Iron oxide nanoparticles-poly-L-lysine complex

SPIO-PLL

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Created: February 20, 2008; Updated: March 26, 2008.

Chemical name:	Iron oxide nanoparticles-poly-L-lysine complex	
Abbreviated name:	SPIO-PLL	
Synonym:	Ferumoxides-PLL, Feridex-PLL, FE-PLL	
Agent category:	Nanoparticle	
Target:	Other	
Target category:	Other	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Iron oxides	
Activation:	No	
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals	No structure is available in PubChem.

Background

[PubMed]

Transplantation of stem cells (SCs) and other mammalian cells into tissues provokes great clinical research interest in developing cell- and gene-therapeutic agents (1). In particular, SCs have unlimited self-renewal capacity, high multilineage differentiation potential, and injury site homing capability (2). Marrow-derived mesenchymal stem cells (MSCs) are multipotent SCs that can differentiate into osteocytes, chondrocytes, tenocytes, and adipocytes (2). MSCs have been widely used in regeneration of connective tissues such as bone, cartilage, and fat. Neural stem cells (NSCs) are another type of multipotent SCs that are generally propagated into floating cell clusters called neurospheres (NSs) and further

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NLM Citation: Zhang H. Iron oxide nanoparticles-poly-L-lysine complex. 2008 Feb 20 [Updated 2008 Mar 26]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

differentiate into neurons, astrocytes, and oligodendrocytes (3). Embryo-derived stem cells (ESCs) are pluripotent SCs that can differentiate into virtually all cells of the human body. ESCs provide a replacement cell source to treat various diseases including age-related functional defects, hematopoietic and immune system disorders, heart failure, chronic liver injuries, diabetes, Parkinson's and Alzheimer's diseases, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers (4). Tracking the migration of transplanted SCs *in vivo* becomes a critical methodology in evaluating the efficacy of SC therapy (5). Among a variety of non-invasive imaging modalities, magnetic resonance imaging (MRI) can monitor the migration of SCs as long as a few months after the transplantation of paramagnetically labeled SCs (6). With its inherent high spatial resolution, MRI provides insight into several cellular processes including localization and migration of the cells, cell survival and proliferation kinetics, and cell differentiation of de-differentiation patterns, which can aid clinical implementation of cell therapy (7).

Most paramagnetic labeling of SCs is carried out with dextran-coated super paramagnetic iron oxide nanoparticles (SPIO) because of their good biodegradability and tolerance (8). Although SPIOs are known to accumulate in Kupffer cells and reticuloendothelial cells in the spleen after intravenous administration, they cannot be used to efficiently label nonphagocytic or non-rapidly dividing mammalian cells *in vitro* (9). SPIOs are internalized through formation of a complex with DNA transfection agents such as poly-L-lysine (PLL) to achieve ~100% efficiency of labeling cells (8). PLL contains positive charges and forms stable complexes with the negatively charged SPIO by simply mixing SPIO with PLL in cell culture media. The formation of the SPIO-PLL complex *via* electrostatic interaction alters the electrostatic interactions between the SPIO and the cell membrane, which initiates endocytosis and/or macropinocytosis of SPIO-PLL complexes (1). PLL (molecular weight ~400 kDa) is available from many manufacturers (8). Ferumoxides (SPIOs) as MRI contrast agents approved by the U.S. Food and Drug Administration for detection of liver lesion are also commercially available, including Feridex and Endorem (8). For having a dextran-coating, these commercial SPIOs can be directly used in the process of cell labeling. This labeling method can upload 10–20 pg Fe to each cell and appears not to affect the cell viability or the proliferation capacity (9). SPIO is usually detected as signal-loss (hypointense) areas in T_2*-weighted images in MRI. The substantial magnetic susceptibility effect of SPIO leads to a hypointense area much larger than the actual size of the particles, known as a "blooming effect" (10). This effect permits detection of ~20 labeled cells per 1,000 cells in a voxel (1 mm slice thickness, ~300 μm in plane resolution) by MRI at 1.5 T (9), the proliferation of labeled cells at up to 18 weeks (6), and a single labeled cell in mice at ultra-high magnetic fields (11).

Synthesis

[PubMed]

