International Journal of Nanomedicine

Dovepress
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REVIEW

The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
31 October 2012
Number of times this article has been viewed

Eleonore Fröhlich

Center for Medical Research, Medical University of Graz, Graz, Austria

Abstract: Many types of nanoparticles (NPs) are tested for use in medical products, particularly in imaging and gene and drug delivery. For these applications, cellular uptake is usually a prerequisite and is governed in addition to size by surface characteristics such as hydrophobicity and charge. Although positive charge appears to improve the efficacy of imaging, gene transfer, and drug delivery, a higher cytotoxicity of such constructs has been reported. This review summarizes findings on the role of surface charge on cytotoxicity in general, action on specific cellular targets, modes of toxic action, cellular uptake, and intracellular localization of NPs. Effects of serum and intercell type differences are addressed. Cationic NPs cause more pronounced disruption of plasma-membrane integrity, stronger mitochondrial and lysosomal damage, and a higher number of autophagosomes than anionic NPs. In general, nonphagocytic cells ingest cationic NPs to a higher extent, but charge density and hydrophobicity are equally important; phagocytic cells preferentially take up anionic NPs. Cells do not use different uptake routes for cationic and anionic NPs, but high uptake rates are usually linked to greater biological effects. The different uptake preferences of phagocytic and nonphagocytic cells for cationic and anionic NPs may influence the efficacy and selectivity of NPs for drug delivery and imaging.

Keywords: endocytosis, plasma membrane, lysosomes, polystyrene particles, quantum dots, dendrimers

Introduction

Nanoparticles (NPs) can be applied in the medical sector as sensors, in cell and organ imaging, drug delivery, implants, and implant coatings. Surface charge is the most important factor affecting NPs in terms of their function in imaging and drug delivery. In these applications, inorganic carbon, metal, metal oxides, and sulfides as well as a variety of organic and biodegradable NPs were used (Table 1). Many NPs are tested in preclinical studies, but only polymer-based, lipid-based, protein-based NPs and nanocrystals are approved for drug delivery, while iron oxide NPs are in clinical use for magnetic resonance imaging and drug delivery. Most approved NP formulations are formulations of conventional compounds for improved drug delivery, particularly in oncology.

Reasons for the relatively low number of approved particles are, among others, problems in reproducibility and long-term stability of NP formulations and lack of guidelines for relevant biological testing. The attachment of functional groups and coatings to prevent uptake by the reticuloendothelial system increases the variety of NP preparations. As each parameter can be varied, a great number of NPs could be designed. For a faster development of efficient particles, it would be very useful to

Correspondence: Eleonore Fröhlich
Center for Medical Research, Medical University of Graz, Stiftingtalstr 24, Graz A-8010, Austria
Tel +43 316 3857 3011
Fax +43 316 3857 3009
Email eleonore.froehlich@medunigraz.at

Table 1 Overview of nanoparticle (NP) formulations in development for imaging and drug delivery with examples for approved drugs, with indication of the most important fields of application[172]

Particle used	FDA-approved drugs
(A) Imaging	
Iron oxide, gold NPs, gadolinium NPs, quantum dots	Endorem/feridex (superparamagnetic iron oxide associated with dextran, http://www.mr-tip.com/serv1.php?type=db1&dbs=Endorem)*
	Cliavist/resovist (superparamagnetic iron oxide, http://www.mr-tip.com/serv1.php?type=db1&dbs=resovist)
	Sinerem/combidex (ultrasmall superparamagnetic iron oxide, http://www.mr-tip.com/serv1.php?type=db1&dbs=sinerem)*
(B) Drug delivery	
Polymer-based	Adagen (PEGylated adenosine deaminase, immune, http://www.rxlist.com/adagen-drug.htm)
Polyacrylamide, polymethylmethacrylate, polyalkylcyanoacrylate, polylactate-*co*-glycolate, phosopholipid, pluronic, poly L-amino acid, cyclodextrins	Cimzia (PEGylated fab fragments of human TNF-α, immune, http://www.rxlist.com/cimzia-drug.htm)
	Copaxone (amino acid polymer, MS, http://www.rxlist.com/copaxone-drug.htm)
	Eligard (leuprolide acetate/poly[D,L-lactide-*co*-glycolide] polymer, onco, http://www.rxlist.com/eligard-drug.htm)
	Genexol-PM (PEGylated poly[lactic acid] micelle formulation of paclitaxel, onco, http://pharmalicensing.com/public/outlicensing/view/4809/genexol-pm)
	Macugen (PEGylated anti-VEGF aptamer, ophth, http://www.rxlist.com/macugen-drug.htm)
	Mircera (methoxy PEG-epoetin beta, onco, http://www.rxlist.com/mircera-drug.htm)
	Neulasta (PEGylated filgrastin, onco, http://www.rxlist.com/neulasta-drug.htm)
	Oncaspar (PEGylated L-asparaginase, onco, http://www.rxlist.com/oncaspar-drug.htm)
	Pegasys (PEGylated interferon α-2a, immune, http://www.rxlist.com/pegasys-drug.htm)
	PegIntron (pegylated interferon α-2b, immune, http://www.rxlist.com/pegintron-and-rebetol-drug.htm)
	Renagel (polymeric amine, dialysis, http://www.rxlist.com/renagel-drug.htm)
	Somavert (PEGylated human hormone receptor antagonist, endo, http://www.rxlist.com/somavert-drug.htm)
	Taxotere (micellar docetaxel, onco, http://www.rxlist.com/taxotere-drug.htm)
Lipid-based	Abelcet (lipid complex of amphotericin B, infection, http://www.rxlist.com/abelcet-drug.htm)
Solid-lipid NPs, nanostructured lipid carriers	AmBisome (liposomal formulation of amphotericin B, infection, http://www.rxlist.com/ambisome-drug.htm)
	Amphocil/Amphotec (micellar amphotericin B, infection, http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=6780)
	Estrasorb (micellar estradiol, endo, http://www.rxlist.com/script/main/rxlist.asp?drug=estrasorb&monotype=rx-desc&monopage=1)
	Definity (liposomal octofluoroproane, cardio, http://www.rxlist.com/definity-drug.htm)
	DaunoXome/Myocet (liposomal formulation of duanorubicin, onco, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000297/human_med_000916.jsp&mid=WC0b01ac058001d124)
	DepoCyt (liposomal formulation of cytarabine, onco, http://www.rxlist.com/depocyt-drug.htm)
	DepoDur (liposomal formulation of morphine, onco, http://www.rxlist.com/depodur-drug.htm)
	Doxil/Caelyx (PEGylated liposomal formulation of doxorubicin, onco, http://www.rxlist.com/doxil-drug.htm)
	Mepact (liposomal mifamurtide, onco, http://www.cancer.gov/dictionary?cdrid=644758)
	Octocog alfa (liposomal factor VIII, hematology http://www.medicines.org.uk/guides/Octocog%20alfa/Haemophilia)
	Visudyne (liposomal formulation of verteporfin, ophth, http://www.rxlist.com/visudyne-drug.htm)
Protein-based	Abraxane (albumin-bound paclitaxel, onco, http://www.rxlist.com/abraxane-drug.htm)
Albumin	
Nanocrystals	Avinza (nanocrystal morphine, pain, http://www.rxlist.com/avinza-drug.htm)
	Cardizem (nanocrystal diltiazem, cardio, http://www.rxlist.com/cardizem-drug.htm)
	Emend (nanocrystal aprepitant, onco, http://www.rxlist.com/emend-drug.htm)
	Focalin (nanocrystal dexmethyl phenidate, ADHD, http://www.rxlist.com/focalin-drug.htm)
	Invega sustenna (nanocrystal paliperidone palmitate, schizophrenia, http://www.rxlist.com/invega-sustenna-drug.htm)
	Megace ES (nanocrystal megestrol, immune, http://www.rxlist.com/megace-drug.htm)

