

Probing the Cytotoxicity of Nanoparticles: Experimental Pitfalls and Artifacts

Jenny Domey, Lisa Haslauer, Ina Grau, Claudia Strobel, Melanie Kettering, and Ingrid Hilger

Abstract Throughout the last years, a huge variety of different nanoparticle formulations have been studied with the aim to assess their harmlessness in biological systems, to elucidate how the morphological features govern their impact on cells, and to develop cell labeling strategies for biomedical purposes. Most of such studies are based on the use of various cell viability assays. Interestingly, different results – even contradictory ones – have been observed between the groups, even though the respective nanoparticle formulations were more or less similar. One possible reason for such discrepancies is the occurrence of specific interactions between the nanoparticles and the ingredients of the respective cell viability assays. A similar situation can be encountered when researchers investigate the labeling of (stem) cells for biomedical purposes. Hereto, different labeling efficiencies were observed with the corresponding effects on cell viability and functionality. Therefore, the present review focuses on potential pitfalls and artifacts associated with the cytotoxicity evaluation of nanomaterials.

Keywords ATP · Cell viability assays · Iron oxide · MTS · MTT · Nanomaterials · Nanoparticles · Toxicity

J. Domey, L. Haslauer, I. Grau, C. Strobel and M. Kettering
Institut für Diagnostische und Interventionelle Radiologie I des Klinikums der Friedrich-Schiller-Universität Jena, Bachstraße 18, D-07740 Jena, Germany

I. Hilger (✉)
Institut für Diagnostische und Interventionelle Radiologie I des Klinikums der Friedrich-Schiller-Universität Jena, Bachstraße 18, D-07740 Jena, Germany

Institut für Diagnostische und Interventionelle Radiologie I des Klinikums der Friedrich-Schiller-Universität Jena, Forschungszentrum Lobeda, Erlanger Allee 101, D-07747 Jena, Germany
e-mail: ingrid.hilger@med.uni-jena.de

Contents

1	Introduction	32
2	Validity of Cell Viability Assays	33
3	Effects of Nanoparticle Labeling Processes on Cells	39
4	Conclusion	42
	References	43

1 Introduction

Nanotechnology has emerged as a promising tool in different branches of science. Investigators are keen to understand the physicochemical properties of nanoscale materials (<100 nm) as a function of their size, shape, surface chemistry, and chemical composition. In the last years, extensive work has been done to elucidate the interactions between differently designed nanomaterials and biological systems. The main aim is to assess the impact of nanoparticle exposure or to identify potential applications in future medicine. In this context, different nanomaterial formulations have been exposed to different cell types, organisms (bacteria), tissues, and animals. As a result, scientists became aware of the complexity of these interactions. Particularly if injected into the blood stream, the adsorption of serum proteins on the nanoparticles' surface [1] is a determining factor governing their fate in the body and the uptake of nanoparticles by the respective target cells.

When analyzing the bio-pathological effects on cells, one of the first processes to consider is nanoparticle adsorption on the cell surface and internalization. Hereto, the nanoparticles' shape seems to be important. In this regard, rods were shown to induce maximum uptake, followed by spherical, cylindrical (more symmetrical than rods), and cubic nanoparticles; this is true for nanoparticles larger than 100 nm [2]. With decreasing size (smaller than 100 nm) spheres are better internalized than rods [3]. The aspect ratio and the composition of the nanoparticles [4] influence the degree of nanoparticle uptake as well. Besides uptake as well as electrostatic interactions, the intracellular degradation processes, which are largely dependent on the composition of the nanomaterial, influence their impact on cell viability. To test cell viability after nanoparticle exposure, different assay systems addressing specific metabolic pathways of the cell have been extensively used for screening purposes with more or less diverging results. The underlying reasons are rather complex and not well understood. A set of examples for the biological impact of specific nanomaterials and the problems associated with their experimental investigation will be detailed below.

Due to their good biocompatibility, iron oxide nanoparticles have been proposed in particular for a great variety of applications in medicine and biology. Examples are their use for cell separation procedures in life-science research or the labeling of stem cells in order to allow for an accurate monitoring of their migration via

magnetic resonance imaging (MRT). One important prerequisite for both applications is that the cells must retain their viability and functionality during these experimental manipulations.

This review focuses on the different aspects which are to be considered when cell viability assays are used to assess the cytotoxicity of nanoparticles and on the potential pitfalls which should be taken into account when cells are labeled with magnetic nanoparticles.

2 Validity of Cell Viability Assays

A huge variety of different cell viability assays have been developed to assess the impact of xenobiotics on cells. One very simple approach is the use of the dye trypan blue. It is a diazo dye which selectively stains dead cells. The dye is unable to pass the cell membrane of viable cells but enters the cytoplasm of dead cells with compromised membranes. The staining method has been described as “dye exclusion method,” since it excludes live cells from staining. After staining, live cells are counted using a microscope. In a similar manner, the dye Evans blue has been used in cell viability assays.