The SPIO-PLL complex is made by simply mixing SPIO and PLL in solution (8). Depending on the cell types, the ratio of SPIO to PLL varies between 1:0.03 and 1:0.06. Frank's group described a detailed procedure of labeling various SCs with commercial Feridex (SPIO) and a 388-kDa PLL as the starting materials (8, 9). For adherent cells such as human cervical carcinoma (HeLa) cells, human MSCs, and rhesus ESCs, or for rapidly growing cells such as small-cell lung carcinoma cells and human marrow-derived neural competent cells, the optimal concentration was 25 μg Fe/ml Feridex and 0.75 μg/ml PLL. For mononuclear cells such as mouse and marmoset T lymphocytes, hematopoietic SCs (CD34+), and mouse Sca1+, the optimal concentration was 25 μg Fe/ml Feridex and 1.5 μg/ml PLL. The labeling efficiency was ~100% for both incubation conditions.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The iron content in various labeled cells was quantified with nuclear magnetic resonance T_2 relaxometry (9). Typically, intracytoplasmic endosomal iron was found to be 1–3 pg/cell in T cells, 4–10 pg/cell in HeLa cells, and 15–20 pg/cell in MSCs, compared to 0.01–0.1 pg/cell in unlabeled cells (endogenous iron) (9). The degradation of SPIO-PLL complexes was examined under various physiological conditions (12). After mixing 25 μg Fe/ml SPIO with 0.75 μg /ml PLL in buffers of different pH levels, the T_2 and Fe(III) concentration were measured at 0, 6, 24, 48, 72, and 96 h as well as at 7, 14, and 21 days. SPIO-PLL complexes appeared to dissolve rapidly in buffers containing sodium citrate at pH 4.5; they were less soluble in the same buffer at pH 5.5, but they were not readily soluble in buffers containing sodium acetate or in cell culture media RPMI-1640 regardless of pH. To assess the stability of SPIO-PLL complexes inside the endosomes/lysomes (12), MSCs labeled with SPIO-PLL were analyzed by electron microscopy, T_2 relaxometry at 1.0 T, and MRI at 1.5 T. MSCs were incubated overnight in RPMI-1640 media containing 25 μg/ml SPIO and 0.75 μg/ml PLL. The labeled cells were collected at different time points from 1 h to 5 days for analysis. Electron microscopic imaging showed degraded SPIO-PLL in some intracellular endosomes/lysosmes between days 3 and 5. Some endosomes containing SPIO-PLL were found to fuse with lysosomes to cause rapid dissociation of SPIO within lysosomes at low pH. The viability of labeled HeLa/MSC was examined at days 1, 3, 7, and 15, and its value was ~100% at day 15. The paramagnetic labeling appeared not to alter the differentiation capacity of MSCs compared with the unlabeled cells.

The SPIO-PLL human central nervous system stem cell–derived NSs (hCNS-NSs) were tested for labeling efficiency, cell proliferation, cell differentiation, and electrophysiological behavior (6). The labeling efficiency was determined with Prussian blue staining, one of the most sensitive histochemical tests for iron-positive cells, which indicated that ~98% of cells were labeled. The cell viability was 92 ± 3% for SPIO-labeled hCNS-NSs compared with 96 ± 2% for unlabeled hCNS-NSs. No difference in the proliferation rate was observed between SPIO-labeled and unlabeled hCNS-NSs. The SPIO content per cell was approximately halved every 3 days in culture, which was

consistent with cell-doubling time. After 10-day differentiation, an average of 35.5 ± 4% of the cells were Nestin-positive, 56.2 ± 7.7% were β-tubulin-positive, and 19.4 ± 3.5% were glial fibrillary acidic protein–positive in the labeled cells. There was no statistically significant difference between the labeled and unlabeled cells. These percentages were found to be similar to the unlabeled cells. After 28-day differentiation, whole-cell patch-clamp recording showed no difference in electrophysiological characteristics for the labeled and unlabeled cells. Cell differentiation was also examined in SPIO-PLL–labeled MSCs (13), which exhibited an unaltered viability, proliferation similarity, normal adipogenic and osteogenic differentiation, but a marked inhibition of chondrogensis. The blocking of chondrogenic activity appeared to be mediated by the SPIO, not by the transfection agent PLL. MSCs of canine, porcine, or human (hMSC) origins demonstrated similar labeling efficiency (13), indicating the non-specific nature of SPIO uptake among different species. The long-term viability, growth rate, and apoptotic indexes of the labeled MSCs appeared to be unaffected by the endosomal incorporation of SPIO compared with the unlabeled cells. In non-dividing hMSCs, endosomal SPIO could be detected after 7 weeks; however, in rapidly dividing cells, intracellular iron disappeared at five to eight divisions (14).

Animal Studies

Rodents

[PubMed]

Yocum et al. studied the effect of SPIO-PLL–labeled MSCs on the physiology of rats after intravenous injection (15). hMSCs were incubated with 25 μg Fe/ml SPIO and 0.75 μg/ml PLL (molecular weight = 388 kDa). After the rats (n = 25, 125–250 g) received 2×10^6 labeled hMSCs (~10 pg Fe per cell) in 0.5 ml of phosphate-buffered saline, a series of measurements were conducted for 42 days, including complete blood counts, liver and renal function tests, and serum electrolyte and iron concentration measurements. The administration of labeled hMSCs appeared not to have a significant effect on hematology, blood chemistry indicators, or organ function. Hemoglobin concentration and mean corpuscular volume were altered significantly. Other properties such as serum electrolyte and iron concentrations changed significantly. To determine the half-life of labeled hMSCs in the circulation, 100 μl of venous blood was collected from the inferior vena cava before injection of the labeled hMSCs and at 1, 5, 15, 30, 60, and 120 min after injection of the labeled hMSCs. Prussian blue–positive MSCs were not detected at 15 min after injection, which was attributed to the homing of hMSCs to the liver, spleen, bone marrow, and other organs.

