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Synthetic and biogenic magnetite nanoparticles for tracking of stem cells and dendritic cells

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ABSTRACT

Accurate delivery of cells to target organs is critical for success of cell-based therapies with stem cells or immune cells such as antigen-presenting dendritic cells (DC). Labeling with contrast agents before implantation provides a powerful means for monitoring cellular migration using magnetic resonance imaging (MRI). In this study, we investigated the uptake of fully synthesized or bacterial magnetic nanoparticles (MNPs) into hematopoietic $Flt3^+$ stem cells and DC from mouse bone marrow. We show that (i) uptake of both synthetic and biogenic nanoparticles into cells endow magnetic activity and (ii) low numbers of MNP-loaded cells are readily detected by MRI.

Engineered magnetic nanoparticles (MNPs) are currently emerging as promising tools in numerous applications for medical diagnosis and therapy, such as drug delivery systems or contrast agents for magnetic resonance imaging (MRI). Cellular therapies using stem cells and immune cells, such as dendritic cells (DC), are increasingly applied in clinical trials. DC are professional antigen-presenting cells that play a key role in the induction of primary immune responses and have been implicated in determining the balance between immunity and tolerance induction [1,2]. This makes DC a particularly attractive target for the development of therapeutics in pathological situations, such as autoimmune diseases, cancer and cardiovascular diseases, and thus DC are readily used as cellular vaccines in clinical trials [3].

Accurate delivery of DC to target organs and migration of the cells for effective antigen presentation and activation of an immune response is essential for the success of such therapies [4]. However, the ability to non-invasively monitor cell trafficking or a specific cellular function at the target site after application is rather limited. MRI is well suited for obtaining three-dimensional (3D) high-resolution images and is now widely used in clinical practice [5]. Stable labeling of cells with contrast agents has proven successful for MRI-based detection of cell deposits and their migration [6–9]. Most applications provide information about the location but not about a cellular functional status. First attempts have been made to develop functionalized MNPs that combine labeling of stem cells and DC for monitoring cellular localization and additional activities (e.g. adjuvant function of DC and measuring of DC function *in vivo*) in one MNP formulation [10,11]. For this purpose two strategies are currently being considered. First, fully synthetic iron oxide-based MNPs are used that allow functionalization by chemical modifications. Second, magnetosomes from magnetotactic bacteria are taken into consideration. Magnetosomes are organelles that comprise nanometer-sized crystals of magnetite enveloped by a biological membrane composed of phospholipids and specific proteins. The magnetosome membrane is critical for magnetosome stability, the control of magnetite crystal size and morphology, and additionally provides a matrix for functionalization of magnetosomes. This can be achieved by chemical modifications but moreover, and potentially superior to chemical approaches, by genetic engineering of the magnetosome membrane proteins.

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We have recently described the synthesis and physicochemical properties of synthetic oleate-stabilized magnetite MNPs [12], where the lipid–shell contributes to improved biocompatibility (in the following referred to as lipid–shell MNPs). Additionally, it has allowed further functionalization, such as biotin-conjugation and binding of the fluorescence tag streptavidin–fluorescein isothiocyanate (FITC) [10].

In this study, we investigated the uptake of fully synthetic lipid–shell MNPs and of magnetosome MNPs into hematopoietic $Flt3^+$ stem cells and DC from mouse bone marrow [13–15]. We show (i) that uptake of both synthetic and biogenic nanoparticles into cells endow magnetic activity on $Flt3^+$ stem cells and DC (ii) that low numbers of MNP-loaded cells are readily detected by MRI.

Superparamagnetic magnetite lipid–shell MNPs were obtained by coprecipitation of Fe^{2+} and Fe^{3+} ions with NH_3 and stabilized with *cis*-9-octadecenoic acid sodium salt (oleate) as described [12]. Magnetosomes, also referred to as biogenic MNPs, were isolated and purified from the magnetotactic bacterium *Magnetospirillum gryphiswaldense* strain MSR-1 (DSM 6361) as described earlier [16]. Sterile solutions of MNPs containing 1 mg Fe/ml were used in 1:50 dilution for cell labeling.

DC were differentiated from hematopoietic $Flt3^+$ stem cells (referred to as $Flt3^+$ stem cells) of bone marrow suspensions from C57BL/6 mice (Charles River, Sulzfeld, Germany) as described [14]. DC were seeded at 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin (all from Gibco-BRL) and 50 μM β-mercaptoethanol (Sigma-Aldrich, Taufkirchen, Germany) containing 200 U/ml of recombinant mouse GM-CSF and were incubated with sterile-filtered and buffered aqueous solutions of magnetosomes or lipid–shell MNPs 24 h before analyses. Cell numbers were determined with an electronic cell counter device (CASY1, Schärfe System, Reutlingen, Germany).

Magnetic resonance (MR) images of agarose phantoms were acquired using a Bruker Biospin 7.0 T scanner (Ettlingen, Germany). MNP-labeled cells were suspended in 10 μl agarose (Sigma-Aldrich) and filled in 3.5 mm diameter drill holes of a 3.5 cm agarose phantom as described before [17]. Dissected lymphnodes (LN) from animal experiments were embedded into 1.6% agarose phantoms. MR images were acquired as described [17]. Phantoms were analyzed with T2*-weighted 3D gradient echo FLASH sequences (TE = 12 ms, TR = 200 ms and TE = 14 ms, TR = 500 ms, respectively; flip angle = 30°) with an isotropic resolution of 78 μm^3 (for cell phantoms) and 55 μm^3 (for LN).