Other assay systems address specific metabolic pathways in the cell. For example, the cellular membrane integrity can be assessed by determining the release of lactate dehydrogenase into the culture medium. The enzyme lactate dehydrogenase is normally localized in the cytosol and is consequently sequestered inside viable cells. A release occurs in damaged cells only. Advantages of this assay are fast performance and simple evaluation [5]. However, nanoparticle incubation is limited to low concentrations of serum proteins, since fetal serum contains distinct amounts of lactate dehydrogenase itself, which might lead to false-positive results. Low serum protein concentrations on the other hand might not mimic the *in vivo* situation appropriately, particularly when assessing the cytotoxic response of blood cells.

Another possibility is to determine the viability of a cell population via the so-called MTS assay. It is based on the reduction of a methyl tetrazolium salt (MTS: [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]) to a water-soluble formazan product in presence of a corresponding amount of the redox coenzymes NADH/NADPH and endogenous dehydrogenases. The amount of converted tetrazolium salt is quantified photometrically (absorbance at 490 nm) and correlates with the number of living cells in the respective culture system [6]. Apart from the MTS, other tetrazolium salts have been proposed for cell viability studies, such as XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide), WSTs (water-soluble tetrazolium salts, e.g., WST-1, WST-8), and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide); MTT was the first compound that was introduced for dehydrogenase-based cell viability assessments and still required an additional solubilization step. Basically, all tetrazolium salt-based cell viability assays measure glycolytic activity. To exclude false-negative readouts particularly when testing viable but metabolically inactive cells, it is important to include untreated controls.

Alternatively, cell viability can be measured by the assessment of cellular ATP levels. This is accomplished by the so-called ATP assay, a luminescence assay system, which measures the metabolic activity of cells via the enzymatic transformation of D-luciferin and intracellular ATP to oxiluciferin and light.

A further endpoint assay to probe cell viability is the assessment of thymidine incorporation during DNA replication. This test system directly measures cell proliferation. When cell density allows for cell proliferation, it can also be used for viability assessments of cells with a short G1-phase as it is particularly the case for permanent cell lines. Experimentally, a specific thymidine analogue like EdU (5-ethynyl-2-deoxyuridine) is added to the culture medium and gets incorporated into newly synthesized DNA. Analysis is performed by a click labeling reaction of the incorporated EdU with a reagent containing copper and Oregon Green® 488 azide. For signal amplification, an anti-Oregon Green® antibody conjugated to the enzyme horseradish peroxidase (HRP) is used which then reacts with a specific substrate and produces a fluorescent product.

Recently, critical debates on the reliability of these testing systems especially for the MTS or MTT assay have been reported [7–10]. According to these reports, the *in vitro* cytotoxicity assays based on intracellular enzymatic conversions are prone to overestimate cell viability when they are incubated with certain nanoparticle formulations. One example is the exposure of rat gliosarcoma cells (9 L/lacZ) or murine macrophages (J774A.1) to carboxymethyl dextran-coated, clustered magnetic (iron oxide) nanoparticles as shown in Fig. 1. Even though care has been taken (e.g., by the incorporation of multiple washing steps or the separation of supernatant from cells) to prevent light absorbance due to the presence of nanoparticles in the sample, an overestimation of the cell viability using the MTS assay compared to the ATP assay is evident (Fig. 1). This overestimation of cell viability leads to erroneous EC₅₀ values (Table 1) and is attributed to the interference of the nanoparticles with ingredients of the MTS assay resulting in artificially higher dehydrogenase activities. Interestingly, it has been shown that mesoporous silica nanoparticles can accelerate the exocytosis of formazan crystals after the particles have been taken up by HeLa cells and astrocytes. The authors attribute this effect to the perturbation of intracellular vesicle trafficking as a result of the nanoparticle uptake [11]. Similar relationships have already been observed for other chemicals as well: Hoskins et al. hypothesize that the false increase of cell viability as measured by MTS might be due to changes in redox metabolism as a response to the presence (uptake) of nanoparticles [8].

With respect to cell culture conditions, it is also well conceivable that the presence of aggregated magnetic nanoparticles on cells (Fig. 2) can bias the status of cell viability, particularly if colorimetric methods based on the light absorbance of enzymatic products are used (e.g., MTS test). It is rather challenging to control nanoparticle-mediated absorbance, since nanoparticles are mostly very prone to adhere to the cell culture ware or they get internalized in cells. Thus, subtraction of the “nanoparticle blank values” (in absence of cells) from those values recorded for cells incubated with nanoparticles would not entirely heal the effect of overestimation. Moreover, the overestimation of cell viability assessed via MTS

