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Synthesis and toxicity characterization of carbon coated iron oxide nanoparticles with highly defined size distributions

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ABSTRACT

Background: Iron oxide nanoparticles hold great promise for future biomedical applications. To this end numerous studies on iron oxide nanoparticles have been conducted. One aspect these studies reveal is that nanoparticle size and shape can trigger different cellular responses through endocytic pathways, cell viability and early apoptosis. However, systematic studies investigating the size dependence of iron oxide nanoparticles with highly defined diameters across multiple cells lines are not available yet.
Methods: Iron oxide nanoparticles with well-defined size distributions were prepared. All samples were thoroughly characterized and the cytotoxicity for four standard cell lines (HeLa Kyoto, human osteosarcoma (U2OS), mouse fibroblasts (NIH 3T3) and mouse macrophages (J7442)) where investigated.
Results: Our findings show that small differences in size distribution (ca. 10 nm) of iron oxide nanoparticles do not influence cytotoxicity, while uptake is size dependent. Cytotoxicity is dose-dependent. Broad distributions of nanoparticles are more easily internalized as compared to the narrow distributions for two of the cell lines tested (HeLa Kyoto and mouse macrophages (J7442)).
Conclusion: The data indicate that it is not feasible to probe changes in cytotoxicity within a small size range (10 nm). However, TEM investigations of the nanoparticles indicate that cellular uptake is size dependent.
General significance: The present work compares narrow and broad distributions for various samples of carbon-coated iron oxide nanoparticles. The data highlights that cells differentiate between nanoparticle sizes as indicated by differences in cellular uptake. This information provides valuable knowledge to better understand the interaction of nanoparticles and cells.

1. Introduction

Magnetic nanoparticles are of great interest for use in a wide range of disciplines and are expected to play an evermore important role in biotechnology and biomedicine. Novel methods in treatment and diagnostics are being developed using nanoparticles (NPs) with the promise of overcoming many of the limitations of current procedures. Nonetheless, dextran coated iron oxide nano-particles are the only form of NPs thus far approved by the US Food and Drug Administration (FDA). The interest in iron oxide NPs lies in their potential as elements for contrast enhancement in magnetic resonance imaging, tissue repair, immunoassay, detoxification of biological fluids, cell separation, drug delivery and hyperthermia [1–7].

The scientific curiosity with regard to nanoparticles stems from their size dependent-properties that mainly originate from the dominance of their large surface area. Moreover, the morphology of NPs can be an intrinsic functionalization in itself [8]. However, the impact of the size and shape of NPs on their toxicological effects is only beginning to be investigated and its understanding is crucial for designing their physical and chemical properties more accurately and according to the requirements. Stoehr et al. [9] found that spherical silver nano- and microparticles exhibit little impact on alveolar epithelial cells whereas wire-shaped silver particles induced a strong cytotoxicity, loss in cell viability, and early calcium influx. Another recent study with silica NPs showed that

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variations in their shape and size can trigger different cellular responses and even influence the cell migration on surfaces [10]. Various other studies hint that NP size and shape can trigger different cellular responses in endocytic pathways, cell viability and early apoptosis [11–14]. Theoretical and experimental studies with iron oxide NPs with large diameter distributions suggest that particles of different diameters could be internalized differently by cells which could lead to different biological responses. However, the mechanisms involved are still unclear [15]. Ying and Hwang investigated the cytotoxicity of iron oxide NPs with different sizes and coatings on A3 human T lymphocytes using commercial NPs for which no information on their diameter distributions was available [16]. Another study compared various metal oxide particles including iron oxide, again using only a single cell line. The diameter distributions of the investigated NPs were very large [17]. Ideally studies with very narrow diameter distributions, so called monodisperse samples, are thought to be preferable so as to avoid any size dependency that may or may not exist within the observed diameter distribution.

To our knowledge no systematic study on size dependency of cytotoxicity using iron oxide NPs with highly defined diameter distributions across multiple cell lines exists so far. Even though iron oxide NPs are regarded as stable [18], there is the risk that NPs in general can react when in direct contact with an external biological environment. This can lead to their degradation resulting in toxic byproducts [19]. A promising means to overcome this problem is to use coatings that can also provide these particles with new and enhanced properties [20]. Some examples include polymers [21,22], surfactants [23] and proteins [24]. A recent study suggests that it is not only the type of molecule used in the bioconjugation that is important, but also how it is distributed on the surface [25]. Even differences in the surface charge of NPs can be recognized by cells [26]. Carbon coatings are attractive because they do not only offer high chemical and thermal stability, but also provide a platform that can be easily functionalized [27–30]. In the case of magnetic NPs (e.g. iron oxide), carbon coatings can also help to reduce magnetic interparticle interactions and thereby hinder agglomeration [31]. Indeed carbon-based nanostructures are potentially attractive for a variety of applications in biomedicine ranging from drug delivery to biosensing [32–36].