	Naprelan (nanocrystal naproxen, immune, http://www.rxlist.com/naprelan-drug.htm)
	Rapamune (nanocrystal sirolimus, immune, http://www.rxlist.com/rapamune-drug.htm)
	Ritalin (nanocrystal methyl phenidate, ADHD, http://www.rxlist.com/ritalin-drug.htm)
	Theo-Dur (nanocrystal theophylline, resp, http://www.rxmed.com/b.main/b2.pharmaceutical/b2.1.monographs/CPS-%20Monographs/CPS-%20%28General%20Monographs-%20T%29/THEO-dur.html)
	Tricor (nanocrystal fenofibrate, cardio, http://www.rxlist.com/tricor-drug.htm)
	Triglide (nanocrystal fenofibrate, cardio, http://www.rxlist.com/triglide-drug.htm)
	Verelan (nanocrystal verapamil, cardio, (http://www.rxlist.com/verelan-drug.htm)
Metallic NPs Gold, iron oxide, quantum dots	Feraheme (ferumoxytol, anemia, http://www.rxlist.com/feraheme-drug.htm)
Polysaccharide-based Chitosan, alginate	None
Dendrimers Poly(amido amine), poly(ethyleneimine)	None
Biological Retrovirus, lentivirus, adenovirus, herpes simplex virus -1, adeno-associated virus	Gardasil/Cevarix (human papilloma virus-like particles, vaccination, http://www.emea.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000721/human_med_000694.sjsp) Engerix/Recombivax (human hepatitis B virus-like particles, vaccination, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Engerix_B/human_referral_000098.jsp&mid=WC0b01ac0580024e9a)
Others Fullerenes, ceramic (silica) NPs, titanium dioxide NPs,	None

Note: *Withdrawn from the market.

Abbreviations: PEG, polyethylene glycol; TNF, tumor necrosis factor; MS, multiple sclerosis; VEGF, vascular endothelial growth factor; onco, oncology; immune, modulation of immune system; endo, endocrine; cardio, cardiology; resp, respiratory problems; ophth, ophthalmology; ADHD, attention deficit hyperactivity disorder.

identify correlations of specific surface properties to cellular effects. Studies on polystyrene particles, where size and charge can be changed in a controlled way, have been widely used as models.

Positively charged constructs are used in nonviral gene transfection, and studies on gene-delivery systems with cationic liposomes and cationic polymers help to understand the role of positive surface charge. Cationic lipid/DNA complexes (lipoplexes) enter cells by endocytosis or direct penetration through the cell membrane after interaction of the cationic lipopolyamines with proteoglycans of the cell membrane. For subsequent delivery of DNA to the nucleus, degradation in the lysosomes is prevented by different mechanisms. Lipoplexes have protonable amine groups that slow down the acidification of endosomes, and thereby slow down endosome–lysosome transition.[1] Xu and Szoka[2] proposed the following mode of action: anionic lipids from the cytoplasmic facing monolayer of the endosome flip-flop in the membrane and diffuse laterally to form charge neutral ion pairs with the lipoplexes. Thereby, the DNA is released from the lipoplex and from the endosome. The mechanism of gene delivery by cationic polymers (polyplexes) is slightly different. Cationic polymers form complexes with the negatively charged DNA, and still possessing a net positive surface charge, bind to the negatively charged plasma membrane of the target cells to a higher degree than negatively charged or neutral molecules.[3] For release of the genetic material, these complexes are transported via the endosomal–lysosomal system into the endosomes where these complexes are cleaved by enzymes into polyamines and DNA. The polyamines buffer H^+ and cause lysosomal Cl accumulation with subsequent osmotic swelling and lysis of the endosomes, thereby preventing degradation of the DNA by lysosomal nucleases. This mechanism is termed the "proton sponge" effect. The released DNA passes to the nucleus and integrates into the nuclear DNA.