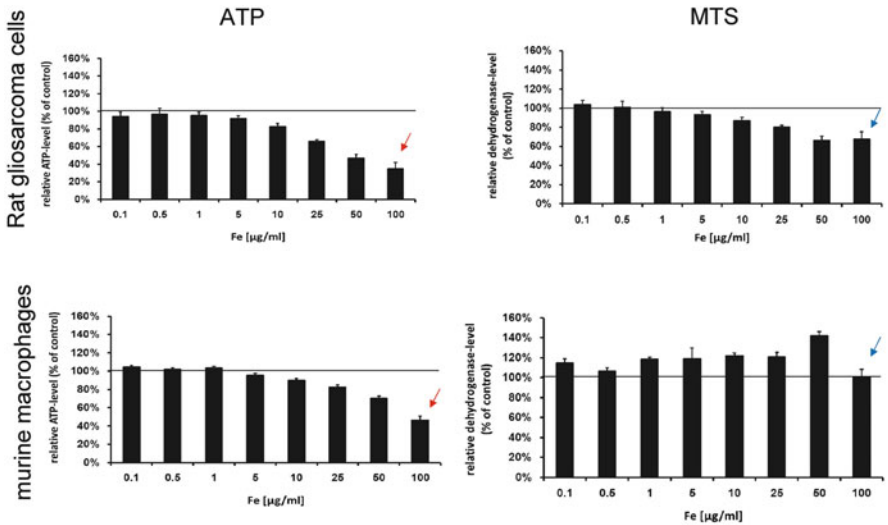


Fig. 1 Impact of nanoparticle exposure depends on the cell type and on the cell viability assay used. Relative ATP level (*left*) and dehydrogenase level (*right*) of rat gliosarcoma cells (9 L/lacZ, *top*) and murine macrophages (J774A.1, *bottom*) after exposure to carboxymethyl dextran-coated iron oxide nanoparticles for 24 h. The cellular ATP and dehydrogenase levels were normalized to the respective levels of untreated control cells (100%). According to DIN EN ISO 10993–5 a nanomaterial can be considered as toxic when cell viability drops below 70% (*dark line*). *Red arrows* indicate the loss of cell viability after nanoparticle incubation (100 µg/mL) which was determined by using a luminescent ATP assay for both cell lines. In contrast to that, *blue arrows* indicate only a slight loss in cell viability for rat gliosarcoma cells and constant cell viability for murine macrophages which is measured by the MTS assay

Table 1 MTS and ATP assays produce different values for the effective concentration EC₅₀ of iron oxide nanoparticles in contact to murine macrophages and rat gliosarcoma cells

Incubation time	Murine macrophages		Rat gliosarcoma cells	
	MTS	ATP	MTS	ATP
24 h	>100*	86	>100*	>100*
48 h	>100*	92	>100*	58

Rat glioblastoma cells (9 L/lacZ) and murine macrophages (J774A.1) were incubated with nanoparticles for 24 and 48 h. EC-50 concentrations as given in the table (in µg/mL) were calculated from data of both assays. >100* = the effective dose is higher than the highest nanoparticle concentration under test (100 µg/mL)

can differ in dependence on the cell line under study. For example, as shown in Fig. 1, overestimation was more pronounced for macrophages than for glioblastoma cells. This observation can be attributed to the fact that the first provide much more intercellular spaces for nanoparticles to hide than the latter. Hence, this would mean that the confluency status of the respective cell population at the time of nanoparticle exposure might also influence the outcome of cell viability assays.

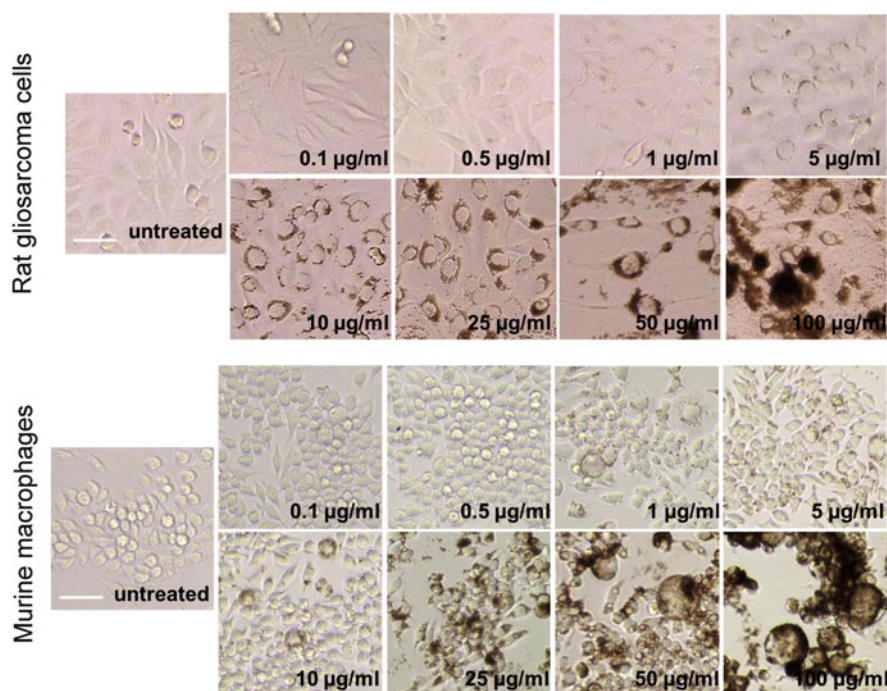


Fig. 2 Aggregation of iron oxide nanoparticles on the cell surface leads to opaque areas which are usually not removed by washing. Rat gliosarcoma (9 L/lacZ) cells and murine macrophages (J7747A.1) were incubated with carboxymethyl dextran iron oxide nanoparticles and examined via light microscopy (scale bar 50 µm)

