Methodology

Dendritic cell uptake of iron-based magnetic nanoparticles

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Abstract

We have investigated the internalization of magnetic nanoparticles (NPs) into dendritic cells (DCs) in order to assess both the final location of the particles and the viability of the cultured cells. The particles, consisting of a metallic iron core covered with carbon, showed no toxic effects on the DCs and had no effect in their viability. We found that mature DCs are able to incorporate magnetic nanoparticles in a range of size from 10 nm to ca. 200 nm, after 24 h of incubation. We describe a method to separate cells loaded with NPs, and analyze the resulting material by electron microscopy and magnetic measurements. It is found that NPs are internalized in lysosomes, providing a large magnetic signal. Our results suggest that loading DCs with properly functionalized magnetic NPs could be a promising strategy for improved vectorization in cancer diagnosis and treatment.

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1. Introduction

The recent advances in synthesis routes and functionalization of magnetic nanoparticles (NPs) have enabled new opportunities for scientific research in the fields of new materials, spintronics, and biomedicine. For the latter, the use of magnetic NPs can be already found in routine laboratory and clinical protocols, such as cell sorting, DNA separation, Magnetic Resonance Imaging (MRI) and gene therapy (Pankhurst et al., 2003; Hafeli et al., 1997; Tartaj et al., 2003). Currently, eukaryotic cells can be easily targeted with magnetic nanoparticles of a given size, since the dimensions of such particles are comparable to any subcellular structure. However, the central question of how a given cell type recognizes and incorporates a passive nanoparticle is not yet completely understood.

Current improvements in this field rely on new functionalization processes of the NPs with specific ligands for targeting specific cell membrane receptors (Jaulin et al., 2000). The use of NPs for targeted delivery of anticancer agents and molecular diagnosis, developed along the last years, has been already reviewed in detail (Fortina et al., 2005). Loading magnetic NPs with specific anticancer drugs and targeting them to specific tumor sites is a promising strategy for detection and elimination of neoplastic cells disregarding their physical size, which in turn could stop metastatic cells from proliferating. However, it is now generally recognized that the reticulo-endothelial system (RES) is a very effective system that detects and phagocytes NPs, preventing their therapeutic function. This high effectiveness makes necessary for any system of NPs to be used as targeting carrier to mimic biological units in order to pass through the RES without being detected.

The use of charged cells as selective biological vectors for in vivo applications is a promising strategy to avoid the subtle problems related to the response of a living organism to alien objects (i.e., NPs-drug assembly). Specifically, the injection of nanoparticle-loaded DCs into the blood system appears as...

Abbreviations: DCs, dendritic cells; NPs, nanoparticles; Fe@C, carbon-coated iron nanoparticles; RES, reticulo-endothelial system; $M_s$, saturation magnetic moment.

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a valuable drug-delivery strategy for tumor targeting, since the cargo is delivered inside a known object (the DCs) and therefore no RES action against these carriers is expected. DCs are the most potent antigen-presenting cells to naive T-cells, triggering antigen-specific immune responses. These cells can be obtained from myeloid or plasmocitoid progenitors (Ito et al., 2005). DCs obtained from myelomonocytic progenitors (MDCs) and primed with tumor antigens have shown antitumor activity when inoculated in animal models (Mayordomo et al., 1995) and humans (Nestle et al., 1998). Recently, the induction of endothelial cell features in MDCs cultured in the presence of angiogenic factors like VEGF has been demonstrated (Conejo-Garcia et al., 2004) and similar cells have been isolated from human tumor vessels (Conejo-Garcia et al., 2005), opening the possibility of targeting MDCs which have incorporated magnetic nanoparticles to tumor sites and use them to visualize tumors by magnetic resonance imaging (MRI) (De Vries et al., 2005) or for oncologic therapy: in this respect, magnetic inductive hyperthermia can effectively kill tumors to which sufficient numbers of nanoparticles have been delivered.

The importance of magnetic NPs for these applications is not only due to their role as passive carriers, but actually on their concurrent multi-tasking capacities such as (a) having a magnetic core for location as MRI contrast agent; (b) having power absorption capacity for drug release or hyperthermic ablation; or (c) having porous surface to store specific drugs for long-term release by magnetic field stimulation. The efficiency of magnetic NPs for a given application will depend strongly on specific characteristics of the constituent material. An important property is related with high magnetic moment required in order to reduce the clinical doses and minimize possible toxicity effects. In this context, iron nanoparticles are promising due to its high Curie temperature (Tc) and saturation magnetic moment (Ms ∼ 211 emu/g). The manufacture of iron magnetic NPs measuring few nanometers (ca. 10 nm) has been developed many decades ago and there are many well established synthesis routes. However, to synthesize these particles keeping the magnetic moment of the bulk material is a difficult challenge because the high surface/volume ratio makes the effects from surface disorder become dominant.

