Open access • Journal Article • DOI:10.1021/CR2002596

Assessing the in vitro and in vivo toxicity of superparamagnetic iron oxide nanoparticles. — Source link

Morteza Mahmoudi, Morteza Mahmoudi, Morteza Mahmoudi, Heinrich Hofmann ...+3 more authors

Institutions: Pasteur Institute of Iran, École Polytechnique Fédérale de Lausanne, Tehran University of Medical Sciences, University of Bern ...+1 more institutions

Published on: 11 Apr 2012 - Chemical Reviews (American Chemical Society)

CHEMICAL REVIEWS

REVIEW

pubs.acs.org/CR

Assessing the In Vitro and In Vivo Toxicity of Superparamagnetic Iron Oxide Nanoparticles

Morteza Mahmoudi,[*,†,‡,§] Heinrich Hofmann,[†] Barbara Rothen-Rutishauser,[∥,⊥] and Alke Petri-Fink[*,∥,#]

[†]Laboratory of Powder Technology, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland (http://ltp.epfl.ch)

[‡]National Cell Bank, Pasteur Institute of Iran, Tehran, Iran (www.biospion.com)

[§]Institute for Nanoscience and Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[∥]Adolphe Merkle Institute, University of Fribourg, 1700 Fribourg, Switzerland (www.am-institute.ch)

[⊥]Respiratory Medicine, Bern University Hospital, Inselspital, Freiburgstrasse, CH-3010 Bern, Switzerland

[#]Department of Chemistry, University of Fribourg, 1700 Fribourg, Switzerland (www.chem.unifr.ch)

CONTENTS

1. INTRODUCTION

Nanotechnology has become a key word of public interest, since people realized the social and economic power of nanotechnology development. Nanotechnology has already become part of our daily life, and it will have an as yet unknown technological impact because it concerns all aspects of human life from novel building materials to electronics, cosmetics, pharmaceutics, and medicine.[1] In recent years, engineered nanoparticles started to become the most important components in nanotechnology. The International Organization for Standardization (ISO) has provided specific definitions in their recent document entitled "Nanotechnologies—Terminology and definitions for nanoobjects—Nanoparticle, nanofibre and nanoplate". As the basis of this review, the following definitions for a nanoparticle (NP) and a nano-object will be used. A nanomaterial is a material with one, two, or three external dimensions in the nanoscale (1−100 nm), whereas a nanoparticle is defined as a material with all three external dimensions in the nanoscale (ISO/TS: 27687:2008). The current choice of available nanoparticles ranges from relatively simple single titania nanoparticles, which are used in modern sunscreens, to highly complex nanoparticle systems such as coated and multiply derivatized superparamagnetic iron oxide nanoparticles (SPIONs) for drug delivery systems.[2−5] All these artificial and engineered nanoparticles have one thing in common: their chemical, physical, and biological characteristics differ considerably from the bulk material properties. For example, the ferromagnetic iron oxides such as maghemite and magnetite lose their permanent magnetization if they are <30 nm in diameter.[6] Therefore, these materials can be considered as entirely new materials whose impact on humans and environment is not yet known in detail.

U.S. and European governments are promoting study programs on the impact of nanotechnology. The key research report "Nanoscience and nanotechnologies: Opportunities and uncertainties" was published by the Royal Society & Royal Academy of Engineering of Britain in 2004. More recently the Swiss Federal Office of Health, together with the Swiss Federal Office of Environment, has published an action plan, "Synthetic Nanomaterials".[7] This action plan enables both researchers and industrial users of nanoparticles to assess the potential risks of nanoparticles. A lot of research is currently ongoing to use the novel characteristics of nanoparticles for pharmaceutical applications,[8,9] especially for targeted drug delivery,[10,11] biomedical imaging,[12] or biosensing.[13] However, biomedical applications require a detailed understanding of interactions between NPs and biological systems. How these particles react with living cells, proteins, hormones, or immune factors is fundamental to the long-term clinical and commercial viability of such nanoscaled products. More significant is how nanoparticles react following biodegradation within the body—and specifically, whether the particles (or their byproducts) are subject to bioaccumulation within cells or organs, inducing intracellular changes or inflammatory responses. Nanoparticles for biomedical applications have undergone numerous studies in vitro and in vivo with varying results.[14,15] However, this is exceptional and data are only available for very specific particles of specific size with well-characterized and defined surfaces if these compounds are to be used in biomedicine. In other words, extensive studies of the safety of nanoparticles are linked to specific products and are performed by the manufacturers of these products as a condition of them being sold. These regulatory bodies are already in place for most industries especially for the pharmaceutical industry. In this field we can profit from the existing severe rules for the approval of new pharmaceuticals. The European Medicines Agency (EMA) has declared regarding guidance for the application of nanomedicine: "Specific guidance on quality, toxicology, clinical development and monitoring aspects may be developed in the future, once sufficient scientific experience has been gained for specifically

Received: July 12, 2011

dx.doi.org/10.1021/cr2002596 | *Chem. Rev.* XXXX, XXX, 000–000

identified sub-technologies within the field of nanomedicines".[16] This means that medical device manufacturers are obliged to carry out an assessment of the risks as defined in the Medical Devices Directive. In particular, they propose a tentative classification rule for "free" nanoparticles in medical devices, based on the principle that "all devices incorporating or consisting of particles, components or devices at the nanoscale are in Class III unless they are encapsulated or bound in such a manner that they cannot be released to the patient's organs, tissues, cells or molecules". From this it is clear that there is no universal "nanoparticle" to fit all the cases; each nanomaterial must be treated individually when health risks are expected. There is no systematic study available so far; toxicological data are difficult to compare because the parameters, investigated cells or animals, and particles in each of the published studies differ substantially. However, the scientific community agrees that the size, the surface (charge), and the colloidal behavior including unspecific protein adsorption play a crucial role.[17,18]

Many applications and investigations use nanoparticles in colloidal suspensions. By tailoring interactions between colloidal particles, one can design stable fluids, gels, or colloidal crystals. Long-range, attractive van der Waals forces are ubiquitous and must be balanced by Coulombic, steric, or other repulsive interactions to engineer the desired degree of colloidal stability.[19] The colloidal behavior of nanoparticles in different cell media or body fluids is almost never considered or related to particle−cell interactions. It has been shown that submicrometer polymeric particles coagulate in the cell medium, whereas colloidal stability in aqueous solution was ensured for several weeks.[20] A recent study by Limbach et al.[21] on the in vitro agglomeration of nanoparticles at low concentrations has revealed the need for thorough particle size measurements and colloidal stability investigations.

In recent years, several studies showed that different test systems exhibit weak points concerning the analytics of nanoparticles and nanomaterials. Interactions of nanosized materials with the analytical system itself, inappropriate choice of reaction conditions, or insufficient pretreatment of the particle dispersion lead to erroneous results or artifacts, which are often very hard to rebut.[22] For example, nanomaterials have been shown to interact or react with certain dyes of a toxicity assay or tightly bind to the analyte molecules.[23,24] Therefore, the application of photometric methods is fairly restricted. Accordingly, it could be shown that the currently used biological/toxicological assays and even existing Organisation for Economic Co-operation and Development (OECD)-guidelines could be inadequate and that their use is doubtful. For example, the lack of a nanoparticle-type positive control questions the suitability of tests to identify genotoxicity of nanomaterials. Although several genetic toxicology tests have been validated for chemicals according to the OECD test guidelines, the relevance of these assays for nanoparticulate materials remains to be determined.[25] Several erroneous measurements have been revealed, which raised the discussion on which tests are applicable at all.

A few years ago, an international group requested a strategy to overcome such an insufficiency in experimental testing. Andrew Maynard and his colleagues[26] clearly demanded to develop and validate methods to evaluate the toxicity of engineered nanomaterials within the next 5−15 years (from 2006 on). "The first challenge in this context is to reach international agreement on a battery of in vitro screening tests for human and environmental toxicity within the first 2 years, and to validate these tests within the following 5 years."[26] This statement is based on the situation regarding the toxicity tests of engineered nanoparticles for technical applications. It is evident that such a statement is also true for nanoparticles designed for medical applications. However, the first step is yet to be completed.

2. DEVELOPMENT AND CHARACTERIZATION OF SPIONS FOR BIOMEDICAL APPLICATIONS

SPIONs offer many applications in biomedicine such as magnetic resonance imaging (MRI) for contrast enhancement, drug delivery, stem cell tracking, heat source in magnetic fluid hyperthermi,a or magnetic separation technologies (e.g., rapid DNA sequencing) and ultrasensitive diagnostic assays.[27−30] Because of their special properties, these particles offer a variety of advantages compared to other tools: (i) the controllable sizes ranging from ∼3 to several hundred nanometers (in beads), and (ii) the tailor-made surface coating, which can be adapted in a way so that the particles can selectively bind to a defined biologic entity (such as cells or degraded extracellular matrix molecules) or deliver molecules and drugs to specific sites. In addition, their outstanding magnetic properties makes them versatile candidates for molecular resonance imaging (MRI) or hyperthermia.[31] Most commercially available particles or beads with modified surfaces show sizes ≫ 150 nm and are used for in vitro separation but are not designed for selective adsorption/uptake into cells or tissue. On the other hand, very small particles (diameter < 30 nm) are commercially available, but only with a limited number of functional surfaces, and were developed for liver and recently also for imaging metastases in lymph nodes by MRI. MRI contrast agents were first introduced in the mid-1980s; there are currently numerous SPION compounds already FDA-approved for use in the clinic as well as other undergoing clinical trials.[32] The number of SPION-related publications has increased strongly over the years.[33] Many investigations are related to synthesis, characterization, and surface properties of the magnetic nanoparticles. Biological issues have been increasingly addressed in the last few years, and toxicity of SPIONs in medical applications has clearly become an issue.[34] Although information about the toxicity of nanoparticles and specifically SPIONs continues to increase, a significant knowledge gap exists on a complete toxicological profile of these promising nanoparticles proposed for safe future use in many aspects of biomedical engineering. Without the data, risk assessment or regulation for safety of the materials suffer significantly.

This review presents a broad overview of currently available in vitro and in vivo toxicity data. The reader will realize that the toxicity data obtained vary significantly depending on size, size distribution, surface (including coating), and subsequent surface derivatization.