In contrast, when using the ATP assay after exposure of the cells to iron oxide nanoparticles, a consistent decrease of viability can be observed with increasing particle concentrations. This effect applies for the two cell lines under investigation (glioblastoma cells and murine macrophages, Fig. 1), demonstrating that the cytotoxic effect is not cell line dependent. This finding does not corroborate with the data gained with the MTS assay under identical experimental conditions (cell lines, nanoparticle concentrations, incubation times; Fig. 1) for the reasons described above. The observations are in agreement with the findings of other authors when data derived from assays based on cellular ATP are closest to the data that were gathered by counting viable cells (Fig. 3) or trypan blue exclusion [2, 3]. Since the ATP assay system measures luminescence, there is no interference with the particles' intrinsic light absorbance, which is particularly influential in the case of iron oxide nanoparticles. If the nanoparticles to be tested emit light by themselves (e.g., due to functionalization with fluorescent dyes for microscopy studies), potential interferences with luminescence from the assay system should be assessed using appropriate controls.

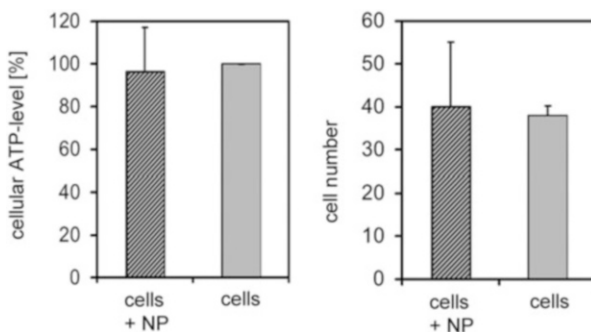


Fig. 3 Comparison of Au@Fe₃O₄ nanoparticle impact on cellular ATP levels and the number of viable cells after exposure. Human microvascular endothelial cells (HMEC-1) were incubated with (“cells+NP”) or without (“cells”) Au@Fe₃O₄ nanoparticles (50 µg/mL Fe (II)/(III)) for 24 h. Cellular ATP values were normalized to control values (no particle exposure, native cells), which were set as 100%. Number of viable cells per section is determined via microscopy (nonviable cells were removed by washing). *NP*, nanoparticles

Interestingly, it was found that certain carbon nanotube formulations are able to convert MTT into its MTT-formazan-insoluble form in the absence of any living organism. For example, polyoxyethylene sorbitan mono oleate-suspended carbon nanotubes (SWCNTs) interfere less with an MTT assay than sodium dodecyl sulfate-suspended ones [12]. Therefore, it is imperative to carefully validate cytotoxicity assays to ensure correct experimental data.

Specific interactions between nanoparticles and certain ingredients of cell viability assays are more likely to occur for some nanoparticle formulations than for others. In this context, SWCNTs appear to interact with some tetrazolium salts such as MTT but not with WST-1 or XTT. These effects are related to the insoluble nature of the MTT-formazan rather than the enzymatic reaction [13]. Particularly multi-walled carbon nanotubes may bind to the insoluble formazan product and prevent its re-solubilization [14]. Conflicting results were observed with several different assays such as neutral red, MTT, or cytokine IL-8 release when assessing carbon nanomaterial cytotoxicity in epidermal cell culture systems [15]. Up to now, there are only hypotheses about the reasons for these different interactions which merit further studies.

Another example of conflicting results is the outcome of the ATP assay reading cellular ATP levels when it is compared to the so-called EdU-incorporation assay after exposure of cells to Au@Fe₂O₃ Janus particles (Fig. 4). Specific reactions between the assay components and the nanoparticles are easily controlled by exposing the nanoparticles alone (without the presence of cells) to the reagents of the assay system. As shown in Fig. 4, no interactions of the nanoparticles with the components of the ATP assay can be detected, indicating that the decrease of cell viability in the presence of particles is reliable. In contrast, when applying the EdU-incorporation assay, distinct fluorescence signals can be detected in the presence of nanoparticles without cells. The explanation is as follows: EdU

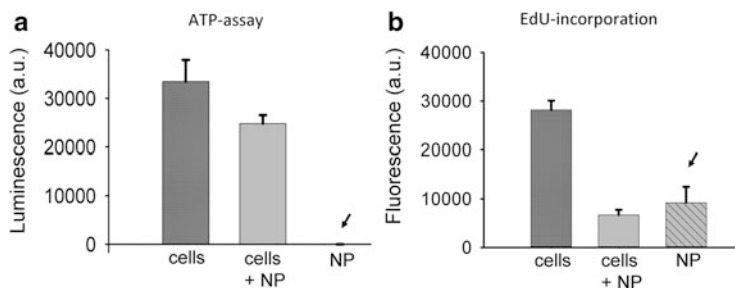


Fig. 4 Outcome of ATP and a thymidine incorporation assays on human endothelial cells after incubation with Au@Fe₃O₄ nanoparticles. Assay ingredients were incubated with HMEC-1 cells only ("cells"), or with cells and magnetic nanoparticles ("cells+NP", 50 µg/mL Fe(II)/(III)), or nanoparticles only (NP). (a) Luminescence-based ATP assay. (b) Thymidine incorporation assay using the analogue EdU. For analysis, a fluorescent substrate is attached to the incorporated uridine. Luminescence and fluorescence are given in arbitrary units. NP, nanoparticles

