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Leading Opinion

Universal cell labelling with anionic magnetic nanoparticles☆

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ABSTRACT

Magnetic labelling of living cells creates opportunities for numerous biomedical applications, from individual cell manipulation to MRI tracking. Here we describe a non-specific labelling method based on anionic magnetic nanoparticles (AMNPs). These particles first adsorb electrostatically to the outer membrane before being internalized within endosomes. We compared the labelling mechanism, uptake efficiency and biocompatibility with 14 different cell types, including adult cells, progenitor cells, immune cells and tumour cells. A single model was found to describe cell/nanoparticle interactions and to predict uptake efficiency by all the cell types. The potential impact of the AMNP label on cell functions, in vitro and in vivo, is discussed according to cellular specificities. We also show that the same label provides sufficient magnetization for MRI detection and distal manipulation.

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1. Introduction

Magnetic labelling of cells raised up increasing interest due to the various biological or medical applications involving magnetism in living organisms. Magnetic forces are widely used to separate cells in vitro [1–3], but also to manipulate or attract cells by an external stimulus with applicability for basic study of cell migration [4,5], for tissue engineering [6,7] or for cell therapy [8,9]. However, the most developed applications concern the use of magnetic resonance contrast agent to identify and track the migration of magnetically labelled cells following infusion or transplantation in vivo [10–12]. In this field, different techniques have been developed to label non-phagocytic cells in culture using magnetic nanoparticles. The main requirement is to supply cells with sufficient magnetization to be detectable by MRI (or manipulated by magnetic forces), while maintaining cell viability and functionalities. Dextran-coated iron oxide nanoparticles (Ultrasmall Superparamagnetic Iron Oxide USPIO) approved for clinical MRI protocols were first experimented for in vitro cell labelling, but showed poor intracellular uptake, especially for cells that lack substantial phagocytic capacity [13–15]. To facilitate cell labelling, different strategies have been developed. The first class of strategies is based on a receptor mediated approach. Immunoglobulins were covalently linked to the dextran polysaccharide coat of the iron oxide in order to induce specific recognition with receptors at the surface of targeted cell and then trigger receptor mediated endocytosis: monoclonal antibody (mab) to the mouse transferrin receptor OX26 [16], human transferrin [17], and anti CD-11 mab for dendritic cells [18]. This strategy is similar to labelling techniques used in magnetic cell sorting applications, although the labelling procedure is modified to promote endocytosis of the magnetic tag. It is species-specific and may suffer from an insufficient number of receptors at the surface.

Coupling the particle surface to a translocation agent which is not dependent on a receptor, as the HIV tat peptide [19], has been shown to improve the cell labelling efficiency [20] with a cell uptake increasing with the tat peptide/particles ratio [21].

The second class of labelling techniques, currently chosen in most of cell imaging assays, involves the use of a transfection agent helping the internalization of the magnetic nanoparticles. This method has applications in the labelling of a wide variety of cells since its mechanism is non-specific. Highly charged macromolecules form large complexes with dextran-coated nanoparticles, adsorb to the cell membrane via electrostatic interactions and induce membrane bending [22] that triggers endocytosis. This strategy is similar to the one used to transfect oligonucleotides into cells. Transfection agents (TAs) include cationic peptides, lipids, polyamines, and dendrimers. It can be directly engineered on the particle surface, as for magnetodendrimers [23], a highly branched regular 3D carboxylated structure on the iron oxide core. More widely, TA is simply added for a given time to dextran-coated SPIO

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suspension to form complexes, whose size, zeta potential, stability in culture medium as well as MR relaxivities and interactions with cells are finely tuned by the nature of the TA and particles/TA ratio [24–26]. Hence, despite its simplicity of use, the control of the complexes formed by TA and nanoparticles and their subsequent properties are not easily achievable. Nevertheless, different TAs, each of them complexed with USPIO ferrumoxides, have been successfully used for efficient magnetic labelling of various cell types with incubation time of at least 6–12 h [27,28]. However, inhibition of the chondrogenic differentiation of mesenchymal stem cells labelled with poly-L-lysine and ferrumoxides was observed [29]. Recently the clinically approved polycationic peptide – protamine sulfate – was proposed as a highly efficient TA to label mature [25] as well as stem cells [30–32] without any effect on their differentiation capacity in vitro or in vivo. The low molecular weight of protamine sulfate leads to smaller and better controlled complexes as compared with PLL. However, recent studies pointed out the possible precipitation of the TA-nanoparticles complexes and adsorption of these complexes on the plasma membrane of cells rather than internalization [26,33].

Also involving electrostatic interactions with cell, another class of efficient magnetic label has emerged in the last few years. It consists of dextran-free iron oxide nanoparticles coated with charged monomers. They are characterized by the absence of polymers, a small size (hydrodynamic diameter < 50 nm), a negative zeta potential and an electrostatic stabilization in colloidal suspension. The anionic citrate-coated USPIO (VSOP-C125 developed by Ferropharm, Germany) was shown to be incorporated by macrophages much faster and with a better efficiency than their carboxy-dextran counterparts [34]. Similar citrate-coated nanoparticles (VSOP-C184) with a very small size (iron core of 4 nm) are now under phase 2 clinical development [35]. At the same time, we demonstrated that anionic nanoparticles coated with dimercaptosuccinic acid (DMSA) were internalized by macrophages and Hela cells [36,37] in much higher amounts than classical dextran-coated nanoparticles. Surface coating was pointed out as a key factor to allow for non-specific interactions with plasma membrane. Since, a wide variety of cells have been labelled after short incubation with anionic monomer-coated nanoparticles without impairment of the cell viability and functionality. The aim of this paper is to review and document the use of anionic monomer-coated maghemite nanoparticles (AMNPs) for cell labelling. This labelling method, which leads to endosomal internalization of the particles, is very simple (no modification of nanoparticles surface, no addition of transfection agent), rapid (20 min to 2 h), efficient and applicable for every kind of cell. Since its mechanism has been fully characterized, the labelling procedure is reliable and uptake efficiency is predictable knowing cell size, incubation time and extracellular iron concentration. Here different aspects are developed concerning the mechanism of cell uptake, the intracellular pathway and biocompatibility of AMNP and the use of AMNP-labelled cells for MRI detection and for magnetic manipulations.