There are an increasing number of publications relating the cytotoxicity levels of nanoparticles to their morphology with special emphasis on size, shape, and surface defects. However this correlation between morphology and cytotoxicity has been mostly investigated for nanoparticles pursuing extreme differences such as comparing 10 nm particles with 100 nm ones or using samples that do not have a homogeneous nature (e.g. different shapes and sizes intermixed within a sample). Here we deliberately focus on a small range of diameters and narrow specific diameter distributions of purely spherical iron oxide nanoparticles. To our knowledge there is still a need to determine if cells are able to discriminate between small diameter variations in the order of 10 nm, namely when differences in the size of NPs start to be significant and measurable when brought together with cells. In this work we aim to investigate a small range of diameters of iron oxide NPs. For this we use a colloidal chemistry route involving the decomposition of iron oleate to synthesize spherical iron oxide NPs [37]. Moreover, the technique inherently leaves a thin carbon rich coating on the surface of the as-produced NPs. The implemented synthesis route is attractive because, apart from yielding NPs with highly defined diameter distributions, it is also possible to tailor the mean diameters of the NPs by varying the reaction conditions. We also show that one can tailor the width of the diameter distribution by altering the purity of the precursors. This allowed us to produce three very narrow size distributions (9.7, 14.8 and 16.8 nm) and full width at half maximum (FWHM) corresponding to 1.4 nm, 1.4 nm, 2.5 nm, respectively. In addition, a fourth sample with a broad diameter distribution (20.3 nm) and FWHM of 5.5 nm spanning the diameters of the initial three samples was prepared. The results are presented in Table 1. These samples enable us to investigate two important questions. Firstly, are the cytotoxicity and cellular uptake influenced by small diameter changes and secondly, are the cytotoxicity and uptake sensitive to broad diameter distributions as compared to narrow diameter distributions? This latter question is important when considering the synthesis of high performance NPs on an industrial scale where a broad diameter distribution usually implies reduced production costs.

The as-produced samples were carefully characterized and their biocompatibility was evaluated. In order to determine the material toxicity and the possible size dependence, four cell lines were incubated with the NPs and the cell viability was measured using the MTT and trypan blue assays. The standard cell lines used in the viability assays were HeLa Kyoto, human osteosarcoma (U2OS), mouse fibroblasts (NIH 3T3) and mouse macrophages (J7442).

2. Experimental

2.1. Iron oxide NP synthesis

The iron oxide NPs were synthesized based on the work of L. M. Bronstein [38], which is now briefly explained. A typical procedure to synthesize iron oxide NPs with highly defined sizes is based on the iron oleate complex serving as a precursor. This complex is formed in the reaction between iron chloride (III) and sodium oleate dissolved in a mixture of ethanol, hexane and distilled water. When heated for 4 h on a hot plate, the metal chloride and the sodium oleate react to form a metal–oleate complex (iron oleate) and sodium chloride (NaCl). The resulting mixture containing the iron oleate is then washed with distilled water to remove the salt byproduct and the oleate is isolated using a separation funnel. The purified and waxy complex is then thermally decomposed by boiling in a high temperature solvent. Following a modified procedure of the original synthesis route [38], the iron oleate complex passes through a two-step process, which allows the formation of NPs with different diameters. As a first step the iron oleate complex is thermally treated in vacuum for 24 h at 30 and subsequently 70 °C. This heating treatment removes crystal hydrate water and at 70 °C dissociates the metal carboxylate bonds [38]. The second step is the thermal decomposition of the iron oleate complex in different solvents. The use of various solvents to decompose the iron oleate complex is important because each solvent has a different boiling temperature at which the decomposition occurs and plays a role in the final monodispersed size of the NPs. In other words the different temperatures influence the nucleation and growth processes of the NPs. The different thermally treated iron-oleate complexes were firstly dissolved in a mixture of oleic acid and different solvents (octadecane, octadecene, eicosane and docosane) and heated up for different periods of time and temperature in a reflux system. The solution, which then contains the iron

Table 1
Main parameters used to synthesized different size iron oxide nano-particles and comparison between LVTEM mean diameter evaluations and diameter estimates using the Scherrer equation.

Sample	Anneal in vac. [°C]	Solvent	Reflux temp. [°C]	Reflux time [min]	TEM diameter [nm]	Scherrer size [nm]	Full width at half maximum [nm]
a	30	Octadecane	318	30	9.7 (±1.4)	12.3 (±4.5)	1.4
b	70	Docosane	365	3	14.8 (±0.8)	14.3 (±4.8)	1.4
c	70	Eicosane	335	30	16.8 (±1.4)	16.7 (±5.3)	2.5
d	–	Octadecene	300	30	20.3 (±2.6)	18.3 (±9.2)	5.5