detection (EdU incorporated into DNA) is based on a click reaction, which is a copper-catalyzed covalent reaction between an azide and an alkyne (see also above). Copper is likely to undergo redox reactions with iron from the iron oxide based nanoparticles, particularly if the shielding of the metallic core by the particle shell is not appropriate [16]. From this observation one can conclude that cell viability assays based on redox reactions can only be performed with (metallic) nanoparticles as long as they are covered by an intact surface shielding.

A similar effect can be found when analyzing the effects of CeO₂ nanoparticles on cells. The differences of the readouts "cellular ATP" and "cellular dehydrogenase activity" as shown in Fig. 5 can be attributed, at least partially, to the adsorption of insoluble formazan products on the CeO₂ nanoparticles with increasing time of incubation. Consequently the cell viability will rather be underestimated. This is in agreement with the observations on carbon nanotubes described above.

Irrespective of the readout used in cell viability assessment, one has to keep in mind that validity of such assays is confined to the *in vitro* situation only. Direct toxicity extrapolations to the *in vivo* situation should be undertaken with great care, since (1) nanomaterials are mostly applied in nonphysiologically high concentrations in *in vitro* experiments; (2) the outcome represents mostly the response of one cell system (exception: co-culture systems), whereas a complex interaction between different cell types takes place in an *in vivo* environment; (3) the absence of an immune system *in vitro*; (4) numerous investigators use serum-free media to prevent nanoparticle agglomeration *in vitro*, a situation which is never encountered *in vivo*; and (5) in the case of complete culture medium, opsonization processes in presence of fetal calf serum do not necessarily represent the situation in humans.

Those studies that report on interferences of nanoparticles with cell viability assays clearly implicate the necessity of standardizing these assays and of including the correct controls. There is also a strong need for reference materials. Additionally, the experience and knowledge gained so far strongly indicates that more than one readout parameter should be considered when the biological response to

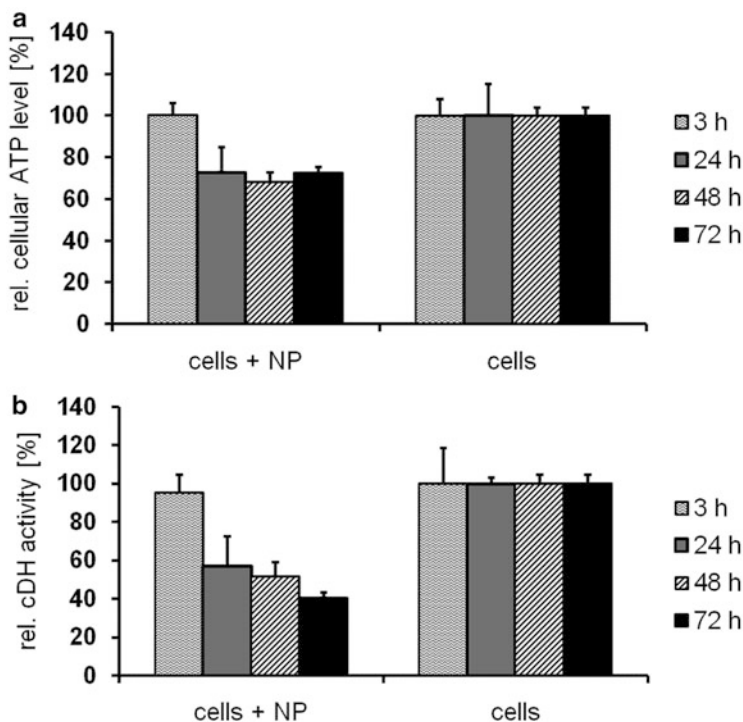


Fig. 5 Outcome ATP and MTS assay for human endothelial cells after incubation with CeO_2 nanoparticles. HMEC-1 were incubated with 100 $\mu\text{g/mL}$ (size: 40 nm) CeO_2 nanoparticles for 3–72 h. (a) ATP assay, (b) MTS assay. NP, nanoparticles

nanomaterials is studied. Particularly helpful and informative are viability assays in combination with assays probing other biological markers of cellular physiology, such as cytoskeleton integrity or the occurrence of reactive oxygen species (ROS) in order to be able to draw adequate conclusions and exclude potential methodological biases. In this context, a very detailed particle characterization including hydrodynamic diameter, polydispersity index, zeta potential, and serum protein opsonization properties is extremely valuable and necessary for accurate data analysis.