In this work, we present results on the inclusion of magnetic nanoparticles into dendritic cells, in order to assess the viability of DCs charged with magnetic NPs. We have found a simple and precise method to detect the incorporation of magnetic NPs into DCs, which has the potential to quantify the amount of magnetic material absorbed.

2. Materials and methods

2.1. Synthesis of nanoparticles

The synthesis route for the magnetic nanoparticles used in this work has been reported elsewhere (Fernandez-Pacheco et al., 2006). Basically, it is based on a modification of the Kratschmer–Huffmann arc-discharge method to prepare iron nanoparticles coated with carbon layer (labeled as Fe@C hereafter). The synthesis is performed at temperatures ca. 3000 °C, at which the iron electrode and the silica powders are sublimed, and then condensed in colder areas of the system. The particles are collected as a deposit on the inner surfaces of the chamber, washed several times and finally different fractions are separated by means of a magnetic field gradient.

2.2. Cell culture and NPs’ loading

Following informed consent, mononuclear cells obtained from 14 ml of blood of healthy volunteers were cultured during seven days with AIM-V media with Interleukin 4 (10 U/ml) and Granulocyte–macrophage colony stimulation factor (10 U/ml). On day 5, 5 µg/ml of media containing lypopolysacharides were added to induce maturation of DCs, that was confirmed by flow cytometry on an aliquot of cultured cells on day 6. On day 6, 15 µl/ml of media of a 5% suspension of iron—carbon (Fe@C) nanoparticles were added to the culture. Finally, cells were collected on day 7, washed twice with PBS and resuspended in the same buffer and centrifugated during 25 min at 400 G with Ficoll histopaque (1.077 g/ml). DCs were isolated from the PBS/Ficoll interface and unincorporated NPs remained at the bottom of the tube (see Fig. 1).

2.3. Processing for confocal microscopy and TEM

Incorporation of magnetic NPs was assessed by confocal microscopy (data not shown) and transmission electron microscopy. For the latter, pelleted cells were fixed in PBS containing 2% glutaraldehyde at room temperature for 30 min and kept overnight at 4 °C. The cells were then washed three times with PBS and treated with 1% OsO4 in PBS for 10 min. All of the samples were washed three times in H2O dehydrated in an ascending series of ethanol solutions. Propyleneoxide (PO) was used as a transitional solvent. The cells were incubated overnight in a solution containing a 1:1 mixture of PO and Epox—Araldite (EA) (Electron Microscopy Sciences). The next day, the mixture was replaced with 100% EA, and the
capsule was placed in a desiccator for 8 h. The sample was then placed in plastic capsules containing EA and polymerized at 60 °C for 48 h. Thin (0.1 μm) sections were cut using a microtome and placed on 200 mesh Cu grids. The samples were stained with 1% aqueous uranyl acetate and Reynolds lead citrate. Sections were examined with a Hitachi 7100 transmission electron microscope (Hitachi High Technologies).

Dendritic cells were cultured in a 12-well plate, and NPs incorporated as described above. Cells were collected from different plates on days 7, 8, 9, 10 and 11 and viability of the DCs was assessed by the exclusion of trypan blue.

2.4. Magnetic measurements

For magnetic measurements, all samples were conditioned in closed containers before quenching the DCs/NPs mixture below its freezing point (~265 K) from room temperature. A commercial SQUID magnetometer was used to perform static measurements as a function field and temperature. Magnetization data were collected in applied magnetic fields up to 1 T, between 5 K and 250 K to avoid the melting of the solid matrix (solvent).

3. Results

The incorporation of NPs into DCs was assessed by electron microscopy. As shown in Fig. 2, NPs can be seen as intra-lysosomal aggregates within dendritic cells. The content of lysosomes in dendritic cells pulsed with iron–carbon NPs includes metallic structures that are absent in the lysosomes of unpulsed dendritic cells.

The fraction of viable cells on days 0, 1, 2, 3 and 4 after NPs’ incorporation was not significantly affected by the incorporation of carbon-coated iron NPs. As shown in Fig. 3, cell viability was 80% on day 0 after incorporation of NPs and decreased slowly so that it remained above 75% for four days. The viability of dendritic cells incorporating Fe–C NPs was identical to unpulsed dendritic cells on day 0, and it decreased slightly faster, but remained above 70% by day 4.