3. SPIONS IN IN VITRO ASSAYS

To obtain reliable and reproducible data from in vitro tests, it is of utmost importance to establish adequate and reproducible analytical environments in terms of, for example, the choice of cells, growing conditions, or sample preparation assay procedures. Determination of the possible toxicity of SPIONs is usually initially determined using in vitro toxicity tests as also described for other nanoparticles,[35] namely, the viability of cells, i.e., cytotoxicity, oxidative stress, inflammatory reactions, and genotoxicity.[36]

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

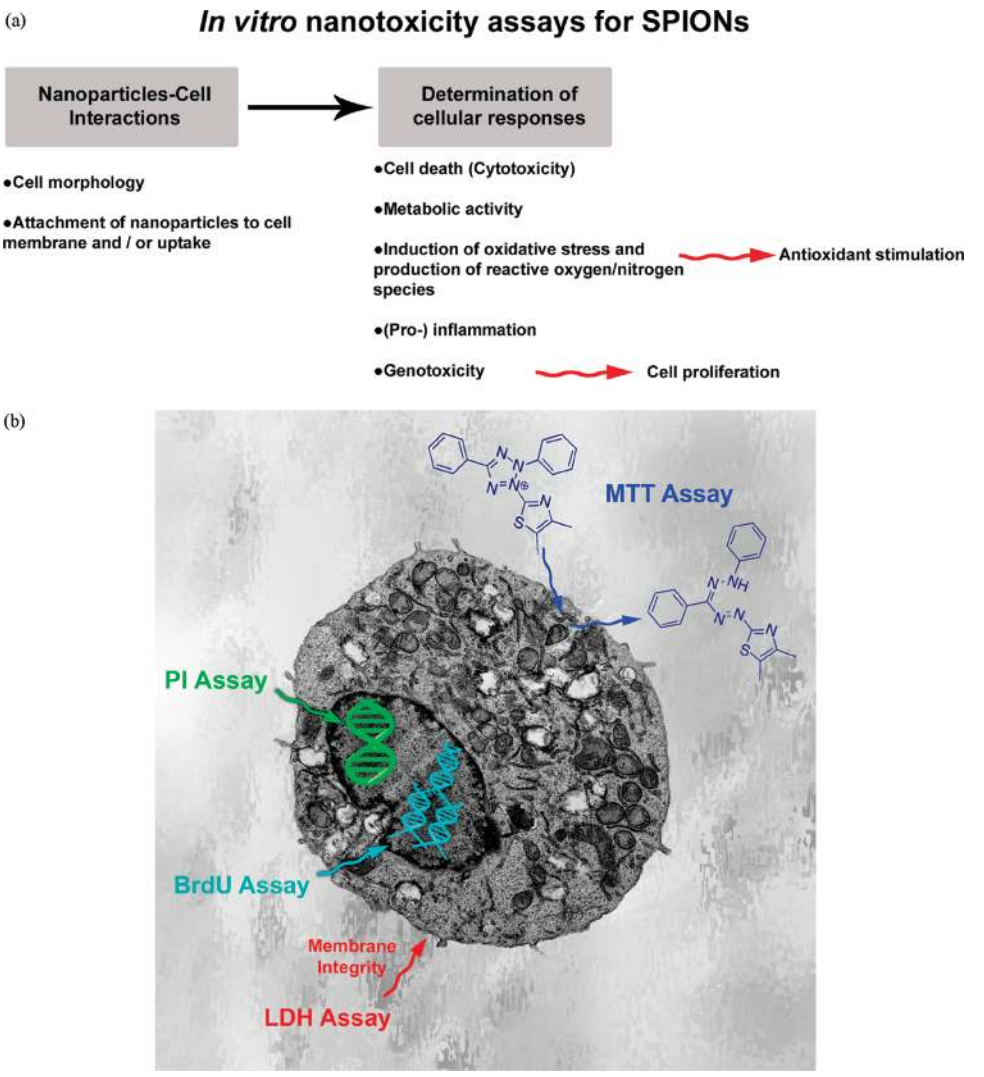


Figure 1. (a) Prominent in vitro assays that are used to probe the impact of nanomaterials on cells; (b) transmission electron micrograph of a human liver cell line (HepG2) showing the mechanism of action of representative cell assays (i.e., MTT, checking mitochondria activity; PI, DNA staining; BrdU, DNA replication staining; LDH, membrane integrity assessment). From unpublished data by Mahmoudi et al.

If the in vitro toxicity tests show only minor or no effects in the concentrations to be used, then the samples will be forwarded to do some in vivo (animal) tests in a relevant (similar to human application) model. If that looks good, then the product should be registered with the FDA for approval for the first clinical human trials.

Nanomaterials often impact on the metabolic activity of cells, membrane integrity of the cells, cell apoptosis, and proliferation. The most prominent assays to determine the predetermined impacts are summarized in Figure 1.

Most of the early studies were done on dextran-coated SPIONs.[37] These first studies were conducted to understand the mechanism of nanoparticle cellular uptake;[37−39] from the results that were obtained, one could conclude that a variety of cells can be efficiently labeled with SPIONs by simple incubation and intracellular labeling may be used for MR imaging of in vivo cell tracking.

In 1996, Mueller et al.[40] reported that some SPIONs showed significant toxicity. Several years later, Berry et al.[41,42] showed that uncoated or dextran-coated SPIONs could cause varying degrees of cell death and were able to induce vacuole formation and clear disruptions in the skeleton of dermal fibroblasts. These observations were reconfirmed by Gupta and co-workers,[32,43,44] who clearly demonstrated cytotoxicity and cytoskeletal disruption by bare SPIONs. In other studies, they tried to prevent the apparent endocytosis-mediated cytotoxic effects by coating SPIONs with different proteins such as lactoferrin and ceruloplasmin, and they showed that the cell response could be directly modulated by the choice of coating.[44−46]

van den Bos et al.[47] presented that Feridex, a dextran-coated SPION, could demonstrate significant toxic effects upon macrophage exposure, including decreased proliferation and cell death. Further investigations revealed that the cause of toxicity was directly attributable to oxidative stress and the generation of free radicals. Similarly, in a study specifically evaluating the effect that magnetic labeling might have upon cells, Stroh et al.[48] confirmed that the large amounts of citrate-coated SPIONs in cells resulted in a significant increase in protein oxidation and oxidative stress. They showed that iron was the source of reactive oxygen species (ROS) by showing a dramatic reduction in these levels via coadministration of an iron chelator.

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

Multiple studies of different SPIONs, which were previously considered innocuous to humans, were compared with nanoparticles of the well-known carcinogen asbestos.[49−51] Thanks to transmission electron microscopy (TEM) and toxicity assays, Soto and co-workers noted that murine macrophage cells exposed to bare SPIONs showed cytoxicities nearing 90% of the asbestos-treated cultures.[49−51] Similarly, in a study by Brunner et al.,[49] SPIONs showed a cell-specific response, clearly showing toxicity equal to that of asbestos toward human mesothelioma cells. They postulated that the EC50 value (∼100 μM), roughly 40 times lower than those published for iron ions, was due to massive Haber-Weiss reactions resulting from the rapid uptake and intracellular transportation of nanoparticles, which is probably very different from aqueous iron ions. In another broad-spectrum toxicity test of metal oxide nanoparticles,[52] bare SPIONs showed significant morphological effects but only modest toxicity upon a neuroblastoma cell line. Jeng and Swanson[52] measured mitochondrial function via the MTT assay, and at the highest concentrations tested ([Fe] ≈ 2.5 mM) showed that SPIONs had a statistically significant effect upon mitochondrial function. Au et al.[53] studied the effects of proprietary commercial SPIONs (NanoSonics (Blacksburg, VA)) upon astrocytes in vitro at a specified concentration (∼175 μM) and also found significant effects upon mitochondrial function as well as decreased cell viability. Similarly, Pisanic et al.[54] also developed a quantifiable model cell system and tested the effect of a well-published anionic SPIONs formulation (coated with dimercaptosuccinic acid) upon the various cell functions of a pheochromocytoma neuronal-type cell line. They found that the particles elicited a dose-dependent ([Fe] = 0.15−15 mM) diminishing ability of the cells to either survive or demonstrate normal biological responses and morphologies.

Table 1 presents the studies where SPION-mediated toxicity in vitro is observed by various cytotoxicity assays. In this table, effects of SPIONs with various physicochemical properties on various cell lines (i.e., human and nonhuman) are fully considered. The viability was mostly measured between 24 and 72 h. The data are categorized according to the tissue and/or cell type used, and in addition, all publications with the same cells are listed together.

There are multiple other in vitro studies that have demonstrated little or no toxicity of SPIONs suspensions;[55−66] many of them are summarized in more general reviews of nanotoxicity.[6,27,67−105]

As mentioned before, the surface coating can have drastic effects on nanoparticle stability, aggregate size, and finally cellular interaction, significantly affecting the fate and extent of SPIONs uptake in intercellular medium.[106] Although Diaz et al.[107] reported significant toxicity and ROS production in response to uptake of SPIONs, like Jeng and Swanson,[52] the results were strongly related to the employed cell type; more specifically, the responses of four tested cell lines to SPIONs were significantly different. It was also shown that the number of nanoparticles per cell (independent of concentration) as well as the number of cells tested might also affect the results of toxicological evaluations. Thus, one can conclude that it may not be possible to find a direct correlation between ROS production and cellular toxicities. Similarly, de la Fuente et al.,[108] in a study of SPIONs coated with different saccharides, showed that even the most seemingly minute changes in SPIONs' coating can drastically affect cell responses and viability.

There are several defenses mechanisms against oxidative stress (e.g., glutathione and antioxidant enzymes) that would be activated in mammalian cellular machinery; however, these defenses can be overcome by the formidable oxidizing capacity of the NPs. There are thought to be at least four primary sources of oxidative stress in response to SPIONs including direct generation of ROS from the surface of the NPs, production of ROS via leaching of iron molecules from the surface degradation of SPIONs by enzymatic degradation, altering mitochondrial and other organelle functions, and induction of cell signaling pathways together with their consequence activation of inflammatory tells, which results in the generation of ROS and reactive nitrogen species (e.g., nitric oxide).[109] It appears that engineered SPIONs have exhibited the potential to induce oxidative stress via all four of these mechanisms.[110]

Several studies have shown direct evidence of ROS damage by SPIONs. For instance, van den Bos et al.[47] reported a dextran-coated SPIONs dose-dependent increase in lipid peroxidation, whereas Stroh et al.[48] measured considerable increases in both lipid and protein oxidation, using citrate-coated SPIONs. Moreover, Alekseenko et al.[111] studied the effects of uncoated SPIONs on neuronal cells; in contrast to others, the authors investigated the effects of ferritin, the natural iron storage protein that exists in cells of all types and contains a 7 nm iron oxide core, surrounded by a protein coat.[112] In their work, it was found that ferritin had a key role for direct generation of ROS in rat synaptosomes; this could eventually lead to neurodegeneration in vivo.

Nanoparticles have been implicated to be in direct contact with, and to produce damage within, mitochondria.[113] Given this proximity to the mitochondria, it is highly likely that the redox-active surface of SPIONs could extensively influence electron flow and act to alter mitochondrial functionality. Therefore, the toxicity assays, which work based on the presence of active reductase enzymes within the mitochondria of living cells (e.g., MTT, MTS, and XTT), may contain large errors. Several studies described above utilized the MTT assay to assess mitochondrial function; however, some authors typically assumed nonviability in those cells exhibiting reduced reductive activity.[44,51,53,71]

The plasma membrane and proteins are recognized as another potential intracellular target for SPIONs-associated toxicity. In addition to induction of cell signaling pathways, SPIONs (both uncoated and coated) induced redox reactions can activate and upregulate plasma membrane proteins (e.g., nicotinamideadenine dinucleotide phosphate oxidase[114] and its analogues[115]), resulting in the generation of the oxidase product. In this case, phagocytic cells (e.g., macrophages[116]) can take up the SPIONs consistently in both in vitro and in vivo environments.[37,38,50,51,106,117−120]

Nuclear factor-kappa B, the oxidative stress response transcription factor found ubiquitously within eukaryotes,[121] has also been shown to be activated by different nanoparticles (e.g., gold) and also results in induction of inflammatory pathways including production of various cytokines such as various interleukins, interferon gamma, tumor growth factor beta, and tumor necrosis factor.[122,123] There has been little direct evidence for the induction of inflammatory pathways and cytokines by engineered SPIONs (both uncoated and coated) in vivo and in vitro. Two recent in vitro studies on the effect of SPIONs loading upon macrophage function have revealed modification of cellular behaviors as well as modulated cytokine expression. Siglienti et al.[124] observed that loading macrophages with uncoated SPIONs resulted in enhanced interleukin-(IL)-10 production and inhibition of tumor necrosis factor-α (TNF-α), indicating potential immunomodulatory capabilities. Hsiao et al.[125] also studied the response of clinically used SPIONs ferucarbotran loading upon macrophages and found that high doses/levels of