3 Effects of Nanoparticle Labeling Processes on Cells

One of the most prominent manipulations of cells using nanoparticles is cell labeling for various biomedical purposes. In biomedical research, specific labeling of cells has been applied frequently to select distinct subpopulations with defined surface expression patterns. For instance, cells are incubated with target-affine and selectively functionalized magnetic particles based on iron oxide.

Labeled cells can be separated selectively from a mixed population by applying magnetic columns which retain labeled cells in the presence of a magnetic field. Elution can easily be performed by removal of the column from the magnetic field.

Another concept is the labeling of stem cells with the aim of monitoring their delivery and migration to target organs that are affected by a specific disease via MR imaging. It is a prerequisite for this approach that those cells retain their normal viability, functionality, and capability of migration during labeling. The labeling of stem cells is mainly based on the cellular mechanisms of endocytosis [17], depending in a complex manner on size, shape, surface charge, and chemistry of the nanoparticles, as well as on the cell type. Additionally, several strategies have been proposed to enhance cellular uptake, such as the use of commercial transfection reagents and nanoparticle functionalization with cell membrane translocating peptides. These strategies have been nicely summarized by Neoh and Kang [18] and Berman et al. [19] among others.

In the literature, different labeling efficiencies have been reported so far. For example, mesenchymal and hematopoietic stem cells are labeled with carboxydextran-coated nanoparticles up to 8.3pg Fe(II)/(III)/cell [20] or just up to 2.6pg Fe(II)/(III)/cell [21], respectively. In contrast epithelial liver carcinoma cells incubated with carboxymethyl dextran nanoparticles have internalized 110pg Fe/cell [22], while lung carcinoma cells took up 202pg Fe(II)/(III)/cell after exposure to aminosilane nanoparticles [23].

These differences in labeling efficiency might be due to the individual uptake activities of cells but also, at least partially, due to methodological artifacts. One challenge is the fact that discrimination between NPs on the surface of the cell and those which have been already internalized is experimentally hard to achieve. We have observed that after incubation of cells with starch-coated iron oxide nanoparticles and subsequent separation of labeled cells from non-labeled ones with the help of a magnetic column, the amount of iron per cell distinctly drops after magnetic separation (Fig. 6). Interestingly, the effect did not depend on whether the cells were labeled by nanoparticle sedimentation compared to magnetic field (83 mT)-mediated nanoparticle encounter. The effect is more striking when cells are exposed to particularly high iron oxide nanoparticle concentrations. The underlying reason for this observation is the presence of nanoparticle aggregates on the cell surface which obviously can be removed during magnetic separation using a magnetic column but not via the regular washing steps with buffer during the labeling procedure per se. Therefore, *in vitro* labeling of cells should be performed with reasonable nanoparticle concentrations in order to avoid excess of nanoparticle association on the cell surface which affects the labeling efficiency.

We could show that the use of magnetic delivery of NPs and magnetic separation of cells do not alter the intracellular compartmentalization of magnetic nanoparticles. In this context, iron oxide nanoparticles were found to be localized in the endosomes and secondary lysosomes in accordance to other reported studies [24–26].

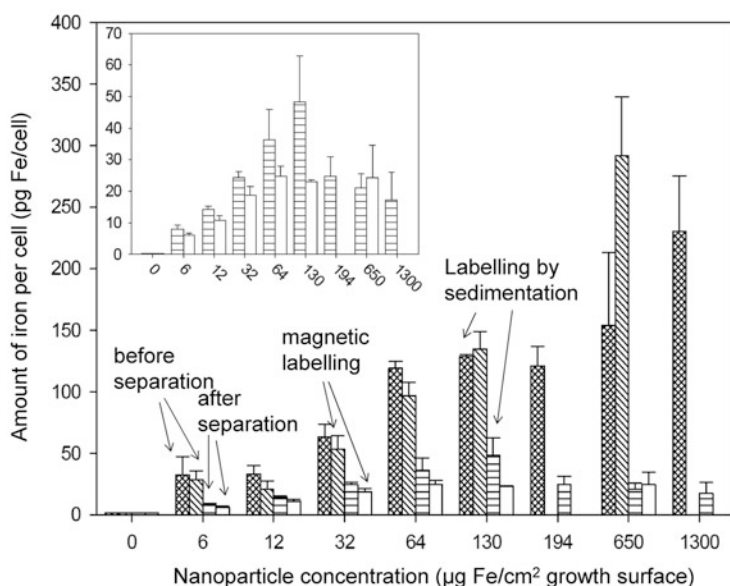


Fig. 6 The labeling efficiency of human adenocarcinoma cells decreases distinctly after separation using magnetic columns. BT-474 cells were exposed for 24 h to iron oxide nanoparticles (up to 1300 µg Fe(II)/(III)/cm² growth surface) without (labeling by sedimentation) or in the presence of a 83 mT magnet (magnetic labeling). After washing off unbound nanoparticles, cells were harvested. Labeled cells were separated from non-labeled ones using a magnetic column. Inset: magnified presentation of data obtained after magnetic separation of labeled cells

Extensive studies have already proven a good biocompatibility of iron nanoparticles. The viability of epithelial liver carcinoma cells (110pg Fe(II)/(III)/cell) or human colon carcinoma cells (7pg Fe(II)/(III)/cell) was 95% of non-treated controls [22, 27] after intracellular NP delivery. Similar results were found for mesenchymal (8.3pg Fe(II)/(III)/cell) or hematopoietic stem cells (2.6pg Fe(II)/(III)/cell) [21]. Nevertheless, labeled cell populations exposed to magnetic separation can experience a drop of their viability as shown in Fig. 7. The drop in viability is particularly visible for labeled cells but independent on the strategy of cell labeling (magnetic delivery compared to sedimentation). Such effects could arise from specific interactions of the cell membrane with the filling material of the columns or the applied shear stress [28].