The separation sequence used for our DCs’ samples allows us to assume that, after centrifugation of charged cells in a density gradient, all the magnetic particles not included into the DCs are located in the pellet. On the other hand, the floating band is composed of charged DCs with magnetic NPs, with eventual small fractions of uncharged cells. Therefore magnetic studies were carried for both the material from the band (DCs with NPs), and from the pellet (NPs only). Fig. 4a shows the magnetic response from DCs as cultured, i.e., before loading the magnetic NPs. The results show a linear dependence with the applied field with negative slope. This is distinctive of a diamagnetic behavior. The magnetic response of the colloidal NPs shown in Fig. 4b is of ferromagnetic type, due to the magnetic iron-based NPs. For the charged cells, the contribution from the particles is clearly seen as a ferromagnetic (FM) S-shaped curve (Fig. 4c). This FM component is superimposed to the (small) diamagnetic signal from the DCs, evidenced in the negative slope of the $M(H)$ curves at high fields. After normalizing both measurements to the total culture volume, we could subtract the DCs’ signal from the as cultured cells to obtain the net magnetic signal, which shows the expected nearly saturation behavior (Fig. 4c).

Fig. 2. Electron microphotographs of DCs without (A) and with (B) Fe–C NPs. Intra-lysosomal NPs’ aggregates like metal shavings inside lysosomes are observed (black arrows). Without NPs, the content of lysosomes is seen as homogeneously white.

Fig. 3. Time evolution of the fraction of viable cells after Fe@C nanoparticles’ incorporation (dark bars), as compared to untreated DCs (light bars).
4. Discussion

Several previous studies have reported on the uptake of different complexes by the cell, or the inclusion of latex-type nanoparticles (Wilhelm et al., 2003; Coester et al., 2006; Lutsiak and Robinson, 2002), proposing also models for the mechanisms of phagocytosis. However, only in recent years the specific case of magnetic NPs, composed of metallic and ceramic phases has attracted some attention (De Vries et al., 2005; Anderson et al., 2005). The magnetic performance of the particles is usually enhanced if materials with large saturating magnetization values are chosen, such as Fe, Ni, Fe₃O₄, etc. One of the key issues that restrict the choice of simply the best magnetic material is related to the toxicity effects that many of these elements (or their oxides) have on the cellular metabolism. Superparamagnetic iron–oxide nanoparticles (called SPION) have a large magnetic moment and low toxicity, and for that reason they are being increasingly used in clinical protocols of magnetic resonance imaging instead of the former gadolinium (Gd)-based contrast agents. Also, the few SPION-based products approved for clinical use have shown better efficiency as negative T2 contrast agents: whereas Gd-based products need dosages of about 100 μmol/kg-dose, the Fe₃O₄ SPION-based colloids are applied within the 10⁻⁵–10⁻⁴ mol Fe/kg-dose. Being antigen-presenting cells, it has been previously seen that dendritic cells can uptake peptides, proteins, messenger RNA and other molecules.

Uptake of magnetic NPs by the DCs is demonstrated in this work both directly from transmission electron microscopy images, and also from indirect magnetic measurement. From the former, it can be observed that the particles are located mainly in the lysosomes, where NPs’ aggregates with high density are observed (black arrows). Without NPs, the content of lysosomes is homogeneously less dense.

From the fit of the isothermal magnetization curves (not shown here), we have observed that both the as prepared ferrofluid and charged DCs display the same magnetic parameters, indicating that the particle size distribution remains essentially unaltered after the incorporation into DCs. From this we conclude that the endocytosis process is not size-selective within the size-range (10⁻²–200 nm) of the present particles. Also, the amount of magnetic material incorporated by the DCs yielded a magnetic signal (10⁻³ emu) that is larger than the diamagnetic signal from biological tissues (10⁻⁵–10⁻⁴ emu for DCs at low fields), and quite above the detection limit of SQUID measurements (10⁻⁷ emu). This situation makes our separation process potentially powerful for the detection of small amounts of all types of targeted cells as, for example, the case of metastatic cells within the blood stream or lymph nodes. It has been recently reported that NPs can be charged with antibodies and other biologically active compounds in order to improve imaging techniques such as magnetic resonance imaging (Lee et al., 2007). Given the functional features of dendritic cells, loading dendritic cells with NPs is a promising way to improve cancer diagnosis and treatment.

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