The use of cationic NPs is limited by their cytotoxicity. For poly(propylene imine) dendrimers, other candidates for nonviral gene transfer, the relation of primary amine groups and toxicity has been clearly shown.[4] Shielding of the amine groups by functionalization decreased the toxicity of these constructs.[5] This review aims to clarify if cationic NPs interact with other cellular targets, act by other cytotoxic mechanisms and use other uptake routes than anionic and neutral NPs.

Cytotoxicity

The cytotoxicity of NPs depends on particle parameters like morphology, such as aspect ratio/shape and size. Hydrophobicity, surface area in terms of roughness and porosity, and surface charge influence the capacity to produce reactive oxygen species (ROS), determine binding sites for receptors, and influence dispersion and aggregation of the particles. Cytotoxicity is also often due to contamination, solubility and release of toxic components, and adsorption of compounds. On the other hand, biological parameters such as cell type used for the study or the culture and exposure conditions (eg, cell density, particle concentration, medium composition, temperature), also influence cytotoxicity.

Main influencing factors for cytotoxicity are material, size, shape, composition, surface charge, and surface hydrophobicity. The correlation of cytotoxic effect and size has been studied in many papers. For nonphagocytic cells, small size correlates with increased cytotoxicity. In vitro experiments showed higher cytotoxicity of well-dispersed mesoporous silica and amorphous silica, dolomite, ZnO, Ni, Ag, and polystyrene NPs compared to the respective microparticles.[6–15] When particles smaller than 100 nm are compared, still-smaller particles act more toxically than larger ones (quantum dots,[16] TiO_2[17]). In contrast to these studies, no differences have been reported for 10–100 nm silica particles compared to 45 μm ones,[18] and for nickel ferrite NPs.[19] Okuda-Shimazaki et al demonstrated the importance of the aggregation state and showed that larger aggregates of TiO_2 NPs acted more cytotoxically than smaller ones.[20]

Phagocytes such as macrophages and monocytes react more strongly to microparticles than to NPs. One study reported higher cell damage for silica microparticles than for NPs,[21] and another study noticed absence of cell damage in THP-1 cells for 30–70 nm silica NPs, while 1000 nm particles acted cytotoxically.[22]

Compared to nonphagocytic cells, THP-1 cells are also much more resistant to 20–200 nm silver and to 21 nm TiO_2 NPs.[23] Size-dependent toxicity studies in vivo are less conclusive: systemic toxicity upon intraperitoneal application of 10 nm and 50 nm iron oxide particles was higher than that after dosing with 1000 nm particles.[24] When applied intraocularly however, 4000 nm magnetic iron oxide particles caused more toxicity than 50 nm particles.[25]

It is also generally accepted that fiber-shaped NPs of a given material are more reactive and toxic compared with spherical particles: carbon nanotubes, for instance, are generally more toxic than fullerenes.[26,27] Hydrophobicity is often linked to surface charge, but at the same surface charge, NPs with hydrophobic surfaces, eg, oleic acid-coated nickel ferrite and stearic acid-coated TiO_2 particles reacted more cytotoxically than the respective noncoated particles.[19,28]

In the following sections, the influence of charge on cytotoxicity and cellular uptake will be described in more detail.

Charge-dependent cytotoxicity

For cytotoxic action, both charge density and charge polarity play a role. Charged NPs, eg, gold particles, are more cytotoxic than neutral forms,[29] and positively charged ZnO, silica, silica-titania hollow, and gold nanoparticles act more cytotoxically than negative variants of similar size in nonphagocytic cells.[30–34] Cytotoxic action of poly(amidoamine) (PAMAM) dendrimers is correlated with the number of primary amino groups,[35] and cytotoxicity of PAMAM dendrimers decreased when amine groups were neutralized with acetyl groups.[36] Also, in in vivo experiments, high numbers of primary amine groups increased the toxicity of dendrimers.[37] This rule, however, does not apply to all NPs. For some NPs, eg, poly(lactic-*co*-glycolic acid) (PLGA) particles, charge appears to play no role,[38] or other parameters, eg, porosity for mesoporous SiO_2 particles, are more important than surface charge.[39] The lack of negative effects of positively charged PLGA particles could be due to the use of chitosan, a polysaccharide with excellent biological properties, as coating material.[38] Shielding of cationic groups by functionalization and polethylene glycol (PEG)ylation decreased both cytotoxicity and efficacy in NPs where efficacy and cytotoxicity were linked to cationic charge.[40]