The interaction of MNPs with cells was first investigated by transmission electron microscopy (TEM) to determine uptake of MNPs into cells, intracellular localization and particle morphology before and after uptake. In aqueous solution, lipid–shell MNPs possess a mean iron core size of 8–10 nm, whereas magnetosomes have a mean iron core size of 40–45 nm (Fig. 1a and b). In initial studies, uptake of MNP into DC was determined since DC are particularly potent in incorporating particles by endocytosis. After co-culture with MNPs for 24 h, DC were extensively washed with phosphate buffered saline (PBS) and fixed with 3% (w/v) glutaraldehyde. Cells were stained with OsO_4 and viewed and recorded with a Philips EM 400 T electron microscope equipped with a CCD camera. Fig. 1c–f reveal that both lipid–shell MNPs and magnetosome MNPs are localized intracellularly in numerous vesicles confined to the cytosol. MNPs are not found at the cell surface or in the nucleus. These results demonstrate effective uptake of both synthetic and biogenic MNPs into DC and suggest an endocytotic uptake mechanism. However, particle size and iron core morphology remained unaltered, 24 h after uptake into DC (Fig. 1a, b, d and f).

We then proceeded to determine whether MNP-loaded $Flt3^+$ stem cells and DC acquired magnetic activity and were retained in a magnetic field. $Flt3^+$ stem cells and DC were treated with increasing concentrations of MNPs or left untreated for 24 h. MNPs exhibited no cytotoxicity on both $Flt3^+$ stem cells and DC (data not shown). Cells were recovered and extensively washed with PBS to remove unbound MNPs and then applied to a magnetic field in Miltenyi MS columns placed in a MiniMACS separator (Miltenyi Biotec, Bergisch Gladbach, Germany). In this assay, only MNP-labeled cells are retained in the magnetic field of the column. Columns were washed with PBS and not-retained cells collected. Retained cells were eluted with elution buffer (PBS containing bovine serum albumin (BSA) and EDTA; Sigma-Aldrich) after withdrawal of magnet. The numbers of both retained and not-retained cells were determined; untreated cells served as control. Unlike control cells, we only observed MNP-loaded $Flt3^+$ stem cells and DC being retained in the magnetic field (Fig. 2a). This was confirmed by Prussian-blue staining for iron in not-retained and retained DC populations after magnetic separation showing complete absence of MNPs in not-retained cells (Fig. 2b). Retention of MNP-labeled cells was dose-dependent (data not shown) and Fig. 2 shows results obtained with optimal conditions for DC labeling with both lipid–shell MNPs and magnetosomes. Under these conditions, the labeling efficiency of MNPs for $Flt3^+$ stem cells yielded lower rates than for DC. Interestingly, magnetosomes and lipid–shell MNPs revealed different labeling potentials for $Flt3^+$ stem cells with a two-fold higher labeling of $Flt3^+$ stem cells by magnetosomes (Fig. 2a). These results suggest different uptake mechanisms for $Flt3^+$ stem cells and DC, which probably critically depends on MNP shell composition.

Next, we determined the intracellular iron concentration after MNP uptake into cells by employing a ferrozine-based colorimetric assay as a direct measure for MNP quantity [18]. $Flt3^+$ stem cells and DC were cultured in the presence of lipid–shell MNPs or magnetosome MNPs comprising a total of 20 μg/ml iron (f.v.) or were left untreated. After 24 h, cells were intensively washed and subjected to magnetic separation with Miltenyi MS columns. MNP-labeled cells were eluted from the columns and then lysed for iron release. Untreated cells were examined accordingly. Ascorbic acid was used to reduce Fe^{3+} to Fe^{2+} ions that form a chelate complex with ferrozine. Absorbance of Fe^{2+}–ferrozine was measured at 550 nm and compared to the absorbance of $FeCl_3$ standards. The minimum detection limit for Fe^{2+} ions in this assay set-up was 250 μmol/l, corresponding to 5 pg of iron per cell. Iron concentrations in untreated $Flt3^+$ stem cells and DC were below the detection limit. The results obtained show a higher uptake capacity of DC for MNPs than $Flt3^+$ stem cells (Fig. 3). The intracellular iron concentration after labeling with lipid–shell MNPs was 77.9 ± 5.8 pg/cell in DC and 13.6 ± 1.6 pg/cell in $Flt3^+$ stem cells, respectively. Interestingly, labeling with magnetosomes resulted in lower iron concentration in DC (31.3 ± 5.4 pg/cell) but led to slightly increased uptake in $Flt3^+$ stem cells (16.8 ± 1.7 pg/cell) concomitant with the higher labeling efficiency of $Flt3^+$ stem cells by magnetosomes (Figs. 2a and 3). These findings further support the notion of different uptake mechanisms for MNPs in $Flt3^+$ stem cells and DC.

Iron oxide nanoparticles are well known to possess longitudinal (T1) and transversal (T2 and T2*) relaxation time shortening effects in magnetic resonance that make them particularly attractive as potential contrast agents for MR imaging [5]. MR relaxometry experiments with the lipid–shell MNPs and bacterial magnetosome MNPs used in this study have been recently performed. Low R1 relaxivities of 4.0 and 3.2 $s^{-1}\,mM^{-1}$ and high R2* relaxivities of 729 and 1198 $s^{-1}\,mM^{-1}$ were reported for lipid–shell MNPs and magnetosome MNPs, respectively, classifying them as potent contrast agents for both T1 and T2*-weighted MR imaging [12,19]. Therefore, we investigated whether both lipid–shell and bacterial MNPs are particularly suitable contrast agents for cell tracking by MRI after uptake. To this end