2. Anionic monomer-coated nanoparticles (AMNPs): synthesis, characterization and cell labelling protocol

The stability of magnetic nanoparticles in colloidal aqueous suspension (ferrofluid) requires repulsive interactions to counterbalance the globally attractive Van der Waals and dipole–dipole interactions. Electrostatic interactions between charged nanoparticles have been proposed by Massart [38] as an alternative to the steric repulsions between polymer coated nanoparticles, which are classically used in commercial ferrofluids. The nanoparticles used in this study are maghemite ($\gamma Fe_2 0_3$) nanoparticles synthesized by alkaline coprecipitation of iron(III) and iron(II) salts. Adsorption of citrate anions to the ferric oxide surface confers to the particles a net negative charge due to carboxylic groups (zeta potential $= -30$ mV) and ensures the colloidal stability in the range of pH from 3 to 11 and for ionic strengths lower than 0.35 mol/L. Alternatively, the particles can be coated with dimercaptosuccinic acid, which possesses thiol groups in addition to carboxylic groups [39]. The mean size of magnetic core is about 8 nm and the size distribution is described by a log-normal distribution with a polydispersity of 0.35. The hydrodynamic size, determined by dynamic light scattering, is about 30 nm. Cell labelling was performed in culture medium (RPMI) without addition of serum, but supplemented with 5 mM sodium citrate to ensure equilibrium between free and particle-bound citrate ions. Iron concentration varied from 0.05 to 20 mM and incubation time from 10 min to 8 h at 37 °C. Unless otherwise stated, the incubation with nanoparticles was followed by two washing steps and by a chase at 37 °C in particle-free culture medium to achieve the complete internalization.

To demonstrate the non-specific nature of labelling with AMNP, this labelling procedure was performed for a wide variety of mammalian cells (see Table 1), including phagocytic and non-phagocytic cells, different species (rat, mouse, human), different

Table 1
Cell types that have been labelled with AMNP

Cell types (origin)	d (μm)	K (μM)$^{-1}$	m_o (pg)	τ_i (h)	Φ_o	m_p (pg)	Ref.
Immune cells							
Raw macrophages (mouse)	11.7 ± 0.8	17 ± 4	6 ± 0.2	1.3 ± 0.2	25 ± 5	33	[37]
Hybridomas (mouse)	12	44 ± 31	2.4 ± 0.2	0.4 ± 0.1	1.5 ± 0.3	5.5	[50]
Dendritic cells (human)	12.2 ± 1.6	29 ± 8	6.6 ± 0.4	0.9 ± 0.1	1.8 ± 0.5	15.2	[42]
OT-1 lymphocytes (mouse)	8.4 ± 0.6	43 ± 26	1.3 ± 0.1	2 ± 0.8	1.1 ± 0.5	2.2	[51]
EL4-B lymphocytes (human)	9.2 ± 1.5	30 ± 10	4.1 ± 0.2	1.4 ± 0.2	1.3 ± 0.4	8	
Tumour cells							
HeLa ovarian carcinoma (human)	20.2 ± 2.6	17 ± 5	18 ± 0.3	1 ± 0.1	2.5 ± 0.2	37.5	[36]
PC3 prostatic carcinoma (human)	14.6 ± 2.3	16 ± 5	8.1 ± 0.2	0.4 ± 0.1	1.8 ± 0.3	13.4	[66,67]
HuH7 hepatic carcinoma (human)	11.6 ± 1.7	27 ± 11	3.4 ± 0.2	1.2 ± 0.1	1.5 ± 0.2	8.3	
Therapeutic adult cells							
Hepatocytes (mouse)	20 ± 4	30 ± 13	21.3 ± 0.8	1.4 ± 0.2	1.9 ± 0.3	49	[68]
Gingival fibroblasts (human)	17 ± 3	24 ± 8	13.5 ± 0.6	1.6 ± 0.5	1.6 ± 1	28	[49]
Smooth muscle cells (rat)	14 ± 1	29 ± 8	5.8 ± 0.2	1.5 ± 0.1	1.9 ± 3	12.4	[47]
Therapeutic stem cells or progenitor cells							
Myogenic precursor cells (pig)	14 ± 2.5	22 ± 11	3.5 ± 0.2	1.8 ± 0.3	1.1 ± 0.3	4.8	[55]
Endothelial progenitor cells (human)	13.8 ± 2.1	39 ± 12	8.9 ± 0.3	1.4 ± 0.2	1.9 ± 0.3	20.5	[48]

The different parameters describing the particle uptake of each cell type are indicated. d is the cell diameter, K is the affinity constant of AMNP for the cell membrane, m_0 is the binding capacity on plasma membrane (in mass of attached particles), τ_i is the characteristic time for internalization, ϕ_0 is the maximal fraction of internalized membrane, m_p is the predicted mass of iron per cell for a labelling condition of [Fe] = 20 mM for 2 h at 37 °C.