In contrast to nonphagocytic cells, phagocytic cells preferentially interact with negatively charged particles, presumably due to the ingestion of bacteria, which also displays a net negative charge.[41] The stronger interaction of phagocytes with negatively charged particles may be the reason for the higher cytotoxicity of anionic cyanoacrylic NPs compared to cationic ones.[42] In line with the low importance of cationic charge for macrophage uptake and cytotoxicity, shielding of the positive surface charge by PEGylation displayed only a small effect on cellular uptake and cytotoxicity in these cells, whereas marked decrease in membrane damage, lipid peroxidation, and oxidative stress were seen in nonphagocytic neuroblastoma cells.[43] It would, however, be oversimplistic to explain these effects only by neutralization of the surface charge, because both functionalization and coating also markedly increase particle size, another key parameter for NP cytotoxicity. Conclusions on surface-charge effects, therefore, are only valid when comparing functionalized or nonfunctionalized particles of similar sizes. When comparing functionalized PLGA NPs with different coatings for tumor targeting, the cationic NPs were slightly more effective than anionic ones, and both accumulated to a higher extent in tumor tissue than bare Pluronic-coated ones.[44]

In general, NPs may interact with a variety of cellular targets to cause adverse effects (Figure 1).

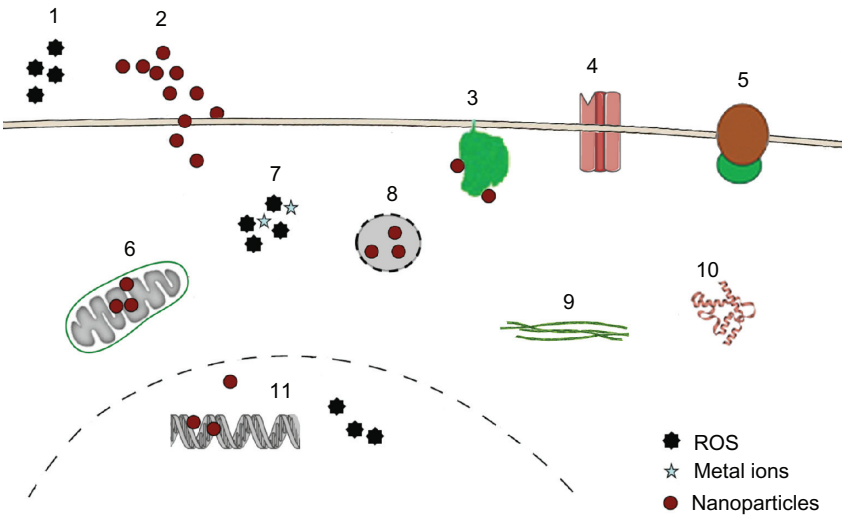

Figure 1 Targets for cytotoxicity of nanoparticles (NPs).
Notes: NPs may act through extracellular generation of reactive oxygen species (ROS) (1), they may physically damage the plasma membrane by causing holes (2) or bind to membrane proteins like nicotinamide adenine dinucleotide phosphate-oxidase (3), Ca^{2+} channels (4), and membrane receptors (5), thereby inducing oxidative signaling, increasing intracellular Ca^{2+} levels and activating second-messenger cascades. Inside the cells, NPs may interfere with mitochondrial metabolism (6), causing generation of radicals and induction of apoptosis. Intracellular ROS generation by NPs or by metals from lysosomal degradation (7) as well as lysosomal disruption (8) and direct binding to components of the cytoskeleton (9) and the induction of structural alterations of proteins (10) are additional modes of toxic actions. In the nucleus, interference with the transcription machinery and oxidative damage of the DNA (11) may occur.

Plasma membrane

NPs may cause focal dissolution of the plasma membrane and hole formation and perturbation of the internal membrane structure. Focal dissolution by carbon particles and loss of membrane folds induced by brookite NPs in exposed cells were observed by electron microscopy.[45,46] Plasma-membrane folds in Madin-Darby canine kidney cells (MDCK) disappeared upon exposure to brookite NPs.[46] The authors speculated that generation of ROS induced lipid peroxide formation in the membranes, thereby decreasing their flexibility. Wang et al,[47] by contrast, showed passive penetration of quantum dots by increasing membrane fluidity. These effects, however, do not inevitably lead to cell death. To repair plasma-membrane damage, either by pore formation through endogenous factors (complement, perforin) or by exogenous factors (bacterial toxins), cells possess several repair mechanisms. Disruption of membrane integrity leads to influx of Ca^{2+} and can be repaired by exocytosis of internal membranes, endocytosis of the permeabilized site, and shedding of the injured membrane through microparticle formation.[48] Repair of the membrane occurs within seconds, and the remodeling of the cortical actin takes a few minutes.[49]

Electrophysiological measurements and studies with unilamellar lipid vesicles indicated the transient disruption of plasma-membrane integrity upon the passive entry of silica NPs into cells.[50,51] Pores < 1 μm in diameter can be closed by sealing the plasma membrane around the hole, and it is likely that plasma-membrane damage by NPs that led to decreased viability exceeded the repair capacity of the cells.

To get insight into the molecular mechanism of plasma-membrane damage, several groups used supported lipid bilayers.[52,53] 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipid bilayers supported on solid substrates do not exactly represent the composition of the plasma membrane in vivo, because they usually consist of only one type of lipid and lack the asymmetric distribution of the lipids and the presence of proteins in real plasma membranes, but they can mimic mechanical processes and metabolism of the plasma membrane quite well.

