# MR Tracking of Dendritic Cells Homing to the Draining Lymph Nodes in Mice

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**Statement of Purpose:** Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. As such they are currently used in clinical vaccination protocols for treatment of various diseases. Correct delivery and subsequent migration of vaccinated DCs to regional lymph nodes is of great importance for effective stimulation of the immune system. MRI is a widely used imaging technology in clinical practice. Using magnetically labeled DCs has proved efficient to monitor the efficacy of vaccination procedures. In this study, we explore to *in vivo* track, with success, the migration of DCs by labeling with Alkyl-PEI2k-IOs, which we synthesized and successfully applied to labeling several other cells as reported previously. The results suggested that Alkyl-PEI2k-IOs provided an important alternative to label DCs at optimized low dosages with high efficiency and low cytotoxcity.

**Materials:** 1-iodododecane , Branched PEI2K, 1,2-hexadecanediol, oleic acid, oleylamine, Iron(III) acetylacetonate, benzyl ether, Poly(allylamine) hydrochloride (PAH, Mw 15kD), Poly(anetholesulfonic acid, sodium salt) (PAS, Mw 10kD) were purchased from Aldrich Chemical Co.. Fetal bovine serum, RPIM 1640 medium, penicillin/streptomycin were purchased from Hyclone, while BALB/c mice from the Center of Laboratory Animal of Sichuan University.

**Methods:** N-alkyl-PEI2k stabilized SPIO nanoparticles were synthesized. DCs were generated and induced from bone marrow cells of mouse following the procedures reported by Lutz. To magnetically label the cells, the SPIO nanocomposites was incubated with DCs overnight. After labeling, assays of labeling efficiency, cell viability and phenotype were performed by Hochest stain, confocal laser scanning microscopy (CLSM), flow cytometry, Perls' staining and transmission electron microscopy (TEM). Then i*n vitro* MRI of SPIO-labeled murine DCs was done to check the MRI sensitivity of the SPIO probe labeling. Finally, *in vivo* MRI was undergone for tracking of DCs home to the draining lymph nodes.

**Results:** Alkyl-PEI2k-IOs were prepared following our previous protocol. Bone marrow-derived DCs (BM-DCs) were induced to differentiate *in vitro* with a yield of about $1\times10^7$ cells/mice. DCs can be sufficiently labeled with SPIO nanocomposites by adding 10 ugFe/ml probes to the culture medium after more than 6 h incubation, which were further confirmed by Perls' staining, CLSM and TEM (Fig. 1 and 2). There was no obvious morphological change between the labeled and unlabeled cells. Hoechst staining also revealed no probe-associated apoptosis introduced by SPIO labeling. The colorimetric ferrozine assay showed the SPIO uptake process in DCs was a time- and dose-dependent behavior. The major surface markers were studied by flow cytometry and compared between labeled and unlabeled DCs. Resultantly, no considerable difference was found between them. *In vitro* MRI demonstrated that T2 values decreased with the increase of cell number. *In vivo* MRI was performed on clinical 3T scanner with an optimized combination of measurement sequences after injection of titration number of DCs into the footpads of the mice. SPIO-labeled DCs were clearly depicted homing into the draining lymph nodes when cell number exceeded $1\times10^5$ (Fig. 3 a-d).

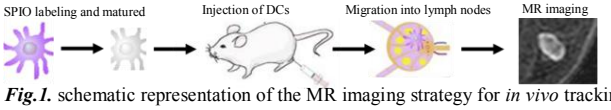


***Fig.1.*** schematic representation of the MR imaging strategy for *in vivo* tracking the process of DC homing into the draining lymph nodes.

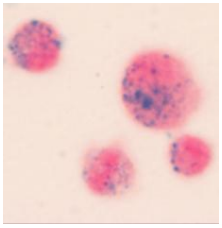

***Fig.2.*** Perls' staining showed the blue-stained iron-contained particles within cytoplasm of DCs.

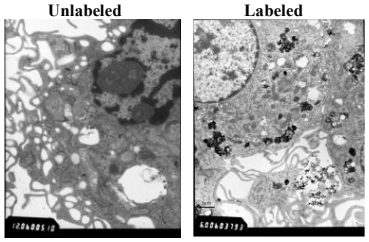


***Fig.3.*** TEM disclosed dispersed intracytoplasm dense deposits representing endocytosed SPIO nanocomposites. The unlabeled DC did not show the same phenomenon.

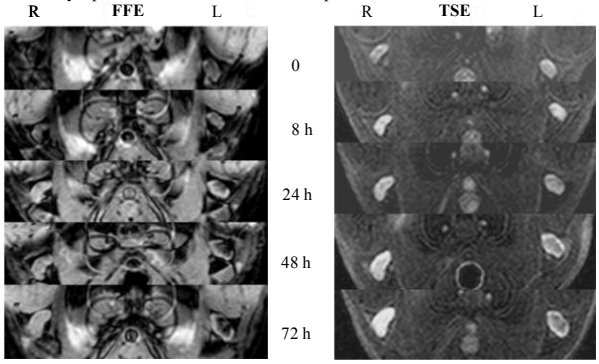


***Fig.4.*** *In vivo* imaging on 3T MR scanner of DC home to the draining lymph node (LN) with FFE and TEE sequence at different time point. MRI clearly captured the signal drop within the left LN after injection of $1\times10^6$ cells. Comparatively, the right control LN did not exhibit this same sign.

**Conclusion:** Alkyl-PEI2k-IOs demonstrate highly efficient labeling of DCs without exhibiting cellular toxicity, making it a potential candidate to visualize the efficacy of DC-based therapy.

**Acknowledgement:** The authors acknowledge the financial support of National Key Basic Research Program of China (2013CB933903)

**References:** 1) Liu G, et al. Biomaterials, 2011, 32:528. 2) Xie J, et al. Acc Chem Res, 2011,44:883. 3) Shan L. http://www.ncbi.nlm.nih.gov/books/NBK52895/.

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