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

Table 1. In Vitro Toxicity Results of Different Cell Types That Have Been Treated with a Variety of SPIONs (Dulbecco's Modified Eagle's Medium (DMEM) Was Used Most in the Majority of Studies)

	cells[a]	average SPIONs size (nm)	coating material[b]	concentration of SPIONs	exposure duration	toxicity evaluation assay[c]	results	ref
fibroblasts	dermal fibroblasts (human)		PEG, onsulin coated	0−1 mg/mL	24 h	MTT	25−50% decrease in viability for bare particles (250 μg/mL); 99% viability for PEG-coated (1 mg/mL) particles	43, 46
	dermal fibroblasts (human)	13.6	sodium oleate	0−1000 μg/mL	24 h	MTT	underivatized SPIONs disrupted the cell membrane and disorganized the cell cytoskeleton; derivatized (lactoferrin or ceruloplasmin) SPIONs attached to the cell membrane	44
	hTERT-BJ1 (human)	15	dextran- and albumin-derivatized and underivatized	0.05 mg/mL	24−72 h	dyes (BrdU)	results showed that uncoated and dextran-coated particles caused cell death; cells exposed to the albumin-coated particles were viable and more densely populated; albumin-coated particles showed cell proliferation, whereas uncoated and dextran-coated particles inhibited proliferation	42
	L929 (mouse)	12.5	PVA	800 mM	72 h	dyes (crystal violet)	confirmed the existence of gas vesicles inside cells	66, 73
	L929 (mouse)	17 samples with various shapes and sizes	PVA	0.2, 1, 5, and 20 mM	3, 24, and 48 h	MTT	toxicity was dependent on nanoparticle morphology and size	60
	L929 (mouse)	82	PEGF and PVA	0.4, 0.8, and 1.6 M	24, 48, and 72 h	MTT	toxicity dependent on nanopartide morphology and size	63, 73
	L929 (mouse)	82 ± 12	PEGF	800 mM	24, 48, and 72 h	dyes (NR)	cells damaged by unsaturated (i.e., without modification with plasma proteins) SPIONs had spherical shapes; in contrast, saturated (i.e., after modification with plasma proteins) SPIONs at the same concentration (i.e., 800 mM) did not change the cell shapes notably and cells appeared not to be damaged	63
	3T3 (mouse)	12		0−30 ppm	72 h	MTT	no or low toxicity	49
	HS68 (human foreskin)	8.7−12	ethylene glycol	1 mg/mL	24 h	MTT	no significant difference in the viability of cells compared to the control group	79
lung cells	A549 (human)	20−30	none	up to 80 μg/mL	18 h	dyes (TB) and ROS	no or low toxicity	104
	A549 (human)	20−30	none	up to 80 μg/mL	4 h	comet	oxidative DNA lesions in cultured A549 cells after exposure to 40 μg/mL and 80 μg/mL SPIONs showed statistically significant ($p < 0.05$) increased levels compared to those of the control at the highest dose	104
	A549 (human)	50	silica	4 mg/mL		MTT	IC_{50} = 4 mg/mL	76
	A549 (human)	20−40		0.01−100 mg/mL	24 h	MTT	cell viability decreased in a dose-dependent manner	85
	H441 (human)	63 ± 36	PEI	90 μg/mL	24−48 h	MTT	toxicity of tested complexes was acceptable (cell viability > 80%)	78

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

Table 1. Continued

	cells[a]	average SPIONs size (nm)	coating material[b]	concentration of SPIONs	exposure duration	toxicity evaluation assay[c]	results	ref
liver cells	BRL 3A (rat)	30 and 47		0, 10, 50, 100, and 250 μg/mL	24 h	MTT	50% decrease in viability (250 μg/mL)	71
	BRL 3A (rat)	30 and 47	none	up to 250 μg/mL	24 h	MTT and LDH	LDH leakage: no cytotoxicity up to 100 μg/mL; however, important toxic effect at 250 μg/mL; different particle sizes (30 and 47 nm) did not produce significantly different results	71
	HepG2 (human)	42	uncoated and bSPIONs-coated Baavi			MTT	no indication of increased cytotoxicity with uncoated or bUSPIO-coated Baavi	75
	HepG2 (human)	61 and 127	amino-surface	3 mg/mL, 300, 30, 3, 0.3, and 0.03 μg/mL	5 days	MTT	LD_{50} of Gal-ASPIO-278 = 1500 μg/mL	82
	HepG2 (human)	61 and 127	amine-surface	0.03, 0.3, 3, 30 300, and 3000 μg/mL	5 days	cytochrome C	cytotoxity is related to the zeta potential of NPs; high positive zeta potential causes severe cytotoxicity, whereas neutral and negative zeta potential values did not show cytotoxic effects	82
	primary hepatocytes (mice)	84 ± 1.4	WSC and LA	100 mg/mL	24 and 48 h	XTT	cells were viable (73%) after incubation with particles for 48 h	94
mesenchymal stem cells	MSC (human)		PLL		1−43 days	MTT	long-term viability, growth rate, and apoptotic indices of the labeled cells were unaffected by the endosomal incorporation of SPIONs	70
	MSC (human)	80−150	none	50−250 μg/mL	24−72 h	comet	results showed that SPIONs at various concentrations in conjunction with protamine sulfate used as cationic transfection agent and treatment times of 24−72 h did not statistically affect the frequency of apoptosis in labeled MSCs ($p > 0.05$)	105
	rMSC (rat) and MSC (human)	7.5 ± 1.2	PDMA	15 μg γ-Fe_2O_3/mL	72 h	WST	toxicity of coated and uncoated nanoparticles was examined and compared to Endorem; the viability of rat and human MSCs labeled with PDMA-coated SPIONs did not markedly decrease compared to the control (i.e., unlabeled MSCs); Endorem incubation resulted in reduced viability (32%); similarly, the viability decreased by 15% using uncoated particles	100
	rMSC (rat)		HEDP	25, 50, and 100 μg iron/ml	48 h	MTS	cell viability was not affected (25 or 50 μg iron/mL); however, viability decreased to 70% at higher concentration (100 μg iron/mL)	91

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

Table 1. Continued

	cells[a]	average SPIONs size (nm)	coating material[b]	concentration of SPIONs	exposure duration	toxicity evaluation assay[c]	results	ref
kidney cells	Cos-7 (monkey)	9		0.92−23.05 mM	4 h	MTT	no toxicity detected with applied dosage (0.92−23.05 mM)	72
	OCTY	14	MPEG-Asp3-NH2-, MPEG−PAA- and PAA	0−400 mg/mL	72 h	MTS	MPEG−Asp3-NH2-coated SPIONs had almost no effect on cell viability compared to the control; MPEG−PAA- and PAA-coated SPIONs markedly reduced cell viability	93
macrophages	J774 (murine)	15−30	Tween 80	25−200 μg/mL; 300−500 μg/mL	1−6 h	MTT	exposure to nanoparticles resulted in enhanced ROS generation, leading to cell injury and death; the cell membrane injury induced by the tested nanoparticles showed concentration- and time-dependent damage	89
	macrophages (human)	120−150	dextran	100 μg/mL	7 days	MTS and dyes (BrdU)	MTS assay of activated macrophages showed that only 20% of macrophages were viable after 7 days; on the basis of their results, the BC (Boyden chamber) experiments were designed to include time points at 24 and 96 h of incubation; the MTS data were validated with the trypan blue exclusion test, which showed a similar survival rate of activated macrophages in vitro	92
	human monocyte macrophage	5 (core), 30 (hydrodynamic)	dextran	1 and 10 mg/mL	up to 14 days	MTT and NBT	only mildly toxic at the highest applied dosage (i.e., particle concentration of 10 mg/mL)	99
nerve cells	astrocytes (human)		none	10 μg/mL	6 h	MTS and LDH	treatment of astrocytes with SPIONs significantly ($p < 0.01$) increased MTS production (100% ± 3.65 vs 112.8% ± 3.23, for control and treated astrocytes, respectively), indicative of alteration in mitochondrial functioning	53
	Schwann (human)		dextran	up to 4 mg/mL	48 h	dyes (PI)	no significant difference in cell viability	103
endothelial cells	BAECs	290 ± 15	none	90 μg/mL	24 h	redox	cell viability was not adversely affected by internalized SPIONs; cell survival of 83 ± 3% relative to untreated cells was observed at the highest applied SPIONs dose (9 μg per well, corresponding to a SPIONs loading of 0.3 ng per cell)	96
cancer cells	HeLa (human cervical)		PLL		1−43 days	MTT	long-term viability, growth rate, and apoptotic indices of the labeled cells were unaffected by the endosomal incorporation of SPIONs	70
	HeLa (human cervical)	4.5−10	dextran, aminodextran, heparin, and dimercaptosuccinic acid	0.05, 0.1, and 0.5 mg/mL	24 h	MTT	viability of cell culture was not significantly affected or modified by the presence of nanoparticles after 24 h incubation (90−100% viability)	86

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

Table 1. Continued

cells[a]	average SPIONs size (nm)	coating material[b]	concentration of SPIONs	exposure duration	toxicity evaluation assay[c]	results	ref
melanoma (human)	14−55	PVA and vinyl alcohol/vinyl amine copolymer	12, 61, and 123 μg/mL	2 and 24 h	MTT	polymer alone (i.e., without SPIONs) was more toxic than polymer-coated SPIONs; positively charged polymers were more toxic than negatively charged or neutral polymers	5
LLC (mouse)	10	poly(TMSMA-*r*-PEGMA)	1, 10, 20, 50, and 100 μg/10^5 cells	12 h	MTT	no toxicity at applied dosage	74
GL261 (mouse brain)	10−100	dextran	1−200 μg/mL	24 h	MTT	nanoparticles functionalized with dendritic guanidines exhibited higher toxicity than those functionalized with dendrons having hydroxyl or amine peripheries	80
K562 (human leukemia)	30	tetraheptylammonium	2.5 μg/mL	72 h	MTT	inhibition rate = 46% for the cell system incubated with Fe_3O_4−PLA	81
K562 (human leukemia)	17 samples with various shapes and sizes	PVA	0.2, 1, 5, and 20 mM	3, 24, and 48 h	MTT	toxicity was dependent on nanoparticle morphology and size	60
K562 and K562/A02 (human leukemia)		ADM conjugated	20 μg/mL and 5 mg/mL	48 h	MTT	nano-Fe_3O_4 combined with DNR could inhibit cell proliferation significantly ($P < 0.001$)	83
B16/DNS and B16/phOx (mouse breast)	39 ± 0.5	DNS hapten covalently attached to CLIO	100 mM	48 h	ATP	DNS−CLIO was nontoxic to B16/DNS (DNS receptor positive) and B16/phOx (control receptor positive) cells	98
SMMC-7721 (human hepatocellular)	13.8 ± 5.3	chitosan	0−123.52 μg/mL	12 h	MTT	FITC−CS@MNPs reduced (∼10%) cell viability at high dose (123.52 μg); bare MNPs significantly reduced (90%) cell viability even at very low concentration (0.01 mg/mL)	87
PC3 (human prostate)	60.8 ± 1.9	TCL-SPIONs	0.1 mg/mL	48 h	MTT	cytotoxicity of Dox-loaded TCL-SPIONs-Apt bioconjugates was comparable to free Dox	84
A2780 (human ovarian) and MCF-7 (human breast)		dextrane and phosphatidylcholine/cholesterol	100 μg/mL	1 to 3 days	MTT	presence of SPIONs in culture medium led to alterations in mitochondria ultrastructural organization and decrease of oxygen uptake by mitochondria in sensitive and anticancer drugs resistant cells	88
SK-MEL-37 (human melanoma)		DMSA, citric acid or lauric acid	up to 840 μg/mL		MTT	IC_{50} = 254, 433, 2260 μg-iron/mL, for lauric acid-, citric acid-, and DMSA-coated nanoparticles, respectively; the cytotoxic response correlated with the hydrodynamic diameter and the zeta potential	58
KB (human carcinoma)	84 ± 1.4	PAMAM and G3	0−80 mg/mL	4 days	XTT	dendrimer-stabilized SPIONs did not display cytotoxicity to KB cells in the predetermined concentration range	94
H184B5F5/M10, SKBR3 (normal breast), MB157, and T47D (human breast cancer)	5.4 and 7.6	none	0.1, 1, 10, and 100 μM	72 h	MTS	no obvious change in cell viability	90