Using bilayer models and computer models of DMPC, the influence of size and surface charge on the interaction with lipids and hole formation was studied and several mechanisms identified.[54] Large cationic G7 PAMAM dendrimers were able to cause holes in intact bilayers, whereas the smaller cationic G5 dendrimers increased the size of preexisting holes but did not generate new holes. Neutral dendrimers adsorbed to the edges of preexisting holes,[55] and removed lipids from the edge of the hole, and formed dendrimer–lipid aggregates.[56] According to Lin et al, cationic gold particles (2.2 nm) can disrupt 20×20 nm lipid bilayers but not 28×28 nm lipid bilayers.[53] The capacity for hole formation was influenced by the density of the particle's cationic charge, the negativity of the lipid bilayer, surface tension, temperature, and salt concentration. Simulation using coarse-grained representations suggests that the degree of gold particle–cell interaction can be tuned by variation of the surface charge. Strongly cationic particles create defective areas across the entire surface of the outer leaflet of the bilayer, and a hydrophilic pore with highly disordered lipids at the edge is formed.[52] In these models, cationic NPs could penetrate better through plasma membranes than anionic particles.

NPs can also cause effects at the plasma membrane by interaction with membrane-bound proteins. Binding to nicotinamide adenine dinucleotide phosphate-oxidase leads to generation of ROS,[57] activation of voltage-gated Ca^{2+} channels to intracellular Ca^{2+} changes,[58] and the activation of membrane receptors to activation of the second-messenger pathways.[59,60] Binding to membrane receptors is intended for therapeutic interventions, eg, the binding of human epidermal growth-factor receptor 2 (HER2)-coated NPs in diagnosis and treatment of HER2 high-expressing breast carcinoma cells.[61] Also, uncoated, nontargeted NPs bind to epithelial growth factor receptor and β1 integrin receptors and activate the respective signaling pathways.[62]

Intracellular targets of NPs are mitochondria, lysosomes, nucleus, and intracellular proteins.

Mitochondria

Swelling of mitochondria occurred after cellular exposure to quantum dots[63] and decrease of the mitochondrial membrane potential has been reported for silver, TiO_2 and alumina NPs.[64–66] The increase in mitochondrial membrane permeability was induced either by disruption of the respiratory chain or by changes in Bax and Bcl-2 expression, which lead to disruption of mitochondrial metabolism, increased ROS production, adenosine diphosphate-induced depolarization, release of cytochrome C, and induction of apoptosis.[67,68] Whereas no obvious morphological damage of lysosomes and mitochondria was reported for carboxyl polystyrene particles of different sizes,[10] amine-functionalized polystyrene particles damaged mitochondria and lysosomes in astrocytoma cells.[69]

Lysosomes

Lysosomes are likely targets for ROS-producing NPs because they are very sensitive to oxidative stress.[70] Healthy lysosomes

may increase the cytotoxicity of NPs by the release of leachable metal ions (eg, from iron oxide NPs[71]), which then generate cellular oxidative stress. Lysosomes as targets for cytotoxicity have been revealed for quantum dots and silicon NPs. Costaining with lysosome markers revealed swollen lysosomes upon exposure to quantum dots.[72] Other groups reported morphological alterations upon exposure to cationic polystyrene particles[69] and cytotoxicity of silicon NPs caused by permeabilization of lysosomes.[73] Especially for cationic NPs and polymers, swelling and disruption of lysosomes due to buffering of H^+ is a major mode of cytotoxic action.[74] When lysosomal membranes are damaged, a high amount of hydrolytic enzymes is released, leading to degradation of intracellular macromolecules. Independent from the release of hydrolytic enzymes, a correlation of cytotoxicity and lysosomal localization has been described for CeO_2 NPs.[75] Anionic CeO_2 NPs were taken up into lysosomes and caused cell death, whereas cationic NPs were localized in the cytoplasm of viable tumor cells. The extent of cellular uptake was not correlated with this cytotoxicity, and it was not clear from this study how lysosomal localization was linked to cytotoxicity.

Autophagy, the intracellular disposal mechanism to remove and degrade undesirable substances, can be activated by cellular stress. The cellular amount of autophagosomes upon exposure to gold NPs, iron oxide NPs, fullerenes, carbon nanotubes, and quantum dots was increased due to oxidative stress, disruption of cytoskeleton, and mitochondrial damage.[76–79] In the absence of metals, either as an integrative part of the particles or as contamination, accumulation of autophagosomes has only been reported in cells exposed to NPs with positive surface charge, cationic polymeric NPs, polyplexes, and cationic dendrimers, and not for anionic NPs.[80–82]

Nucleus

NPs may inhibit cell division and arrest cytokinesis, an action often seen in combination with other effects on DNA. Many NPs (<50 nm) can get into the nucleus,[67,83] but localization in the nucleus is not a prerequisite for action on the DNA because intracellular NPs can gain access to the genetic material during mitosis when the nuclear membrane breaks down. In earlier descriptions of the nuclear pores, passage of particles as large as 25 nm has been reported.[84] Later studies report the nuclear pore as an hourglass-like channel with a diameter of 45–70 nm.[85] In both studies, the dynamic size of the pore was mentioned, which also allows the entry of larger (90 nm) nuclear-targeted NPs into the nucleus.[86] The access to the nucleus, in addition to size, depends on surface charge: noncharged silica NPs can enter the nucleus, whereas the same particles are retained in the cytoplasma when they are functionalized with amine or carboxyl groups.[87]

Studies on isolated DNA revealed thermal stabilization by cationic but not by anionic poly(L-lysine) NPs.[88] This interaction may present a mechanical obstacle to polymerase motion along the DNA chain, leading to inhibition of transcription.[89] Also, aberrant clusters of topoisomerase I induced by SiO_2 NPs can cause alterations in DNA transcription.[90] Genotoxic effects by NPs occur either directly or by oxidative damage of DNA. The consequences of ROS in the nucleus are point mutations in the DNA and double-strand breaks, which have been accused of causing alterations of DNA structure, mitosis, and transcription. High surface activity in the form of ROS generation or through Ti–O or Ti–N bonds could cause DNA alterations induced by Ag and TiO_2 NPs.[67,91] Neither cationic nor anionic polystyrene particles interacted with chromosome reorganization.[92] Extranuclear inhibition of translation can occur through interference of NPs with mRNA-stabilizing proteins.