Ben-Hur et al. conducted a biodynamic study of the migration of transplanted cells to injured sites in chronic experimental autoimmune encephalomyelitis (EAE) mice (n=8) (16). Syngeneic mouse NSs (SM-NSs) or human ESC-derived NSs (hESC-NSs) were incubated with 25 μg Fe/ml Feridex and 0.375 μg/ml PLL (molecular weight = 388 kDa) and differentiated *in vitro* for 5 days. The lineage fate was immunostained with a lineage-

specific marker to verify that the paramagnetic labeling did not affect multipotency of the cells. Transplantation of the labeled cells was carried out on day 6 after EAE induction. Using a stereotaxic device, 150 SM-NSs (~100 cells per sphere) in 5 μl were transplanted into the lateral ventricle of eight EAE mice, and 250 hESC-NSs (~3,000 cells/sphere) were transplanted into both lateral ventricles of another eight EAE mice. MRI was performed at 4.7 T on days 1–2, 6–7, 13–14, and 30 after the transplantation to assess the different stages of disease. The results demonstrated that the labeled cell migration occurred along with white matter tracts (especially the corpus callosum, fimbria, and internal capsule), predominantly early in the acute phase of disease in an asymmetric manner. The distance of cell migration correlated well with clinical severity of disease and the number of microglia in the white matter tracts.

Guzman et al. examined the migration patterns of SPIO-labeled hCNS-NSs after they were transplanted into the brains of newborn and adult (injured and uninjured) rodents (mice and rats) by MRI at 4.7 T (6). Compared with the unlabeled cells, the SPIO-labeled hCNS-NSs appeared to have similar extent of survival, migration, integration, and differentiation after transplantation. All measurements of viability were performed in vitro. The test to check if the cells were alive was conducted through the test of differentiation then followed by histological analysis. In neonatal NOD-SCID mice (n = 16), the migration of SPIO-labeled hCNS-NSs spread along the natural tracts and differentiated into neurons and glia. hCNS-NSs were detected with MRI in the ventricular system immediately after transplantation. The labeled hCNS-NSs were found to distribute into the lateral and the fourth ventricle 3 weeks later and to migrate from the subventricular zone to the corpus callosum and olfactory glomerulus 9 and 12 weeks later. The migration remained visible at up to 18 weeks, which was further confirmed by double immunofluorescence stainings using human-specific and neuronal-specific antibodies. In stroke-injured rat brains (n = 10), transplanted SPIO-labeled hCNS-NSs were detected on T_2*-weight images at up to 5 weeks. To validate the MRI signal of labeled cells, dead and viable labeled cells were implanted on the left and right striatum of immunosuppressed rats, respectively. MRI was performed from day 2 to day 35. The day 2 images exhibited bilateral hypointense areas, which represented the grafts. Whereas minimal signal changes over 35 days were observed on the side with viable cells, a marked reduction in signal strength and graft volume appeared on the side with dead cells. For the nanoparticles in the dead cells, they could be endocytosized by macrophages, generating less intense contrast change than groups of viable cells containing many packed nanoparticles (11).

Amsalem et al. tracked the migration of SPIO-PLL–labeled MSCs in rats (n = 29) with myocardial infarction on a 0.5 T imager (17). Rat MSCs (rMSCs) were isolated from bone marrow of rats and labeled with SPIO-PLL. Seven days after the induction of myocardial infarction, 2×10^6 SPIO-labeled MSCs were injected into the anterior wall of the left ventricle. MRI was performed at 1, 2, and 4 weeks after cell delivery. Hypointensities induced by labeled rMSCs were visible at up to 4 weeks. Ju et al. conducted intrasplenic transplantation of 6×10^6 labeled MSCs cells into rats (n = 6) with liver damage (18). MRI was performed at 1.5 T to track the migration of the cells. The contrast/noise ratio on T_2*-weighted images of the liver appeared to decrease significantly 3 h after injection of the

labeled MSCs; this ratio returned gradually to the level achieved without labeled cell injection in 14 days. Ko et al. reported a migration study of scaffold model in mice (n = 30) at 1.5 T (10). Gelatin sponge that contained SPIO-PLL–labeled hMSCs was implanted in nude mice (6-week-old BALB/C-nude mice). The hypointense areas were observable at up to 4 weeks on T_2^*-weighted images.

Other Non-Primate Mammals

[PubMed]

Kraitchman et al. used MRI at 1.5 T to track the migration of labeled MSCs in swine (sMSCs) with myocardial infarction (19). sMSCs were incubated with 25 μg Fe/ml Feridex and 375 ng/ml PLL (molecular weight = 275 kDa) for 2 h. The labeled sMSCs were injected intramyocardially into swine (n = 5, 25–35 kg), guided by x-ray fluoroscopy. The injected animal had a volume of 4–16 ml at a concentration of 7×10^6 to 1×10^7 cell/ml. The total number of cells injected ranged from 2.8×10^7 to 1.6×10^8. At 24 h after injection, images of the heart exhibited ovoid hypointense lesions with sharp borders at the injection sites. One week later, the delineation of the borders became less clear. The hypointense lesions were expanded by 15% with a 24% deduction in contrast.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

NS 045062, HL 63439, HL 04193, HL 45090, HL 65455, NS 27292, NS37520

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