dx.doi.org/10.1021/cr2002596 |*Chem. Rev.* XXXX, XXX, 000–000

Table 1. Continued

	cells[a]	average SPIONs size (nm)	coating material[b]	concentration of SPIONs	exposure duration	toxicity evaluation assay[c]	results	ref
	B16F10 (mouse breast)	23.8	CMC	1, 2.5, and 5 mM	24 h	XTT	after incubation with NPs for 24 h, the CMC-coated SPIONs showed no toxicity at highest applied NP concentrations (i.e., 5 mM); after 48 h, cell viability was reduced (81%) at concentrations >1 mM	95
mesothelioma cells	MSTO-211H (human)	12		0−30 ppm	72 h	MTT	viability of MSTO cells was decreased at 3.75 ppm; no toxic effect on 211H cells at applied dosage	49
	HMMs (human)	30	dextran	0−10 mg/mL	24, 48, 72 h	MTT	not toxic at particle concentration of 1 mg/mL and mildly toxic at particle concentration of 10 mg/mL after 72 h	77
keratinocytes	HaCaT (human)	20−40		0.01−100 mg/mL	24 h	MTT	cell viability decreased in a dose-dependent manner	85
smooth muscle cells	A10 (rat)	185 ± 3; 240 ± 19	polylactide	10−50 μg/mL	72 h	redox	no toxicity detected	97
lymphocytes	T lymphocyte cell line (rat)	6	scAbCD3	0.15 μg	48 h	WST	polyplexes based on these targeted delivery agents exhibited not only high efficacy of gene transfection in cells but also low cytotoxicity	101
pancreas	human islet	60	dextran	280 μg/mL	48 h	dyes (PI)	results showed that the viability of labeled islets were similar to the control islets	102

[a] Abbreviations: hTERT-BJ1, Infinity Telomerase Immortalized primary human fibroblasts; L929, mouse fibroblast cell; rodent 3T3, Swiss mouse fibroblast cells; HS68, human foreskin fibroblast cells; A549, human lung adenocarcinoma epithelial cells; H441, human lung adenocarcinoma epithelial cells; BRL 3A, rat liver cells; HepG2, human liver hepatocellular cells; MSCs, mesenchymal stem cells; rMSCs: rat mesenchymal stem cells; Cos-7, obtained by immortalizing a CV-1 cell line derived from kidney cells of the African green monkey; OCTY, mouse kidney cells; J774, murine macrophage cells; Schwann, principal glia of the peripheral nervous system; BAECs, bovine aortic endothelial cells; HeLa, cervical cancer cells; LLC, mouse Lewis lung carcinoma; GL261, mouse brain tumor cells; K562, human immortalized myelogenous leukemia cells; k562/A02, human leukemia cells; B16, mouse melanoma cells; B16/DNS, mouse melanoma cells with DNS receptor positive; B16/phOx, mouse melanoma cells with control receptor positive; SMMC-7721, human hepatocellular carcinoma cells; PC3, human prostate cancer cells; A2780, human ovarian cancer cells; MCF-7, human breast cancer cells; SK-MEL-37, human melanoma cells; KB, human epithelial carcinoma cells; H184B5F5/M10, normal breast epithelial cells; B16F10, SKBR3, MB157, and T47D, three types of breast cancer cells; MSTO-211H, human lung mesothelioma cells; HMMs, human malignant mesothelioma cells; HaCaT, human keratinocyte cells; A10, rat aortic smooth muscle cells. [b] Abbreviation of coatings: PEG, poly(ethylene glycol); PEGF, poly(ethylene glycol-*co*-fumarate); PLL, poly(L-lysine); PVA, poly(vinyl alcohol); PEI, polyethyleneimine; ADM, adriamycin; TCL-SPIONs, thermally cross-linked SPIONs; DMSA, *meso*-2,3-dimercaptosuccinic acid; HEDP, 1-hydroxyethylidene-1.1-bisphosphonic acid; PAA, poly(acrylic acid); MPEG, methoxypoly(ethylene glycol)−oligo(aspartic acid)); WSC, water-soluble chitosan; LA, linoleic acid; PAMAM, dendrimer-stabilized (carboxyl-functionalized poly(amidoamine); G3, dendrimers of generation 3; CMC, carboxymethyl Curdlan; CLIO, cross-linked iron oxide; PDMA, poly(*N*,*N*-dimethylacrylamide); scAbCD3, nonviral gene delivery agent bearing CD3 single chain antibody. [c] Abbreviation of toxicity methods: MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide); MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium); XTT, (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide); BrdU, bromodeoxyuridine; LDH, lactate dehydrogenase; ATP, adenosine triphosphate; NBT, Nitroblue tetrazolium; WST, water-soluble tetrazolium; PI, propidium iodide.

dx.doi.org/10.1021/cr2002596 |*Chem. Rev.* XXXX, XXX, 000–000

SPIONs induced the secretion of TNF-α and resulted in production of nitric oxide. Similarly, Naveau et al.[126] showed that coated anionic SPIONs labeling of human gingival fibroblasts resulted in significant signs of inflammation, including increased expression of IL-1 and -4, as well as secretion of matrix metalloproteinases.

Recently, Radu et al.[127] investigated the effects of SPIONs on lipid peroxidation and the antioxidative system in MRC-5 lung fibroblast cells following exposure for 24, 48, or 72 h. Exposure to α-Fe_2O_3 nanoparticles increased lipid peroxidation, and conversely, the reduced glutathione concentration decreased. In addition, an increase of the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, and glutathione reductase within the interval between 48 and 72 h was noticed. Taking into account that the reduced glutathione level decreased and the malondialdehyde level, a lipid peroxidation product, remained highly increased up to 72 h of exposure, it would appear that the MRC-5 antioxidant defense mechanisms did not efficiently counteract the oxidative stress induced by exposure to hematite nanoparticles. Choi et al.[128] presented in vitro cytotoxicity of iron oxide Fe_3O_4 and manganese oxide MnO using live/dead cell assay, lactate dehydrogenase assay, and reactive oxygen species detection with variation of the concentration of nanoparticles, incubation time, and different human cell lines (lung adenocarcinoma, breast cancer cells, and glioblastoma cells).

Variation of cell medium components and cytotoxicity due to the interactions with nanoparticles were analyzed using ultraviolet and visible spectroscopy (UV/vis) and the MTT assay method.[63,64] The toxicity has been traditionally identified by changes in pH and composition in cells and DMEM due to the tendency of SPIONs to adsorb proteins, vitamins, amino acids, and ions. For in vitro toxicity assessments, a new surface passivation procedure is proposed that can yield more reliable quantitative results. It is shown that a more reliable way of identifying cytotoxicity for in vitro assessments is to use particles with saturated surfaces via interactions with DMEM before usage.

These results show that the assays currently used for toxicity evaluation of nanoparticles are still not well adapted for these measurements, and many results are published based on unreliable methods, i.e., lacking reliability and/or unrealistic test conditions, i.e., overdose situations.[22] Especially in the case of cytotoxicity, the different tests can give very inconsistent results. The reason for these different results is not yet understood, especially because similar tests (MTT and MTS) lead to very different results.[129] However, such models offer an inexpensive and high-throughput alternative to in vivo research strategies. It is of utmost importance to enhance quality and reliability of in vitro studies with nanoparticles in general.

4. IN VIVO TOXICITY OF SPIONS

The physical and chemical characteristics of SPIONs (e.g., surface morphology, surface charge density, coating material, particle size, and size distribution) are considered as crucial factors to determine pharmacokinetics, toxicity, and biodistribution of magnetic nanoparticles. Natarajan et al.[130] employed magnetic nanoparticles with diameters of 20, 30, and 100 nm and evaluated their application for alternating magnetic field therapy and their in vivo performance depending on their size. The nanoparticles were conjugated to ^{111}In-DOTA-ChL6, a radio-immunoconjugate by carbodiimide chemistry. The radio-immuno-NPs were purified and characterized by polyacrylamide gel electrophoresis (PAGE), cellulose acetate electrophoresis, live cell binding assays, and pharmacokinetics in athymic mice bearing human breast cancer xenografts. The radio-immuno-NPs were administered, and blood and tissue data were evaluated at different time points. The results showed that tumor targeting and heating capacity depended on the size of the radio-immuno-NPs.

SPIONs are often classified as biocompatible, showing no severe toxic effects in vivo.[77,131,132] Jain et al.[131] have shown that in vivo administration of SPIONs did not cause a negative effect in liver function. It is noteworthy to mention that the precise prediction of the biological fate of SPIONs is strongly dependent to the composition and amounts of associated proteins at the surface of NPs;[28] the composition and amounts of associated proteins could be defined by exact knowledge of the physicochemical properties of the particles.[28] For instance, a majority of oleic acid/pluronic-coated SPIONs (i.e., 55% of the intravenous injected dose) were accumulated in the liver of rats; however, elimination of dextran-coated SPIONs, via urine and feces, was around 25% of injected dosage in the same animal model.[133] In addition, another report shows the elimination of ~20% of the injected dextran-coated SPIONs (with different size in comparison with ref 133) through urine and feces, in different animal models.[134] As predetermined, these differences in elimination of the NPs could be explained by variation of their protein corona compositions. Because of the physiological iron metabolism of cells, the surface degradation of SPIONs would be processed after their entrance to the intercellular medium, resulting in a temporary increase in iron amounts in the serum.[133] These free iron ions have significant capability for induction of oxidative stress;[135] thus, for in vivo administration of SPIONs, injection of high doses showed be prevented.

In addition to physicochemical properties of NPs, cell type is also recognized as a crucial factor for cellular uptake, intracellular fate, and toxic response of NPs. For instance, Mahmoudi et al.[110] showed that SPIONs with various surface chemistries (uncoated and cyanoethyltrimethoxysilane (CAES)- and aminopropyltriethoxysilane (APTES)-coated) had toxic effects on human brain cells at iron concentrations above 2.25 mM, whereas the same concentration of NPs were compatible with human kidney cells (see Figure 2). Confocal microscopy (see Figure 2d) confirms the impact of various SPIONs on different cell types. For instance, negatively charged SPIONs did not produce significant changes on the actin cytoskeleton of heart cells. However, the same particles showed severe disruption of the actin cytoskeleton in kidney and brain cells. Similar results were observed in vivo. Hanini et al.[132] tested SPIONs in vivo and could confirm that SPIONs induced toxicity in the liver, kidneys, and lungs; however, the brain and heart organs remained unaffected.