Intracellular proteins

NPs have a high affinity to macromolecules, particularly to proteins. This binding may increase protein stability, decrease it and interfere with protein function, or have no effect on the protein.[93] Intracellular TiO_2 NPs induced conformational changes in tubulin and inhibited tubulin polymerization,[94] and thereby could impair cell division, cellular transport, and cell migration. NPs such as CeO_2, quantum dots, copolymer particles, and carbon nanotubes may also lead to protein aggregation and fibrillation.[95] The formation of protein aggregates may promote the development of several neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and prion diseases. Fullerenes, polymeric NPs, and quantum dots have also been reported to prevent the formation of protein aggregates in diseases like Alzheimer's, and potentially could be useful for the prevention of these diseases.[96–98]

Dendrimers, carbon nanotubes, alumina NPs, and chitosan NPs modulate the architecture of intercellular tight junctions by disruption and thereby decrease the transepithelial electrical resistance of cell monolayers.[64,99–101] Lipid NPs do not affect tight junction proteins, and silver NPs increase the barrier function of endothelial monolayers.[102,103] The role of surface charge on these effects is largely unknown.

The different sensitivity of plasma membrane and intracellular organelles to NPs' surface charge may lead to charge-dependent modes of cytotoxicity. On this topic, however, few studies are available.

Charge-dependent differences in the mode of cytotoxic action

It appears that positively charged NPs either directly or by detachment of adsorbed polymers (eg, polyethylenimine) cause membrane damage, whereas anionic particles cause intracellular damage. Although the mechanism of damage by anionic particles is not clear, a correlation of lysosomal localization and cytotoxicity has been identified for nanoceria particles.[75] In one study, where variations of size (30 nm, 150 nm, 500 nm) and surface charge (cationic, anionic, neutral) were evaluated, the relation of surface charge to cytotoxicity was more complex. In the 30 nm and 500 nm zeolite particles, surface charge had only a small effect on cytotoxicity, but marked differences between positively and negatively charged 150 nm zeolite particles were seen in epithelial (human embryonic kidney cells).[104] This may be due to the fact that 150 nm particles possessed the highest charge densities. Amine-functionalized NPs acted more by disruption of membrane integrity, whereas carboxyl-functionalized ones induced apoptosis to a greater extent. In macrophages (RAW cells); however, the 150 nm carboxyl-functionalized particles showed more membrane disruption and more apoptosis than the ones with amine and thiol surface functionalization, corroborating the specific role of anionic charge for macrophages.

Serum effects

Coating with bovine serum albumin (BSA) or the presence of serum in the incubation medium reduced cytotoxicity for many NPs. Polystyrene particles, PLGA particles, polysaccharide NPs, and iron oxide NPs acted less cytotoxically on nonphagocytic cells in the presence of serum.[3,9,105,106] Particularly, serum reduces the effects on membrane integrity. Potential causes for the mitigating effect of protein include instability of the suspension in the presence of proteins and masking of the reactive surface of the NPs, avoiding the interaction of the NPs with the plasma membrane and the generation of ROS. The decreased cytotoxicity in the presence of serum was usually correlated with a lower cellular uptake.[105] In phagocytic cells, where increased cytotoxicity in the presence of serum was reported,[107] serum coating is known to increase the cellular uptake of particles.[108]

Cellular uptake

Similar to cytotoxicity, cellular uptake is influenced by size, shape, material, surface charge, and surface hydrophobicity. Nonphagocytic cells take up spherical NPs between 20 and 50 nm at the highest rates.[61,109–112] Enterocytes are an exception to this rule, because they preferentially ingest particles in the range between 100 and 200 nm.[113] Phagocytic cells, by contrast, preferentially ingest particles between 2 and 3 μm,[114] and phagocytose NPs to a lower extent. Phagocytes contain a higher amount of small supermagnetic iron oxide particles than of ultrasmall supermagnetic iron oxide particles,[115] and they phagocytose particles < 300 nm less well than 5 μm particles.[116] Well-dispersed 20–200 nm silver particles are taken up by phagocytic (THP-1) cells to a lower degree than by nonphagocytic (A549 and HepG2) cells.[23] Aggregates of silver NPs, however, are taken up by phagocytes to a higher extent.[117]

For iron oxide particles, size appears to be a stronger determinant for uptake than surface charge.[118]

Charge-dependent cellular uptake and intracellular localization

Studies on the effect of charge density and of the kind of charge (positive, negative) in nonphagocytic cells showed that charged polystyrene and iron oxide particles are taken up better than their uncharged counterparts.[119–121] When charged groups on the surface were present, positively charged particles were generally better taken up than negatively charged ones. Cells ingest positively charged gold and silver particles, superparamagnetic iron oxide particles, hydroxylapatite, silicon dioxide, lipid particles, poly(lactic acid), chitosan, polymeric particles, and polystyrene particles to a higher extent than the respective anionic ones.[122–131]

Lunov et al studied the preferential uptake of anionic particles by phagocytic cells in more detail.[132] They compared the uptake of polystyrene particles in differentiated macrophages to that of monocytes and observed a preferential uptake of the carboxylated particles by macrophages and a higher uptake of amino-functionalized particles in monocytes. Macrophages have a higher phagocytic activity towards many bacteria than monocytes,[133] and if the preference for anionic particles is linked to phagocytic activity, are expected to display a greater uptake than the less phagocytic monocytes.

