N-Alkyl-polyethylenimine 2 kDa–stabilized superparamagnetic iron oxide nanoparticles for MRI cell tracking

Alkyl-PEI2k/SPIO

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Chemical name:	*N*-Alkyl-polyethylenimine 2 kDa–stabilized superparamagnetic iron oxide nanoparticles for MRI cell tracking	
Abbreviated name:	Alkyl-PEI2k/SPIO	
Synonym:	*N*-alkyl-PEI2k/SPIO, Alkyl-PEI/SPIO	
Agent Category:	Nanoparticles	
Target:	Others (mesenchymal stem cell labeling)	
Target Category:	Non-targeted	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Superparamagnetic iron oxide (SPIO)	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

N-Alkyl-polyethylenimine 2 kDa (PEI2k)–stabilized superparamagnetic iron oxide (SPIO) nanoparticles, abbreviated as alkyl-PEI2k/SPIO, is a contrast agent that was synthesized by Liu et al. for stem cell labeling and tracking with magnetic resonance imaging (MRI) (1).

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MRI of SPIO-labeled stem cells has been considered to be the best choice for *in vivo* cell tracking because of its ability to follow the labeled cells for months as well as to provide detailed anatomic information with high resolution and soft tissue contrast (2, 3). Cells are labeled *in situ* by direct injection of the SPIO-based agents into the tissue area of interest; cells are labeled *in vivo* by intravenous administration of the agents (4, 5). In both preclinical and clinical situations, cells are typically labeled *in vitro* for cell-tracking studies (4). Generally, SPIO nanoparticles need to be coated with organic polymers or other materials for efficient cell labeling. This surface coating increases the stability of SPIO nanoparticles and allows further chemical modifications with ligands (1, 4). In addition, the surface coating could influence the SPIO distribution within cells, which would thus influence the T2 relaxivity. However, there are still a lot of challenges to turn MRI cell tracking into a robust technique either in preclinical settings or in clinical applications. For example, it is impossible to discriminate live cells from dead cells, and to differentiate transplanted cells from macrophages. MRI quantification of both iron oxide concentration and cell number is also not reliable.

It has been shown that SPIO particles clustered in dense vacuoles yield better local contrast enhancement than SPIO particles distributed in cytoplasm (1, 6). Controlled clustered SPIO nanoparticles can greatly shorten T2 relaxation time in comparison with single SPIO nanoparticles at the same iron concentration (1, 6). *N*-Alkyl-polyethylenimine 25 kDa (PEI25k) is a commercial reagent used for gene transfection. Wang et al. applied PEI25k to stabilize SPIO, and the generated nanoparticles exhibited a controlled clustering structure, efficient cell uptake, and high T2 relaxivity when they were used to label mesenchymal stem cells (MSCs) (6). However, PEI25k has been found to be toxic to labeled cells, resulting in cell death, apoptosis, and differentiation inhibition. In comparison, lower molecular weight PEI, such as PEI2k, is more biocompatible. Liu et al. applied PEI2k to form stable nanocomplexes with SPIO (1). The generated nanoparticles, alkyl-PEI2k/SPIO, could hold multiple SPIO particles with a controlled clustering structure. Labeled MSCs showed no evident toxic effect on their viability, proliferation, and differentiation capacity.

Related Resource Links:

- Chapters on cell tracking in MICAD
- Clinical trials for cell tracking in ClinicalTrials.gov

Synthesis

[PubMed]

Liu et al. described the synthesis of alkyl-PEI2k/SPIO in detail (1). Branched PEI2k was reacted with 1-iodododecane in ethanol to generate alkylated PEI2k, which was confirmed with ^{1}H nuclear magnetic resonance with ~11% dodecyl substitution. The SPIO nanoparticles dispersed in chloroform were then mixed with alkylated PEI2k at different polymer/SPIO mass ratios to generate alkyl-PEI2k/SPIO. The alkyl-PEI2k/SPIO

nanoparticles were characterized with dynamic light scattering (DLS), zeta potential, atomic force microscopy (AFM), and transmission electron microscopy. T2 relaxivity was measured at room temperature with 3 T and spin-echo sequence (TR = 5,000 ms, TE = 10–500 ms).