Even though iron oxide nanoparticles are considered to be of no harm in terms of viability and functionality of stem cells, in several cases an inhibition of chondrogenesis [29, 30], a decrease in migration capacity, colony formation ability [31], and inhibition of osteogenic differentiation [32] were observed. Accordingly, specific manipulation procedures necessary for cell labeling require an adequate postlabeling analysis to ensure cell viability and functionality after the nanoparticles are attached to their target.

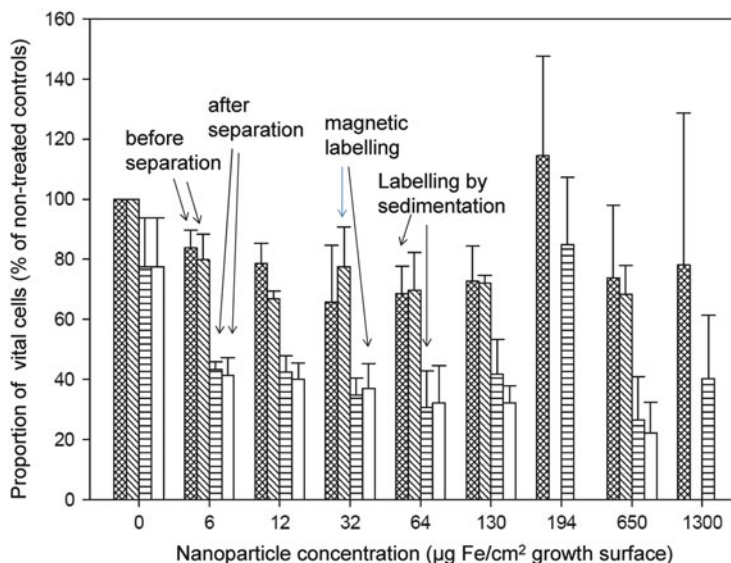


Fig. 7 Impact of cell labeling on the viability of human adenocarcinoma cells. BT-474 cells were exposed for 24 h to iron oxide nanoparticles (up to 1300 $\mu\text{g Fe(II)/(III)}/\text{cm}^2$ growth surface) without (“labeling by sedimentation”) or in the presence of an 83 mT magnet (“magnetic labeling”). After removing unbound nanoparticle cells were harvested. Labeled cells were separated from non-labeled ones using a magnetic column. After elution from the column, labeled cells were seeded again on a cell culture plastic matrix and were assessed for cell viability

4 Conclusion

In conclusion, experimental data from different research groups have shown that specific interactions between nanomaterials and ingredients of cell viability assays can lead to distinct over- or underestimations of cytotoxicity. This data suggests that there is a need to undertake multiple different assays in parallel with appropriate controls and the respective nanomaterials should be adequately characterized in terms of their morphology and chemical composition. Additionally, the limitations of extrapolations to the *in vivo* situation should be taken into account. With respect to labeling of cells for biomedical applications, reasonable nanoparticle concentrations should avoid overestimation of labeling efficiency due to the formation of large nanoparticle aggregates on the cell surface. The retention and elution of labeled cells from magnetic columns is prone to affect viability of cells. These artifacts exemplify potential pitfalls which could hinder the progress of biomedical applications of nanomaterials in the future.

Acknowledgements This work was supported by the German Research Foundation (DFG) under the contract number HI 698/7-2, HI 698/8-2, and HI 698/11-2 and the Federal Ministry of Education and Research (BMBF, Project “NanoMed”). The own data on potential cell viability assay artifacts are based on nanoparticles synthesized by Chemicell, Berlin, Germany, Dr. Rudolf

Herrmann, University of Augsburg, Germany, Oskar Köhler (M.S.) University of Mainz, Germany, and Isabel Schick (M.S.), University of Mainz, Germany. We gratefully acknowledge valuable contribution of Susann Burgold and Julia Göring.