The role of surface charge of polystyrene particles and quantum dots on cellular uptake is controversial. Carboxylated 1 μm and 50 nm polystyrene particles were ingested to a higher degree by alveolar type I cells,[134] whereas Fazlollahi et al[135] showed preferential uptake of cationic polystyrene particles in MDCK cells. For quantum dots, some groups reported preferential uptake of anionic quantum dots,[136,137] and others that of positively charged quantum dots.[138] Ryman-Rasmussen et al[139] did not find any differences between the uptakes of positively and negatively charged quantum dots.

Different degrees of hydrophobicity of the functionalized particles may be one reason for the disparate results. Bu et al also assessed the surface hydrophobicity of the quantum dots they used and speculated that the increased uptake of anionic particles may be caused by a higher hydrophobicity of these particles compared to the corresponding neutral and positive ones.[140] When studying the uptake of polystyrene particles in alveolar macrophages, Makino et al suggested that the preference of cells to ingest charged particles in their study could also be due to the greater softness of amine and carboxyl-functionalized particles compared to plain ones.[141]

Serum effects

Both positively and negatively charged NPs bind serum and albumin, but coverage differs between the particles. The change-dependent coverage of carboxylated polystyrene particles with serum was higher than that of positively charged ones,[142] whereas positively charged CeO particles bound BSA better than negatively charged ones.[143] Similarly, reports on the effect of BSA and serum on cellular uptake showed controversial findings: cells ingested BSA precoated NPs to a lower degree than uncoated ones, as reported by Baier et al,[105] but absorbed serum-coated cationic CeO and mesoporous silicon particles to a higher extent, according to data from other groups.[142,144]

Mechanisms of cellular entry

Under physiological conditions, NPs may enter the cells via passive and active transport. Passive transport of NPs into cells is relatively rare (eg, gold particles[52,145]), and most NPs enter cells by endocytosis. The mechanisms for passive uptake have only partly been identified. Arviso et al suggested perturbation of the membrane potential by positively charged gold particles with flipping of membrane areas as the mode of uptake.[146] An orderly arrangement of hydrophilic and hydrophobic ligands at the particle surface facilitates passive entry for gold NPs and lipid particles. When the ligands were arranged as stripes, the particles were able to translocate easily across the membrane, while in the random arrangement endocytosis occurred.[147–149]

Endocytosis serves to absorb molecules from the extracellular space by invagination of the plasma membrane and formation of intracellular vesicles. The first type of endocytosis discovered was clathrin-mediated endocytosis, but in the meantime several additional types of endocytosis have been identified. For the study of NPs, in general, a simplified classification into the four routes of clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis, and clathrin-independent and caveolae-independent pathways is used[150–153] (Figure 2, adapted from Perez-Martinez et al[154]).

The endocytic routes described so far are receptor-mediated and transport with high efficiency. Bulk flow of substances from the extracellular space, by contrast, occurs through fluid-phase endocytosis, formerly termed pinocytosis.[155] This transport is nonsaturable and has a low capacity. Most endocytic pathways include lysosomes, where a variety of macromolecules can be degraded. If substances, however, enter by caveolae-mediated endocytosis, they may also be delivered to the endoplasmatic reticulum and to the Golgi apparatus, thereby avoiding degradation in lysosomes.[156]

In addition to transcellular transport, NPs can use the paracellular route to pass epithelial monolayers. Opening of the tight junctions by NPs may present an option for drug delivery across the blood–brain barrier but surface charge appears not to play the most important role. Although cationic albumin particles were able to cross this barrier, tight junctions remained intact.[157] Studies using dendrimers of different sizes with cationic and anionic charge also suggest that