Chertok et al.[136] explored the possibility of using SPIONs as a drug delivery vehicle for minimally invasive, MRI-monitored magnetic targeting of brain tumors. The in vivo effect of magnetic targeting on the extent and selectivity of nanoparticle accumulation in tumors of rats harboring orthotopic 9 L-gliosarcomas was quantified with MRI. Animals were intravenously injected with nanoparticles (12 mg Fe/kg) under a magnetic field density of 0 T (control) or 0.4 T (experimental) applied for 30 min. Following their results, accumulation of SPIONs in gliosarcomas can be significantly enhanced by magnetic targeting and successfully quantified by MR imaging with no toxicity observation. Yu et al.[137] reported that the excellent passive tumor targeting efficiency of thermally cross-linked (TCL)-SPIONs allowed detection of tumors by MR imaging and at the same time delivery of sufficient amounts of anticancer drugs that in turn were released

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

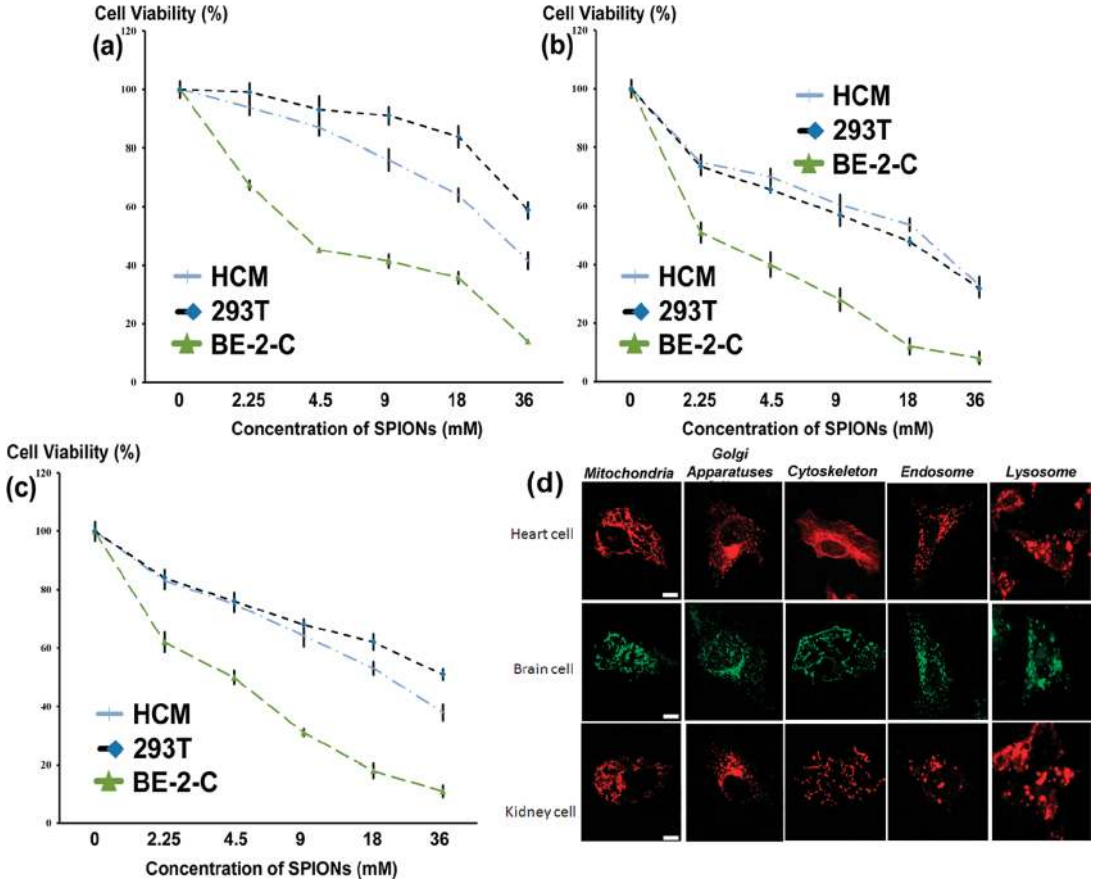


Figure 2. Cell viability of MTT assay results for (a) SPIONs−COOH, (b) uncoated SPIONs, and (c) SPIONs−NH2 samples on heart (HCM), brain (BE-2-C), and kidney (293T) cell lines. (d) Confocal microscopy images showing the effects of the exactly identical amount of negatively charged SPIONs on cellular cytoskeleton as well as compartments of various cell lines (i.e., heart, brain, and kidney). Reprinted with permission from ref 110. Copyright 2011 American Chemical Society.

from the nanoparticles to exhibit anticancer activity. Consequently, doxorubicin@TCL-SPIONs showed exceptional antitumor effects without any systemic toxicity.

After animal studies, Feridex (Endorem) was probed in humans and recognized as safe and efficient.[138] The most frequent side effect was focused on back pain, which was detected in nine patients (4%) and required interruption of the infusion of ferumoxides in five of these. Although lumbar pain has been associated with administration of a variety of colloids and emulsions, the physiological causes are unknown, because no significant changes in chemistry values, vital signs, and electrocardiographic findings were found. It is worthy to note that limitations may also arise in extrapolating from animal models to humans. There are many physiological parameters to consider, ranging from variations in weight, blood volume, cardiac output, and circulation time to tumor volume/location/blood flow, complicating the extrapolation of data obtained in animal models.[139−141] Low toxicity of SPIONs coated with dextran was observed by morphological studies. Minor variations in histology of both spleen and liver were observed at most of the highest concentrations (i.e., 200× higher doses than that used for MR imaging).[142,143]

Lübbe et al.[144] observed no obvious toxicity due to the intravenous injection of magnetic nanoparticles (i.e., with diameter of 100 nm) into mice. The critical matter that would be important in toxicity response is the concentration of ferrofluid; therefore, due to their acute iron overload,[145] high amounts of SPIONs showed a toxic effect.[146] However, the lower amount of the same material was fully biocompatible.[140] De Vries et al.[147] showed that in vivo magnetic resonance tracking of magnetically labeled cells is feasible in human for detection of very low numbers of dendritic cells in conjunction with detailed anatomical information. Autologous dendritic cells were labeled with a clinical superparamagnetic iron oxide formulation or ^{111}In-oxine and were coinjected intranodally in melanoma patients under ultrasound guidance. In contrast to scintigraphic imaging, magnetic resonance imaging (MRI) allowed assessment of the accuracy of dendritic cell delivery and of inter- and intranodal cell migration patterns. Following their results, MRI cell tracking using iron oxide appears clinically safe and well suited to monitor cellular therapy in humans.

Finally, the last form of toxicity evaluation that is becoming increasingly popular is computer simulation processing.[148] Although this method is not routinely integrated in toxicology assessment, it is becoming a useful technique to look at the toxicity of drugs even before their synthesis during drug discovery. Dames et al.[149] showed theoretically by computer-aided simulation, and for the first time experimentally in mice, that targeted aerosol delivery to the lung can be achieved with aerosol droplets comprising SPIONs in combination with a target-directed magnetic gradient field. They suggested that nanomagnetosols may be useful for treating localized lung disease, by targeting foci of bacterial infection or tumor nodules.

dx.doi.org/10.1021/cr2002596 |*Chem. Rev.* XXXX, XXX, 000–000

5. CONCLUSION AND OUTLOOK

For the application of nanoparticles with unique physical properties such as, for example, superparamagnetic particles, the very high but absolutely acceptable barrier given by the regulatory bodies has to be overcome. It has to be approved that particles are harmless by fulfilling the conditions for a device of class III. This demanding directive was installed because the behavior of nanoparticles in living systems is only partially known or predictable and is far from a detailed understanding. On the other hand, a huge and increasing number of publications exists dealing exactly with the previously mentioned subjects. The publications are often based on one type of particles, whereas a particle is defined as a more or less complex system of an inorganic core particle, a layer of, e.g., a biocompatible polymer and additionally linkers and molecules such as proteins, peptides, drugs expressing a biological function, or simply a fluorophore for optical detections. These particles are then tested in vitro with one or several cell lines. Additionally, different methods are used to investigate one or several types of toxicity, such as cytotoxicity or viability, oxidative stress and/or genotoxicity. This short nonexhaustive enumeration shows the very large number of possibilities to investigate in vitro the biocompatibility of nanoparticles for medical applications, an important and mandatory work before final animal tests are planned and carried out. In this review, we tried to focus our work on cytotoxicity of nanoparticles with iron oxide as core and a biocompatible polymer as "first layer". Additional modifications with functional groups to control the surface potential or further derivatization with fluorophors or biomolecules are also incorporated into this study. However, there is not one example of a case study that was carried out in the past, where the same particles were investigated with the same cell lines using the same method/protocol, which would allow comparison and estimation of the experimental error between different research groups.

A very severe problem lies also in the often insufficient characterization of the tested particles and the sparse information on coating and further surface functionalization. For nanotoxicological investigations, Krug and Wick[150] have tried to structure the investigations (transport, surface, and materials) and have defined guidelines for the minimum information, which needs to be provided in a relevant publication. They claim that the scientific community cannot ensure the readers of the quality of studies unless two major aspects are considered: nanomaterials that are to be tested must be sufficiently characterized beforehand and enough information on validity and suitability of the selected test methods must be provided. An additional aspect that was mentioned is the fact that mostly negative effects of nanoparticles were reported and therefore often the employed nanoparticles' doses are too high. To investigate potential applications of nanoparticles, these rules have to be applied but additional information regarding dose, concentration, solvent, application method, and rate are necessary. Our review shows that very few publications fulfill all these conditions. For example, dose and concentration should be mentioned, e.g., per volume or per cell, to allow comparison with other experiments and/or research groups. Additionally, each type of test requires different particle concentrations to address very specific questions. For example, genotoxicity is assayed with much smaller doses that are not cytotoxic to avoid DNA damage by other mechanism (see also manuscript by Krug and Wick).[22] Finally, interference of the nanoparticles with the test system itself should be taken into account in any case, and measurements have to be taken if such an interaction takes place.

The further development in the field of inorganic core/organic shell nanoparticles for medical applications will depend in an important way on a deeper understanding of the nanoparticle/protein interaction. The interaction between a nanoparticle and the cell membrane is mainly controlled by the adsorbed protein at the nanoparticle. The type of protein, the amount, and also conformation depends, on the other hand, strongly on the prepared nanoparticle surface properties, such as diameter, charge, functional groups, and derivatized biomolecules. Additionally, the colloidal behavior, agglomeration rate, and stability have to be taken into account. We believe, therefore, that oversimplified models are not helpful for the estimation of the behavior of the particles in vitro and especially in vivo. This fundamental challenge in understanding the behavior of nanoparticles in complex biological fluids exists also in the field of toxicology of engineered nanoparticles, with the latter being even more complicated because very often the surface composition is not known in detail and the uptake by humans or animals is much less controlled. As discussed in several conferences, a much closer cooperation between the experts has to be encouraged in the future to shorten the time to develop regulations and to bring safe nanoparticles to the market.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mahmoudi@biospion.com, alke.fink@unifr.ch.