References

1. Tenzer S et al (2011) Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. *ACS Nano* 5 (9):7155–7167
2. Gratton SEA et al (2008) The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A* 105(33):11613–11618
3. Chithrani BD, Ghazani AA, Chan WCW (2006) Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett* 6(4):662–668
4. Wang J et al (2010) The complex role of multivalency in nanoparticles targeting the transferrin receptor for cancer therapies. *J Am Chem Soc* 132(32):11306–11313
5. Decker T, Lohmann-Matthes ML (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J Immunol Methods* 115(1):61–69
6. Buttke TM, McCubrey JA, Owen TC (1993) Use of an aqueous soluble tetrazolium formazan assay to measure viability and proliferation of lymphokine-dependent cell-lines. *J Immunol Methods* 157(1–2):233–240
7. Hong SC et al (2011) Subtle cytotoxicity and genotoxicity differences in superparamagnetic iron oxide nanoparticles coated with various functional groups. *Int J Nanomedicine* 6:3219–3231
8. Hoskins C et al (2012) Dilemmas in the reliable estimation of the in-vitro cell viability in magnetic nanoparticle engineering: which tests and what protocols? *Nanoscale Res Lett* 7:1–12
9. Wang PW, Henning SM, Heber D (2010) Limitations of MTT and MTS-based assays for measurement of antiproliferative activity of green tea polyphenols. *PLoS One* 5(4)
10. Monteiro-Riviere NA, Inman AO, Zhang LW (2009) Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicol Appl Pharmacol* 234(2):222–235
11. Fisischella M et al (2009) Mesoporous silica nanoparticles enhance MTT formazan exocytosis in HeLa cells and astrocytes. *Toxicol In Vitro* 23(4):697–703
12. Belyanskaya L et al (2007) The reliability and limits of the MTT reduction assay for carbon nanotubes-cell interaction. *Carbon* 45(13):2643–2648
13. Worle-Knirsch JM, Pulskamp K, Krug HF (2006) Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett* 6(6):1261–1268
14. Cheng C et al (2009) Toxicity and imaging of multi-walled carbon nanotubes in human macrophage cells. *Biomaterials* 30(25):4152–4160
15. Monteiro-Riviere NA, Inman AO (2006) Challenges for assessing carbon nanomaterial toxicity to the skin. *Carbon* 44(6):1070–1078
16. Gao LZ et al (2007) Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat Nanotechnol* 2(9):577–583
17. Conner S, Schmid S (2003) Regulated portals of entry into the cell. *Nature* 422:37–44
18. Neoh KG, Kang ET (2012) Surface modification of magnetic nanoparticles for stem cell labeling. *Roy Soc Chem* 8:2057–2069
19. Berman SM, Walczak P, Bulte JWM (2011) Tracking stem cells using magnetic nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 3(4):343–355

20. Ittrich H et al (2007) In vivo magnetic resonance imaging of iron oxide-labeled, arterially injected mesenchymal stem cells in kidneys of rats with acute ischemic kidney injury: detection and monitoring at 3T. *J Magn Reson Imaging* 25(6):1179–1191
21. Daldrop-Link HE et al (2005) Hematopoietic progenitor cells from umbilical cord blood and from peripheral blood for subsequent in vivo tracking in a xenotransplant mouse model XXX. *Acad Radiol* 12(4):502–510
22. Sun R et al (2005) Physical and biological characterization of superparamagnetic iron oxide- and ultrasmall superparamagnetic iron oxide-labeled cells – a comparison. *Invest Radiol* 40(8):504–513
23. Ma YJ, Gu HC (2007) Study on the endocytosis and the internalization mechanism of aminosilane-coated Fe_3O_4 nanoparticles in vitro. *J Mater Sci Mater Med* 18(11):2145–2149
24. Kettering M et al (2007) Magnetic nanoparticles as bimodal tools in magnetically induced labelling and magnetic heating of tumour cells: an in vitro study. *Nanotechnology* 18:175101
25. Jordan A et al (1999) Endocytosis of dextran and silan-coated magnetite nanoparticles and the effect of intracellular hyperthermia on human mammary carcinoma cells in vitro. *J Magn Mater* 194(1–3):185–196
26. Daldrop-Link HE et al (2003) Targeting of hematopoietic progenitor cells with MR contrast agents. *Radiology* 228(3):760–767
27. Pinkernelle J et al (2005) Imaging of single human carcinoma cells in vitro using a clinical whole-body magnetic resonance scanner at 3.0T. *Magn Reson Med* 53(5):1187–1192
28. Seidl J, Knuechel R, Kunz-Schughart LA (1999) Evaluation of membrane physiology following fluorescence activated or magnetic cell separation. *Cytometry* 36(2):102–111
29. Kostura L et al (2004) Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis. *NMR Biomed* 17(7):513–517
30. Bult JWM et al (2004) Chondrogenic differentiation of mesenchymal stem cells is inhibited after magnetic labeling with ferumoxides. *Blood* 104(10):3410–3412
31. Schafer R et al (2009) Labeling of human mesenchymal stromal cells with superparamagnetic iron oxide leads to a decrease in migration capacity and colony formation ability. *Cytotherapy* 11(1):68–78
32. Chen YC et al (2010) The inhibitory effect of superparamagnetic iron oxide nanoparticle (Ferucarbotran) on osteogenic differentiation and its signaling mechanism in human mesenchymal stem cells. *Toxicol Appl Pharmacol* 245(2):272–279

Measuring Biological Impacts of Nanomaterials

Wegener, J. (Ed.)

2016, IX, 214 p. 100 illus., 56 illus. in color., Hardcover

ISBN: 978-3-319-24821-9