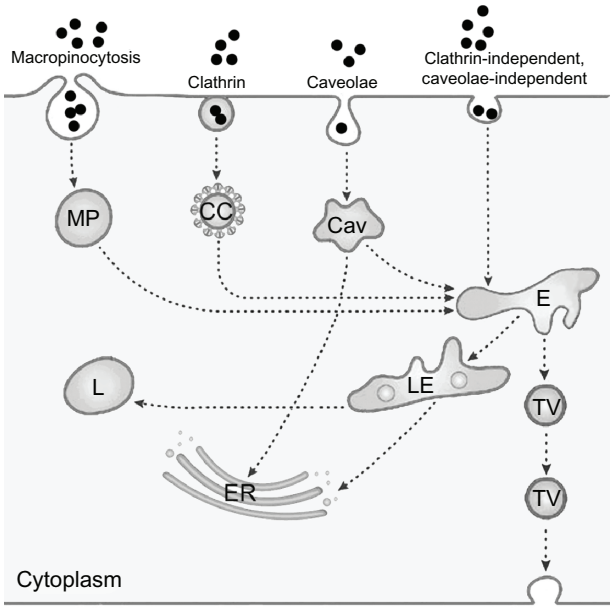

Figure 2 Simplified representation of active uptake mechanisms in nonphagocytic cells.
Notes: Nanoparticle (•) uptake has been evaluated mainly according to macropinocytosis, represented here as only one route, through macropinosome (MP), clathrin-mediated uptake by clathrin-coated pits (CC), and caveolae-dependent uptake by caveosomes (Cav). Uptake by clathrin-independent caveolae-independent endocytosis, which includes flotillin-, Arf6-, Cdc42-, and RhoA-dependent uptake, is also presented as only one route. Fluid-phase endocytosis, which mainly uses the clathrin-coated pits, is not depicted as a separate route. All pathways deliver their content to endosomes (E), late endosomes (LE) and lysosomes (L); the content of caveolosomes may also be delivered to the endoplasmic reticulum (ER) and the Golgi apparatus. Vesicular transport through the cell occurs through transcytotic vesicles (TV). © 2012, Elsevier. Reproduced with permission from Fröhlich E, Roblegg E. Models for oral uptake of nanoparticles in consumer products. *Toxicology*. 2012;291(1–3):8.[179]

(large) size is more relevant for opening of tight junctions than surface charge.[158]

Table 2 presents an overview of studies on surface-dependent particle uptake and shows that no general rules have been identified so far. When positively and negatively charged chitosan and poly(lactic acid) particles were compared in the same study, both types of particles used the same (clathrin-mediated) uptake mechanism.[159,160] Quantum dots can be ingested by clathrin and clathrin-independent caveolae-independent endocytosis.[173,174] Controverse findings were also reported for cationic polystyrene NPs. Clathrin-mediated[175], macropinocytototic[177] and caveolae-dependent[178] routes were described. Plain polystyrene NPs used clathrin-independent endocytosis, whereas positively charged NPs are taken up via clathrin-coated vesicles.[175] The authors also showed that upon inhibition of the clathrin-mediated uptake, plain NPs were ingested by macropinocytosis as an alternative route.[119] The influence of size is obvious in the uptake of 24 nm and 43 nm anionic polystyrene particles: the smaller particles were taken up by the clathrin-independent caveolae-independent route, whereas the larger ones were ingested by clathrin-mediated endocytosis.[176] Cell-specific differences also play a role: cationic polystyrene particles were taken up by LAMP-1-positive endosomes in the macrophage cell line RAW 264.7 and by caveolae in BEAS-2 cells.[162] Similar differences were also reported for dendrimers, which were taken up by clathrin-mediated endocytosis in Caco-2 cells[163] and by macropinocytosis in A549 cells.[164] Foster et al reported very different rates of particle uptake in the respiratory cell lines A459 and Calu-3.[165] For the interpretation of these data, problems related to working with uptake inhibitors have to be taken into account. This includes inhibition of more than one route due to low specificity of the inhibitors, uptake by compensatory mechanisms when one route is blocked, alterations of plasma-membrane proteins, disruption of the cortical actin cytoskeleton, and inhibition of vesicle trafficking, etc.[166]

Table 2 Routes of endocytic uptake in nonphagocytic cells

Particle	Charge	Uptake route	References
Quantum dots	Anionic	Clathrin	173
	Anionic	Clathrin-independent, caveolae-independent	174
Polystyrene	Anionic	Clathrin	119
	Plain	Macropinocytosis	119
	Cationic	Clathrin	175
	Plain	Clathrin-independent	175
	Anionic	Clathrin (43 nm)	176
		Clathrin-independent, caveolae-independent (24 nm)	176
	Cationic	Macropinocytosis	177
		Caveolin	178
Chitosan	Anionic	Clathrin	159
	Cationic	Clathrin	159
Poly(lactic acid)	Cationic	Clathrin	129,160
	Anionic	Clathrin, clathrin-independent	160

The cell-specific expression of endocytic routes may explain the observed differences in the endocytic routes used, in the amount of particle uptake, and in the velocity of this uptake. It is, for instance, known that smooth-muscle cells, fibroblasts, adipocytes, and endothelial cells have an incredible amount of caveolae,[167] and therefore preferentially use this route. This leads not only to a different intracellular localization but also to different velocity of uptake. The clathrin-mediated pathway is faster than the clathrin-independent caveolin-independent uptake,[160] and therefore particles using this route accumulate faster in cells. Asati et al propose to exploit differences in the uptake routes between normal and tumor cells to develop cytostatic NP-based drugs.[75]

Not only cell entry by different uptake routes but also the escape of cationic particles from the endosomal–lysosomal system could explain the charge-dependent differences in the intracellular localization of anionic PLGA and mesoporous and chitosan particles.[129,130,168]

The predictive value of the aforementioned surface charge-dependent cellular studies is currently not clear. For chemicals, a large multicentre evaluation study identified a rather good correlation ($R^2 = 0.77$) between IC_{50} values in cytotoxicity screening assays and human acute poisoning with various chemicals.[169] For NPs, few comparative data are available, which suggests a low predictive value for inhalation exposure[170] and a good correlation for parenteral exposure.[171]

Conclusions

Cationic surface charge for most NPs correlates with higher cellular uptake and greater cytotoxicity in nonphagocytic cells. Cationic NPs appear to cause plasma-membrane disruption to a greater extent and anionic NPs apoptosis. Anionic NPs are better ingested and act more cytotoxically in phagocytic cells. The presence of serum appears to reduce NP uptake in nonphagocytic cells, but increases it in phagocytic cells. The differences between phagocytic and nonphagocytic cells have to be taken into account in the design of medical NPs.

Acknowledgments

Financial support by the Austrian Research Science Grant P22576-B18 is gratefully acknowledged. The author thanks Gabriella Salas for English-language editing.

Disclosure

The author reports no conflicts of interest in this work.

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