BIOGRAPHIES

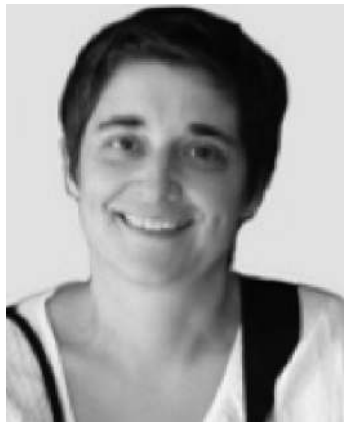
Dr. Morteza Mahmoudi obtained his Ph.D. in 2009 from Sharif University of Technology with specialization on the cytotoxicity of superparamagnetic iron oxide nanoparticles (SPIONs). He has received many awards such as the Shahid Chamran Award (National Endowment for the Elite, 2011), Distinguished Researcher at Pasteur Institute of Iran (2010), the Dr. Mojtahedi Innovation Award for Distinguished Innovation in Research and Education at Sharif University of Technology (2010), and Kharazmi Young Festival Award (2009). His current research involves the magic SPION for simultaneous diagnosis and therapeutic applications (http://www.biospion.com). He is the author of the book entitled "Superparamagnetic iron oxide nanoparticles for biomedical applications", which is published by Nova Science Publishers, NY, USA. He was a visiting scientist at Laboratory of Powder Technology (LTP) and Center for BioNano Interactions (CBNI) at Swiss Federal Institute of Technology

dx.doi.org/10.1021/cr2002596 |Chem. Rev. XXXX, XXX, 000–000

(EPFL) and University College of Dublin (UCD) under the supervision of Professor Heinrich Hofmann and Professor Kenneth A. Dawson, respectively.

Prof. Dr. Heinrich Hofmann got his Ph.D. in Material Science with a thesis prepared at the Powder Metallurgy Laboratory at the Max Planck Institute in Stuttgart. In 1985 he joined the R&D center of Alusuisse-Lonza Services AG, at Neuhausen-am-Rheinfall. In 1993 he joined the Swiss Federal Institute of Technology as Professor and Director of the Powder Technology Laboratory at the Department of Materials Science and Engineering. His research area includes the synthesis of nanostructured materials based on nanoparticles and the modification of surfaces with nanoparticles using colloidal methods. The fields of application of such materials are medical and biological (drug delivery, hyperthermia, cell separation, and biosensors), electronics, and sensors. Recently he started an EU project dealing with SPIONs for molecular imaging, and he is active in several national research programs in the field of nanotoxicity.

Prof. Dr. Barbara Rothen-Rutishauser received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a postdoctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH where she developed and characterized cell culture models for drug transport studies. In 2000 she joined Prof. Peter Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. During the period of her research, Dr. Rothen-Rutishauser has become an expert in the field of cell−nanoparticle interactions in the lung, with a special focus on lung cell culture models and various microscopy techniques such as laser scanning and transmission electron microscopy. In addition, she has overlooked the establishment of a wide variety of commonly used and specialist cell biology assay methods within her research group. Since 2011 she is an independent group leader at the Respiratory Medicine, Department of Clinical Research and Bern University Hospital, Switzerland, and since the first of July 2011 she is the new chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland; the position is shared equally with Prof. Alke Fink.



Prof. Dr. Alke Petri-Fink studied chemistry at the University of Ulm, Germany, where she also obtained her Ph.D. in inorganic chemistry in 1999. After a postdoctoral visit at the Engineering Research Center for Particle Science and Technology, University of Florida, she joined the Institute of Materials Science at the École Polytechnique Fédéral Lausanne (EPFL) in 2000. There she established nanomaterials as a new research field, initiated various national and international projects, and fostered contacts to many industrial partners. In 2009, she established her own research group in the Chemistry Department at the University of Fribourg, and since July 2011 she is full professor and the new chair of BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland. She shares this position equally with Prof. Barbara Rothen- Rutishauser. The BioNanomaterials group envisages all concepts of nanoscience. By combining various aspects of this emerging scientific discipline, the group studies and develops bio(nano)materials from their initial synthesis and characterization to thorough understanding of how they may interact with biological systems.

ACKNOWLEDGMENT

M.M. thanks the European Science Foundation (for the activity entitled "Mapping the detailed composition of Surface-Absorbed Protein Layers on Biomaterials and Nanoparticles") for their financial support. A.P.-F. thanks the Swiss National Science Foundation (PP00P2-123373/1), the University of Fribourg, and FriMat for their financial support. H.H. and A. P.-F. also thank the European Commission ("Nanodiara" Project number NMP4-LA-2009-228929) for generous funding. B.R.-R. is supported by the Swiss National, the Adolphe Merkle and the German Research Foundation.

REFERENCES

(1) Maynard, A. D. *Ann. Occup. Hyg.* **2007**, *51*, 1.
(2) Guo, P.; Coban, O.; Snead, N. M.; Trebley, J.; Hoeprich, S.; Guo, S.; Shu, Y. *Adv. Drug Delivery Rev.* **2010**, *62*, 650.

dx.doi.org/10.1021/cr2002596 |*Chem. Rev.* XXXX, XXX, 000–000

(3) Romero, E. L.; Morilla, M. J. *Adv. Drug Delivery Rev.* **2010**, *62*, 576.
(4) Vega-Villa, K. R.; Takemoto, J. K.; Yáñez, J. A.; Remsberg, C. M.; Forrest, M. L.; Davies, N. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 929.
(5) Petri-Fink, A.; Chastellain, M.; Juillerat-Jeanneret, L.; Ferrari, A.; Hofmann, H. *Biomaterials* **2005**, *26*, 2685.
(6) Mahmoudi, M.; Milani, A. S.; Stroeve, P. *Int. J. Biomed. Nanosci. Nanotechnol.* **2010**, *1*, 164.
(7) Fereral Office of Public Health FOPH. http://www.bag.admin.ch/themen/chemikalien/00228/00510/index.html?lang=en, 13.11. Accessed 2011.
(8) Liu, Y. Y.; Miyoshi, H.; Nakamura, M. *Int. J. Cancer* **2007**, *120*, 2527.
(9) Peteiro-Cartelle, J.; Rodrıguez-Pedreira, M.; Zhang, F.; Rivera Gil, P.; del Mercato, L.; Parak, W. J. *Nanomedicine* **2010**, *4*, 967.
(10) Dhar, S.; Reddy, E. M.; Shiras, A.; Pokharkar, V.; Prasad, B. L. *Chem.—Eur. J.* **2008**, *14*, 10244.
(11) Joshi, H. M.; Bhumkar, D. R.; Joshi, K.; Pokharkar, V.; Sastry, M. *Langmuir* **2006**, *22*, 300.
(12) Jain, P. K.; Huang, X.; El-Sayed, I. H.; El-Sayed, M. A. *Acc. Chem. Res.* **2008**, *41*, 1578.
(13) Olofsson, L.; Rindzevicius, T.; Pfeiffer, I.; Kall, M.; Hook, F. *Langmuir* **2003**, *19*, 10414.
(14) Almeida, J. P. M; Chen, A. L.; Foster, A.; Drezek, R. *Nanomedicine* **2011**, *6*, 815.
(15) Clift, M. J. D.; Gehr, P.; Rothen-Rutishauser, B. *Arch. Toxicol.* **2011**, *85*, 723.
(16) European Medicines Agency. www.ema.europa.eu. Accessed Nov 13, 2011.
(17) Nel, A.; Xia, T.; Mädler, L.; Li, N. *Science* **2006**, *311*, 622.
(18) Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. *Nat. Mater.* **2009**, *8*, 543.
(19) Belyanskaya, L.; Manser, P.; Spohn, P.; Bruinink, A.; Wick, P. *Carbon* **2007**, *45*, 2843.
(20) Herzog, E.; Davoren, M.; Lyng, F. M.; Byrne, H. J.; Chambers, G. *Carbon* **2007**, *45*, 1425.
(21) Limbach, L. K.; Li, Y. C.; Grass, R. N.; Brunner, T. J.; Hintermann, M. A.; Muller, M.; Gunther, D.; Stark, W. J. *Environ. Sci. Technol.* **2005**, *39*, 9370.
(22) Krug, H. F.; Wick, P. *Angew. Chem., Int. Ed.* **2011**, *50*, 1260.
(23) Wörle-Knirsch, J. M.; Pulskamp, K.; Krug, H. F. *Nano Lett.* **2006**, *6*, 1261.
(24) Guo, L.; von dem Bussche, A.; Buechner, M.; Yan, A.; Kane, A. B.; Hurt, R. H. *Small* **2008**, *4*, 721.
(25) Warheit, D. B.; Donner, E. M. *Nanotoxicology* **2010**, *4*, 409.
(26) Maynard, A. D.; Aitken, R. J.; Butz, T.; Colvin, V.; Donaldson, K.; Oberdörster, G.; Philbert, M. A.; Ryan, J.; Seaton, A.; Stone, V.; Tinkle, S. S.; Tran, L.; Walker, N. J.; Warheit, D. B. *Nature* **2006**, *444*, 267.
(27) Mahmoudi, M.; Hosseinkhani, H.; Hosseinkhani, M.; Boutry, S.; Simchi, A.; Journeay, W. S.; Subramani, K.; Laurent, S. *Chem. Rev.* **2011**, *111*, 253.
(28) Mahmoudi, M.; Lynch, I.; Ejtehadi, R.; Monopoli, M.; Laurent, S. *Chem. Rev.* **2011**, *111*, 5610.
(29) Mahmoudi, M.; Sahraian, M. A.; Shokrgozar, M. A.; Laurent, S. *ACS Chem. Neurosci.* **2011**, *2*, 118.
(30) Mahmoudi, M.; Simchi, A.; Imani, M. *J. Iran. Chem. Soc.* **2010**, *7*, S1.
(31) Neuberger, T.; Schöpf, B.; Hofmann, M.; Hofmann, H.; von Rechenberg, B. *J. Magn. Magn. Mater.* **2005**, *293*, 483.
(32) Gupta, A. K.; Gupta, M. *Biomaterials* **2005**, *18*, 3995.
(33) ISI Web of Knowledge database; search on September 20, 2011 with "iron oxide" or "SPION" and "medical application".
(34) ISI Web of Knowledge database; search on September 20, 2011 with "iron oxide" or "SPION" and "medical application" and "toxicity".
(35) Clift, M. J.; Gehr, P.; Rothen-Rutishauser, B. *Arch. Toxicol.* **2011**, *85*, 723.
(36) Smith, M. T. *Environ. Health Perspect.* **1996**, *104*, 1219.
(37) Weissleder, R.; Cheng, H. C.; Bogdanova, A.; Bogdanov, A. *J. Magn. Reson. Imaging* **1997**, *7*, 258.
(38) Moore, M.; Weissleder, R.; Bogdanov, A. *J. Magn. Reson. Imaging* **1997**, *7*, 1140.
(39) Neuwelt, E. A.; Weissleder, R.; Nilaver, G.; Kroll, R. A.; Romangoldstein, S.; Szumowski, J.; Pagel, M. A.; Jones, R. S.; Remsen, L. G.; McCormick, C. L.; Shannon, E. M.; Muldoon, L. L. *Neurosurgery* **1994**, *34*, 777.
(40) Mueller, R. H.; Maassen, S.; Weyhers, H.; Specht, F.; Lucks, J. S. *Int. J. Pharm.* **1996**, *138*, 85.
(41) Berry, C. C.; Wells, S.; Charles, S.; Aitchison, G.; Curtis, A. S. G. *Biomaterials* **2004**, *25*, 5405.
(42) Berry, C. C.; Wells, S.; Charles, S.; Curtis, A. S. G. *Biomaterials* **2003**, *24*, 4551.
(43) Gupta, A. K.; Berry, C.; Gupta, M.; Curtis, A. *IEEE Trans. Nanobiosci.* **2003**, *2*, 255.
(44) Gupta, A. K.; Curtis, A. S. G. *Biomaterials* **2004**, *25*, 3029.
(45) Gupta, A. K.; Naregalkar, R. R.; Vaidya, V. D.; Gupta, M. *Nanomedicine* **2007**, *2*, 23.
(46) Gupta, A. K.; Wells, S. *IEEE Trans. Nanobiosci.* **2004**, *3*, 66.
(47) van den Bos, E. J.; Wagner, A.; Mahrholdt, H.; Thompson, R. B.; Morimoto, Y.; Sutton, B. S.; Judd, R. M.; Taylor, D. A. *Cell Transplant.* **2003**, *12*, 743.
(48) Stroh, A.; Zimmer, C.; Gutzeit, C.; Jakstadt, M.; Marschinke, F.; Jung, T.; Pilgrimm, H.; Grune, T. *Free Radical Biol. Med.* **2004**, *36*, 976.
(49) Brunner, T. J.; Wick, P.; Manser, P.; Spohn, P.; Grass, R. N.; Limbach, L. K.; Bruinink, A.; Stark, W. J. *Environ. Sci. Technol.* **2006**, *40*, 4374.
(50) Soto, K. F.; Carrasco, A.; Powell, T. G.; Garza, K. M.; Murr, L. E. *J. Nanopart. Res.* **2005**, *7*, 145.
(51) Soto, K. F.; Garza, K. M.; Murr, L. E. *Acta Biomater.* **2007**, *3*, 351.
(52) Jeng, A.; Swanson, J. *J. Environ. Sci. Health, Part 1* **2006**, *41*, 2699.
(53) Au, C.; Mutkus, L.; Dobson, A.; Riffle, J.; Lalli, J.; Aschner, M. *Biol. Trace Elem. Res.* **2007**, *120*, 248.
(54) Pisanic, T. R.; Blackwell, J. D.; Shubayev, V. I.; Finones, R. R.; Jin, S. *Biomaterials* **2007**, *28*, 2572.
(55) Sun, R.; Dittrich, J.; LE-Huu, M.; Mueller, M. M.; Bedke, J.; Kartenbeck, J.; Lehmann, W. D.; Krueger, R.; Bock, M.; Huss, R.; Seliger, C.; Grone, H. J.; Misselwitz, B.; Semmler, W.; Kiessling, F. *Invest. Radiol.* **2005**, *40*, 504.
(56) Auffan, M.; Decome, L.; Rose, J.; Orsiere, T.; DeMeo, M.; Briois, V.; Chaneac, C.; Olivi, L.; Berge-Lefranc, J. L.; Botta, A.; Wiesner, M. R.; Bottero, J. Y. *Environ. Sci. Technol.* **2006**, *40*, 4367.
(57) Hu, F. X.; Neoh, K. G.; Cen, L.; Kang, E. T. *Biomacromolecules* **2006**, *7*, 809.
(58) de Freitas, E. R. I.; Soares, P. R. O.; Santos, R. D.; dos Santos, D. L.; da Silva, J. R.; Porfirio, E. P.; Bao, S. N.; Lima, E. C. D.; Morais, P. C.; Guillo, L. A. *J. Nanosci. Nanotechnol.* **2008**, *8*, 2385.
(59) Petri-Fink, A.; Steitz, B.; Finka, A.; Salaklang, J.; Hofmann, H. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 129.
(60) Mahmoudi, M.; Shokrgozar, M. A.; Simchi, A.; Imani, M.; Milani, A. S.; Stroeve, P.; Vali, H.; Hafeli, U. O.; Bonakdar, S. *J. Phys. Chem. C* **2009**, *113*, 2322.
(61) Mahmoudi, M.; Simchi, A.; Imani, M. *J. Phys. Chem. C* **2009**, *113*, 9573.
(62) Mahmoudi, M.; Simchi, A.; Imani, M.; Hafeli, U. O. *J. Phys. Chem. C* **2009**, *113*, 8124.
(63) Mahmoudi, M.; Simchi, A.; Imani, M.; Milani, A. S.; Stroeve, P. *Nanotechnology* **2009**, *20*, 225104.
(64) Mahmoudi, M.; Simchi, A.; Imani, M.; Shokrgozar, M. A.; Milani, A. S.; Hafeli, U. O.; Stroeve, P. *Colloids Surf., B* **2010**, *75*, 300.
(65) Mahmoudi, M.; Simchi, A.; Milani, A. S.; Stroeve, P. *J. Colloid Interface Sci.* **2009**, *336*, 510.
(66) Mahmoudi, M.; Simchi, A.; Vali, H.; Imani, M.; Shokrgozar, M. A.; Azadmanesh, K.; Azari, F. *Adv. Eng. Mater.* **2009**, *11*, B243.
(67) Lewinski, N.; Colvin, V.; Drezek, R. *Small* **2008**, *4*, 26.
(68) Mahmoudi, M.; Azadmanesh, K.; Shokrgozar, M. A.; Journeay, W. S.; Laurent, S. *Chem. Rev.* **2011**, *111*, 3407.

