at, as well as having a more powerful machine in order to see smaller aggregates," he says.

McClelland's current research involves quantifying cell sizes or cell aggregates to determine if they can be detected using MRI at various power ratings.

"Right now, we are using a 7 Tesla level and can pick up aggregates of about 150 microns. But if we decrease that to a clinical machine – a 1.5 Tesla level – there is no way we are going to see it," he says. "We have to figure out a way to get these high-powered machines into the clinical atmosphere and be sure that the contrasts are not mistaken for something else."

Multi-Modality Imaging

Imaging modalities, such as MRI, can achieve higher spatial resolutions for better delineation of tissue anatomy, despite lower sensitivity than radionuclide imaging.

According to Acton and Zhou, it is probable that one optimal imaging modality could be used for monitoring each aspect of stem cell grafting, so that multiple image modalities can be used jointly to obtain a complete picture of stem cell survival, differentiation and therapeutic benefit. Preliminary efforts using radionuclide imaging for cell tracking and high-resolution MRI for evaluation of cardiac function suggest that a mutli-modality approach holds much promise for *in vivo* monitoring of stem cell grafts.

"Multi-modality imaging uses the best features of each modality – spatial resolution with MRI and sensitivity with PET – to give the maximum amount of information on the cell implants," Acton says, "not only to track the cells, but to monitor function and correlate with clinical improvement."

An important recent development, Acton and Rhou say, is the multi-modality fusion reporter system, which can be studied using optical and radionuclide imaging. The fusion reporter could provide the imaging technique that best suits the application – fluorescence for studying individual cells or for cell sorting, bioluminescence for high-sensitivity *in vivo* imaging in small animals, and PET or SPECT when quantitative accuracy is important or for the translation to humans.

"We can't rely on just one type of imaging modality because there are errors in everything we do," says McClelland. "We have to make sure that we concurrently look at other imaging possibilities of the same type of stem cells. Take micro PET, for example. Even though we don't have details in terms of looking at small structures with micro PET, at least we can get the spatial locations using radioactive isotopes and tags to tell us that the stem cells have landed in particular parts of the body or moved to the brain, the lungs or other areas."

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