Negatively Charged MRI Nanoprobes for Dendritic Cell Labeling and *in Vivo* Tracking

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Statement of Purpose: Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. DC based cell therapies are currently approved or under clinical trials for treatment of cancer, diabetes and other diseases. Efficient delivery and subsequent migration of vaccinated DCs to regional lymph nodes is of great importance for effective stimulation of the immune system. However, monitoring of cell trafficking inside body is not feasible without imaging techniques. Magnetic resonance imaging (MRI) is a widely used imaging technology in clinical practice. DCs labeled with imaging probes provides opportunity for one to track them in vivo noninvasively and dynamically. In this study, we explore to monitor the migration of DCs *in vivo* by labeling with a negatively charged MRI probe, poly (aspartic acid)-b-poly(ε-caprolactone)/super paramagnetic iron oxide (PAsp-PCL/SPIO).

Methods: 1) The amphiphilic block copolymer PAsp-PCL was synthesized by ring-opening polymerization. Then, organic phase SPIO nanoparticles were prepared by high temperature decomposition method,[1] and transferred into aqueous phase with the help of amphiphilic polymer PAsp-PCL to obtained PAsp-PCL/SPIO nanoprobe. 2) DCs were seeded at 2×10^5 cells/mL, and incubated with PAsp-PCL/SPIO nanocomposites (10 μg Fe/mL) for 24 h, and maturated with LPS and TNF-α.[2] Labeling efficiency, cell viability and phenotypes were performed by measuring intracellular iron content, DNA fluorometric assay and fluorescence activated cell sorter (FACS). 3) TNF-α (30 ng/leg) was injected into hind legs footpads of BALB/c mice. 24 h later, the mice were injected in the left footpads with 1×10^6 labeled DCs and in the right footpads with the same number of unlabeled DCs. MRI was performed at different time points using the 3 T scanner.

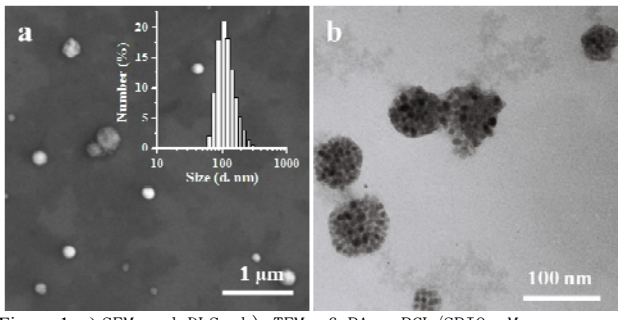

Figure 1. a) SEM and DLS, b) TEM of PAsp-PCL/SPIO. Mean diameter was 123.9 ± 41.0 nm in DLS.

Results: In Figure 1, SEM shows that Obtained PAsp-PCL/SPIO nanocomposites have spherical shape and good dispersion. TEM shows that SPIO nanocrystal are largely presenting as isolated clusters with dense packing. Mean zeta potential was -27 mV. Results of intracellular iron content show that PAsp-PCL/SPIO displayed excellent labeling efficiency for DCs. In cytotoxicity study, DCs and Raw 264.7 viability are all surpassing 90% even in a high iorn concentration (40 μg/mL). In flow cytometry detection (Table 1), high expression of CD11c confirm the purity of DC. Molecular markers that represent cellular maturation (CCR7, MHC-II, CD80 and CD86) were all expressed with a similar level between the unlabeled and labeled DCs, and both presented obviously increased expression comparing to the immature DCs.

Table 1. Flow cytometry analysis of molecular makers in dendritic cells.

	Molecular makers				
	CD11c (%)	CCR7 (%)	MHC-II (%)	CD80 (%)	CD86 (%)
a	87.9	38.4	93.3	55.1	15.2
b	92.0	80.4	99.3	87.2	51.0
c	94.0	81.7	98.9	80.1	54.8

a, immature DCs; b, unlabeled and mature DCs; c, labeled and mature DCs

The migration process of DCs *in vivo* was monitored by MRI. In TSE images, we can see significantly signal intensity reduction with time in central areas of left (L) lymph node, while there was obvious changes in right (R) lymph node. T_2-map images showed that T_2 values of right lymph node gradually increased, while T_2 values of central areas of left lymph node significantly reduced with time. Results indicated labeled DCs migrated from footpad to the draining lymph node, and induced MRI signal intensity changes and T_2 time reduction.

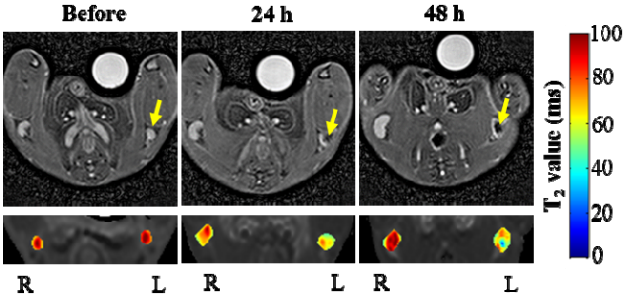

Figure 2. *In vivo* imaging of DC home to the draining lymph node under a 3.0 T MR scanner. Above: T_2-weighted TSE images; below: T_2-map images

Conclusions: Negatively charged MRI nanoprobe, PAsp-PCL/SPIO, has efficient DCs labeling capability and low cytotoxicity. DCs labeled with this probe can migrate to the draining lymph nodes from the side of injection, and this lymph node signal change was captured under MRI.

References: 1. Sun, S. *J. Am. Chem. Soc*. 2004; 126: 273. 2. Martín-Fontecha, *A. J. Exp. Med*. 2002; 198: 615