(69) Mahmoudi, M.; Sant, S.; Wang, B.; Laurent, S.; Sen, T. *Adv. Drug Delivery Rev.* **2011**, *63*, 24.
(70) Arbab, A. S.; Bashaw, L. A.; Miller, B. R.; Jordan, E. K.; Lewis, B. K.; Kalish, H.; Frank, J. A. *Radiology* **2003**, *229*, 838.
(71) Hussain, S. M.; Hess, K. L.; Gearhart, J. M.; Geiss, K. T.; Schlager, J. J. *Toxicol. in Vitro* **2005**, *19*, 975.
(72) Cheng, F. Y.; Su, C. H.; Yang, Y. S.; Yeh, C. S.; Tsai, C. Y.; Wu, C. L.; Wu, M. T.; Shieh, D. B. *Biomaterials* **2005**, *26*, 729.
(73) Mahmoudi, M.; Serpooshan, V.; Laurent, S. *Nanoscale* **2011**, *3*, 3007.
(74) Lee, H.; Lee, E.; Kim, D. K.; Jang, N. K.; Jeong, Y. Y.; Jon, S. *J. Am. Chem. Soc.* **2006**, *128*, 7383.
(75) Raty, J. K.; Liimatainen, T.; Wirth, T.; Airenne, K. J.; Ihalainen, T. O.; Huhtala, T.; Hamerlynck, E.; Vihinen-Ranta, M.; Narvaren, A.; Yla-Herttuala, S.; Hakumaki, J. M. *Gene Ther.* **2006**, *13*, 1440.
(76) Kim, J. S.; Yoon, T. J.; Yu, K. N.; Mi, S. N.; Woo, M.; Kim, B. G.; Lee, K. H.; Sohn, B. H.; Park, S. B.; Lee, J. K.; Cho, M. H. *J. Vet. Sci.* **2006**, *7*, 321.
(77) Muller, K.; Skepper, J. N.; Posfai, M.; Trivedi, R.; Howarth, S.; Corot, C.; Lancelot, E.; Thompson, P. W.; Brown, A. P.; Gillard, J. H. *Biomaterials* **2007**, *28*, 1629.
(78) Mykhaylyk, O.; Antequera, Y. S.; Vlaskou, D.; Plank, C. *Nat. Protocols* **2007**, *2*, 2391.
(79) Lee, K. J.; An, J. H.; Shin, J. S.; Kim, D. H.; Kim, C.; Ozaki, H.; Koh, J. G. *Nanotechnology* **2007**, *18*, 465201.
(80) Martin, A. L.; Bernas, L. M.; Rutt, B. K.; Foster, P. J.; Gillies, E. R. *Bioconjugate Chem.* **2008**, *19*, 2375.
(81) Lv, G.; He, F.; Wang, X.; Gao, F.; Zhang, G.; Wang, T.; Jiang, H.; Wu, C.; Guo, D.; Li, X.; Chen, B.; Gu, Z. *Langmuir* **2008**, *24*, 2151.
(82) Huang, G.; Diakur, J.; Xu, Z.; Wiebe, L. I. *Int. J. Pharm.* **2008**, *360*, 197.
(83) Chen, B. A.; Dai, Y. Y.; Wang, X. M.; Zhang, R. Y.; Xu, W. L.; Shen, H. L.; Gao, F.; Sun, Q.; Deng, X. J.; Ding, J. H.; Gao, C.; Sun, Y. Y.; Cheng, J.; Wang, J.; Zhao, G.; Chen, N. N. *Int. J. Nanomed.* **2008**, *3*, 343.
(84) Wang, A. Z.; Bagalkot, V.; Vasilliou, C. C.; Gu, F.; Alexis, F.; Zhang, L.; Shaikh, M.; Yuet, K.; Cima, M. J.; Langer, R.; Kantoff, P. W.; Bander, N. H.; Jon, S.; Farokhzad, O. C. *Chem. Med. Chem.* **2008**, *3*, 1311.
(85) Horie, M.; Nishio, K.; Fujita, K.; Kato, H.; Nakamura, A.; Kinugasa, S.; Endoh, S.; Miyauchi, A.; Yamamoto, K.; Murayama, H.; Niki, E.; Iwahashi, H.; Yoshida, Y.; Nakanishi, J. *Chem. Res. Toxicol.* **2009**, *22*, 1415.
(86) Villanueva, A.; Caete, M.; Roca, A. G.; Calero, M.; Veintemillas-Verdaguer, S.; Serna, C. J.; Del Puerto Morales, M.; Miranda, R. *Nanotechnology* **2009**, *20*, 1.
(87) Ge, Y.; Zhang, Y.; He, S.; Nie, F.; Teng, G.; Gu, N. *Nanoscale Res. Lett.* **2009**, *4*, 287.
(88) Yurchenko, O. V.; Todor, I. N.; Khayetsky, I. K.; Tregubova, N. A.; Lukianova, N. Y.; Chekhun, V. F. *Exp Oncol.* **2010**, *32*, 237.
(89) Naqvi, S.; Samim, M.; Abdin, M.; Ahmed, F. J.; Maitra, A.; Prashant, C.; Dinda, A. K. *Int. J. Nanomed.* **2010**, *16*, 983.
(90) Huang, J. H.; Parab, H. J.; Liu, R. S.; Lai, T. C.; Hsiao, M.; Chen, C. H.; Sheu, H. S.; Chen, J. M.; Tsai, D. P.; Hwu, Y. K. *J. Phys. Chem. C* **2008**, *112*, 15684.
(91) Delcroix, G. J. R.; Jacquart, M.; Lemaire, L.; Sindji, L.; Franconi, F.; Le Jeune, J. J.; Montero-Menei, C. N. *Brain Res.* **2009**, *1255*, 18.
(92) Pawelczyk, E.; Arbab, A. S.; Chaudhry, A.; Balakumaran, A.; Robey, P. G.; Frank, J. A. *Stem Cells* **2008**, *26*, 1366.
(93) Wan, S.; Huang, J.; Guo, M.; Zhang, H.; Cao, Y.; Yan, H.; Liu, K. *J. Biomed. Mater. Res., Part A* **2007**, *80*, 946.
(94) Shi, X.; Thomas, T. P.; Myc, L. A.; Kotlyar, A.; Baker, J. R., Jr. *Phys. Chem. Chem. Phys.* **2007**, *9*, 5712.
(95) Lee, C. M.; Jeong, H. J.; Kim, E. M.; Cheong, S. J.; Park, E. H.; Kim, D. W.; Lim, S. T.; Sohn, M. H. *Macromol. Res.* **2009**, *17*, 133.
(96) Polyak, B.; Fishbein, I.; Chorny, M.; Alferiev, I.; Williams, D.; Yellen, B.; Friedman, G.; Levy, R. J. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 698.
(97) Chorny, M.; Polyak, B.; Alferiev, I. S.; Walsh, K.; Friedman, G.; Levy, R. J. *FASEB J.* **2007**, *21*, 2510.
(98) Cheng, C. M.; Chu, P. Y.; Chuang, K. H.; Roffler, S. R.; Kao, C. H.; Tseng, W. L.; Shiea, J.; Chang, W. D.; Su, Y. C.; Chen, B. M.; Wang, Y. M.; Cheng, T. L. *Cancer Gene Ther.* **2009**, *16*, 83.
(99) Muller, K.; Skepper, J. N.; Tang, T. Y.; Graves, M. J.; Patterson, A. J.; Corot, C.; Lancelot, E.; Thompson, P. W.; Brown, A. P.; Gillard, J. H. *Biomaterials* **2008**, *29*, 2656.
(100) Babic, M.; Horok, D.; Jendelova, P.; Glogarova, K.; Herynek, V.; Trchova, M.; Likavoanova, K.; Lesny, P.; Pollert, E.; Hajek, M.; Sykova, E. *Bioconjugate Chem.* **2009**, *20*, 283.
(101) Chen, G.; Chen, W.; Wu, Z.; Yuan, R.; Li, H.; Gao, J.; Shuai, X. *Biomaterials* **2009**, *30*, 1962.
(102) Toso, C.; Vallee, J. P.; Morel, P.; Ris, F.; Demuylder-Mischler, S.; Lepetit-Coiffe, M.; Marangon, N.; Saudek, F.; Shapiro, A. M. J.; Bosco, D.; Berney, T. *Am. J. Transplant.* **2008**, *8*, 701.
(103) Dunning, M. D.; Lakatos, A.; Loizou, L.; Kettunen, M.; Ffrench-Constant, C.; Brindle, K. M.; Franklin, R. J. M. *J. Neurosci.* **2004**, *24*, 9799.
(104) Karlsson, H. L.; Cronholm, P.; Gustafsson, J.; Muller, L. *Chem. Res. Toxicol.* **2008**, *21*, 1726.
(105) Omidkhoda, A.; Mozdarani, H.; Movasaghpoor, A.; Fatholah, A. A. P. *Toxicol. in Vitro* **2007**, *21*, 1191.
(106) Raynal, I.; Prigent, P.; Peyramaure, S.; Najid, A.; Rebuzzi, C.; Corot, C. *Invest. Radiol.* **2004**, *39*, 56.
(107) Diaz, B.; Sanchez-Espinel, C.; Arrueho, M.; Faro, J.; De Miguel, E.; Magadan, S.; Yague, C.; Fernandez-Pachecco, R.; Ibarra, M. R.; Santamaria, J.; Gonzales-Fernandez, A. *Small* **2008**, *4*, 2025.
(108) de la Fuente, J. M.; Alcantara, D.; Penades, S. *IEEE Trans. Nanobiosci.* **2007**, *6*, 275.
(109) Risom, L.; Moller, P.; Soft, S. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2005**, *592*, 119.
(110) Mahmoudi, M.; Laurent, S.; Shokrgozar, M. A.; Hosseinkhani, M. *ACS Nano* **2011**, *5*, 7263.
(111) Alekseenko, A. V.; Wassem, T. V.; Fedorovich, S. V. *Brain Res.* **2008**, *1241*, 193.
(112) Theil, E. C.; Matzapetakis, M.; Liu, X. F. *J. Biol. Inorg. Chem.* **2006**, *11*, 803.
(113) Li, N.; Sioutas, C.; Cho, A.; Schmitz, D.; Misra, C.; Sempf, J.; Wang, M.; Oberley, T.; Froines, J.; Nel, A. *Environ. Health Perspect.* **2003**, *111*, 455.
(114) Amara, N.; Bachoual, R.; Desmard, M.; Golda, S.; Guichard, C.; Lanone, S.; Aubier, M.; Ogier-Denis, E.; Boczkowski, J. *Am. J. Physiol., Lung Cell Mol. Physiol.* **2007**, *293*, L170.
(115) Arimoto, T.; Kadiiska, M. B.; Sato, K.; Corbett, J.; Mason, R. P. *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 379.
(116) Park, J. B. *Exp. Mol. Med.* **2003**, *35*, 325.
(117) Ruehm, S. G.; Corot, C.; Vogt, P.; Kolb, S.; Debatin, J. F. *Circulation* **2001**, *103*, 415.
(118) Zhang, Y.; Kohler, N.; Zhang, M. Q. *Biomaterials* **2002**, *23*, 1553.
(119) Kooi, M. E.; Cappendijk, V. C.; Cleutjens, K.; Kessels, A. G. H.; Kitslaar, P.; Borgers, M.; Frederik, P. M.; Daemen, M.; van Engelshoven, J. M. A. *Circulation* **2003**, *107*, 2453.
(120) Storm, G.; Belliot, S. O.; Daemen, T.; Lasic, D. D. *Adv. Drug Delivery Rev.* **1995**, *17*, 31.
(121) Schreck, R.; Albermann, K.; Baeuerle, P. A. *Free Radic. Res. Commun.* **1992**, *17*, 221.
(122) Schins, R. P. F; Donaldson, K *Inhalation Toxicol.* **2000**, *12* (Suppl. 3), 317.
(123) Albrecht, C.; Born, P. J. A.; Unfried, K. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2004**, *552*, 23.
(124) Siglienti, I.; Bendszus, M.; Kleinschmitz, C.; Stoll, G. *J. Neuroimmunol.* **2006**, *173*, 166.
(125) Hsiao, J. K.; Chu, H. H.; Wang, Y. H.; Lai, C. W.; Choii, P. T.; Hsieh, S. T.; Wang, J. L.; Liu, H. M. *NMR Biomed.* **2008**, *21*, 820.
(126) Naveau, A.; Smirnov, P.; Menager, C.; Gazeau, F.; Clement, O.; Lafont, A.; Gogly, B. *J. Periodontol.* **2006**, *77*, 238.
(127) Radu, M.; Munteanu, M. C.; Petrache, S.; Serban, A. I.; Dinu, D.; Hermenean, A.; Sima, C.; Dinischiotu, A. *Acta Biochim. Pol.* **2010**, *57*, 355.