The alkyl-PEI2k/SPIO nanoparticles displayed positive charges at ~40 mV, which was not significantly affected by the PEI2k polymer/SPIO mass ratios and the micelle size. The T2 relaxivity values were 345, 121, and 84 Fe $mM^{-1}s^{-1}$ for the nanoparticles synthesized at polymer/SPIO ratios of 0.6, 1.2, and 2.5, respectively. At the polymer/SPIO mass ratio of 0.6, the generated alkyl-PEI2k/SPIO nanoparticles were well dispersed without obvious aggregation and each nanoparticle was composed of a cluster of a few closely-packed SPIO particles. The diameters of the alkyl-PEI2k/SPIO nanoparticles were 54.7 ± 9.5 and 65 ± 17.2 nm as determined with DLS and AFM, respectively. The sensitivity limit was ~2 µg Fe/ml, which is defined as the alkyl-PEI2k/SPIO concentration at which MRI signal intensity decreases to 50% of that for pure water in T2-weighted images. The alkyl-PEI2k/SPIO nanoparticles with the *N*-alkyl-PEI2k/SPIO mass ratio of 0.6 were selected by Liu et al. for further testing because of the lower PEI requirement and controlled clustering of SPIO nanoparticles with high T2 relaxivity and stability (1).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

MSCs were labeled with alkyl-PEI2k/SPIO for cell tracking studies (1). MSCs were collected from femurs of male Balb/C mice (18–20 g) and were cultured for three to five passages before use. MSCs were incubated with alkyl-PEI2k/SPIO at different concentrations (0.5, 1, 3.5, and 7 µg Fe/ml) for different incubation periods (1, 6, and 24 h). Cells without exposure to alkyl-PEI2k/SPIO were used as controls.

The SPIO uptake in MSCs was time- and dose-dependent. Prussian blue staining showed that nearly 100% of the MSCs were labeled at a concentration of 7 µg Fe/ml for 24 h incubation, which was further confirmed with flow cytometry (93 ± 2%). Cells labeled with a concentration of 0.5 µg Fe/ml for 24 h had iron content similar to that observed at a concentration of 7 µg Fe/ml after 1 h incubation ($P > 0.05$). The uptake quantities sufficient for cellular MRI (>2 pg Fe/cell) were achieved when labeling at a concentration of 1 µg Fe/ml for 24 h and the cellular iron content increased to 7.1 pg/cell in MSCs treated with 7 µg Fe/ml for 24 h incubation.

Cells incubated with 0.5, 1, 3.5, and 7 µg Fe/ml exhibited viability comparable to that of unlabeled control cells based on cell viability assays. There was no obvious morphological difference between labeled and unlabeled cells. No significant apoptosis was induced by SPIO labeling. The alkyl-PEI2k/SPIO nanoparticles were observed in the intracellular space, not in the cell nuclei.

The alkaline phosphatase activity of the labeled cells was 32.5 ± 1.8 U/l, similar to that of the control cells (34.4 ± 1.9 U/l) ($P > 0.05$), indicating that the labeling didn't compromise

the function of osteogenic differentiation, which was also demonstrated with alizarin red staining. The chondrogenic differentiation capability was also unaffected by SPIO labeling as indicated by the staining of proteoglycan-rich extracellular matrix with safranin O and toluidine blue. SPIO labeling led to a significant decrease of the transferrin receptor (TfR) mRNA expression in labeled MSCs as early as 1 d after labeling compared with unlabeled MSCs. A slight recovery of the TfR expression was then observed after the first passage (3 days after labeling), possibly due to feedback of the intracellular iron. For ferritin, after treatment with indicated concentrations of SPIO for 1 d or 3 d, an increasing trend was observed in its mRNA level, which was significantly higher in labeled MSCs than in control cells. Downregulation of TfR and upregulation of ferritin in labeled cells may protect cells from possible cytotoxicity induced by higher intracellular iron concentrations (1, 7).

Animal Studies

Rodents

[PubMed]

Labeled and unlabeled MSCs in collagen hydrogels were separately injected subcutaneously into the flank of mice and monitored for 19 days with MRI (1). T2-Weighted MRI showed that the labeled MSCs were visible as a hypointense area with sharp borders at the injection site. T2 values decreased from 163 ms to 46 ms as the number of cells increased (10^4–10^6). The signal intensity ratios of the control/labeled cells were 30.1, 15.3, 11.7, and 8.9 on days 1, 3, 7, and 19 after injection, respectively. The hypointensity of the labeled MSCs remained visible for at least 19 days. *Ex vivo* hematoxylin and eosin staining of the cells demonstrated that both labeled and unlabeled cells were alive 19 days after implantation. Prussian blue staining showed that a large number of blue-positive cells were viable at the injection site. These results indicate that the hypointense MRI images were induced by SPIO labeling.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

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