(128) Choi, J. Y.; Lee, S. H.; Na, H. B.; An, K.; Hyeon, T.; Seo, T. S. *Bioprocess Biosyst. Eng.* **2010**, *33*, 21.
(129) Wang, P.; Henning, S. M.; Heber, D. *PLoS One* **2010**, *5*, e10202.
(130) Natarajan, A.; Gruettner, C.; Ivkov, R.; Denardo, G. L.; Mirick, G.; Yuan, A.; Foreman, A.; DeNardo, S. J. *Bioconjugate Chem.* **2008**, *19*, 1211.
(131) Jain, T. K.; Reddy, M. K.; Morales, M. A.; Leslie-Pelecky, D. L.; Labhasetwar, V. *Mol. Pharmaceutics* **2008**, *5*, 316.
(132) Hanini, A.; Schmitt, A.; Kacem, K.; Chau, F.; Ammar, S.; Gavard, J. *Int. J. Nanomed.* **2011**, *6*, 787.
(133) Weissleder, R.; Stark, D. D.; Engelstad, B. L.; Bacon, B. R.; Compton, C. C.; White, D. L.; Jacobs, P.; Lewis, J. *Am. J. Roentgenol.* **1989**, *152*, 167.
(134) Bourrinet, P.; Bengele, H. H.; Bonnemain, B.; Dencausse, A.; Idee, J. M.; Jacobs, P. M.; Lewis, J. M. *Invest. Radiol.* **2006**, *41*, 313.
(135) Puntarulo, S. *Mol. Aspects Med.* **2005**, *26*, 299.
(136) Chertok, B.; Moffat, B. A.; David, A. E.; Yu, F.; Bergemann, C.; Ross, B. D.; Yang, V. C. *Biomaterials* **2008**, *29*, 487.
(137) Yu, M. K.; Jeong, Y. Y.; Park, J.; Park, S.; Kim, J. W.; Min, J. J.; Kim, K.; Jon, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 5362.
(138) Ros, P. R.; Freeny, P. C.; Harms, S. E.; Seltzer, S. E.; Davis, P. L.; Chan, T. W.; Stillman, A. E.; Muroff, L. R.; Runge, V. M.; Nissenbaum, M. A.; Jacobs, P. M. *Radiology* **1995**, *196*, 481.
(139) Lubbe, A. S.; Alexiou, C.; Bergemann, C. *J. Surg. Res.* **2001**, *95*, 200.
(140) Lubbe, A. S.; Bergemann, C.; Riess, H.; Schriever, F.; Reichardt, P.; Possinger, K.; Matthias, M.; Durken, B.; Herrmann, F.; Gürtler, R.; Hohenberger, P.; Haas, N.; Sohr, R.; Sander, B.; Lemke, A. J.; Ohlendorf, D.; Huhnt, W.; Huhn, D. *Cancer Res.* **1996**, *56*, 4686.
(141) Lubbe, A. S.; Bergemann, C.; Brock, J.; McClure, D. G. *J. Magn. Magn. Mater.* **1999**, *194*, 149.
(142) Okon, E. E.; Pouliquen, D.; Pereverzev, A. E.; Kudryavtsev, B. N.; Jallet, P. *Tsitologiya* **2000**, *42*, 365.
(143) Okon, E. E.; Pulikan, D.; Pereverzev, A. E.; Kudriavtsev, B. N.; Zhale, P. *K voprosu o toksichnosti magnetit-dekstranovykh chastits: morfologicheskoe issledovanie* **2000**, *42*, 358.
(144) Lübbe, A. S.; Bergemann, C.; Huhnt, W.; Fricke, T.; Riess, H.; Brock, J. W.; Huhn, D. *Cancer Res.* **1996**, *56*, 4694.
(145) Paruta, S.; Horl, W. H. *Kidney Int.* **1999**, *55*, 125.
(146) Van Hecke, P.; Marchal, G.; Decrop, E.; Baert, A. L. *Invest. Radiol.* **1989**, *24*, 397.
(147) De Vries, I. J. M.; Lesterhuis, W. J.; Barentsz, J. O.; Verdijk, P.; Van Krieken, J. H.; Boerman, O. C.; Oyen, W. J. G.; Bonenkamp, J. J.; Boezeman, J. B.; Adema, G. J.; Bulte, J. W. M.; Scheenen, T. W. J.; Punt, C. J. A.; Heerschap, A.; Figdor, C. G. *Nat. Biotechnol.* **2005**, *23*, 1407–1413.
(148) Yu, H.; Adedoyin, A. *Drug Discovery Today* **2003**, *8*, 852.
(149) Dames, P.; Gleich, B.; Flemmer, A.; Hajek, K.; Seidl, N.; Wiekhorst, F.; Eberbeck, D.; Bittmann, I.; Bergemann, C.; Weyh, T.; Trahms, L.; Rosenecker, J.; Rudolph, C. *Nat. Nanotechnol.* **2007**, *2*, 495.
(150) Krug, H. F.; Wick, P. *Angew. Chem., Int. Ed.* **2011**, *50*, 2.