CHAPTER 9

Cell-nanoparticle interactions

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A considerable increase in research using nanotechnology for biomedical applications including diagnostics, drug delivery systems, imaging agents, implant coatings, and other therapeutic techniques has occurred since the beginning of this century [1–4]. The ability of nanoparticles (NPs) to mediate biological effects due to their distinctive physicochemical properties makes them attractive in medicine and nanomedicine (e.g., increase in implant performance by nano-textured surfaces, improvement in NP delivery of therapeutics into tumors by the enhanced permeability and retention (EPR) effect, enhancement of MRI contrast by superparamagnetic NPs) [5–7]. In addition, commercialization of technology is another unique benefit of NPs (e.g., rediscovery of drugs that may have been clinically unsuccessful in the past, give greater intellectual property protection, allow novel drug indications) [1, 8–10].

Examining how nanomaterials interact with biological systems (i.e., nano-bio interaction) has been a new direction in nanotechnology. Designing a series of NPs in which only a single parameter is altered experimentally has been done by investigators. Exposure of these NPs to animals, plants, cells, or tissues and measurement of a biological effect (e.g., toxicity) are then performed [11, 12]. This systematic assessment of various NPs enables ascertaining whether a particular biological effect is due to one or multiple NP parameters. Determining and creating design regulations that guide nanodevice engineering have been a major outcome of these studies [13–16].

Researchers have analyzed the fundamental principles regarding the interaction of living organisms with engineered NPs as a result of advances in nanotechnology and nanomedicine. Aside from interacting with biological structures including tissues and organs, NPs, like viruses and large agglomerates of macromolecules, can cross cellular barriers and be taken up by cells via different endocytosis mechanisms. There have been

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concerns about their possible adverse effects, even though NPs can traverse biological barriers for various therapeutic uses [17–20].

Translating NPs into clinical applications has seen limited success in spite of increased funding and research into their biomedical uses [21, 22]. A large discrepancy between the quantity of research output and NP formulations that actually progress to clinical trials continues to be seen. Many reasons lead to this gap, including obstacles in comprehending and manipulating the most basic interactions of NPs with the biological environment. For evaluating the impact of physico-chemical properties on biological behavior, regulatory agencies need greater physico-chemical characterization and deeper understanding as an increasing number of nanomedicines progress to clinical trials [23–25]. A complete understanding of how interactions and responses of cells are affected by NP properties, especially colloidal behavior in biological solutions (e.g., body fluids or cell culture medium), is, therefore, essential [26, 27].

The dearth of appropriate user-friendly techniques may account for the paucity of exhaustive in vitro and in vivo studies into the degree and nature of interactions between cells and NPs. Adequate resolution, quantitation, evaluation of NP localization in cells at the individual cell level, high sample throughput, and minimal sample preparation are characteristics of an ideal technique. Furthermore, the technique should preferably not need fluorescent or radioactive NP labeling and not be dependent on material properties. Promising analytical methods for examining and quantifying interactions between cells and NPs are described in this chapter.

The degree of NP-biological interactions (e.g., with organisms, tissues, cells) and processes behind these interactions should be scrutinized to evaluate nano-formulation toxicity or efficacy [28, 29]. The extent and essence of nano-bio interactions are dependent on intrinsic NP characteristics (e.g., crystallinity, shape, size), the biological system's properties that NPs are introduced to, and NP alterations during the experiment (e.g., protein corona formation), so achieving this objective is not trivial [30, 31]. Quantitative data on interactions between NPs and cells are seldom reported with nanotoxicological or nanomedical results, even though many investigators admit that these interactions are important [32–36]. The volume, mass, or surface area-based exposure concentrations are mentioned in the majority of studies which include the in vitro response to NPs. Because evaluating the concentration of NPs needed for medical efficacy is the aim of in vivo studies, they have placed stronger emphasis on assessing NP biodistribution and uptake.

A desire to comprehend the processes that occur in NP-biological system interaction has followed the increasing application of nanomaterials for biomedical and industrial purposes. The small size of NPs allows them to have numerous biological responses which are different from larger particles made of similar materials, including uptake [37–39]. Toxicity may result from these effects; on the other hand, these effects may also be utilized in nanomedicine based on the NP properties (e.g., composition, shape, size, surface charge). Characterizing NP-cellular interactions and correlating these

interactions with ensuing cellular responses have been focal points in the field. Developing nanomaterial theranostics that are synthesized rationally and improved understanding of NP toxicology can be achieved through characterizing interactions between NPs and cells [40–42].

NP-cell interactions are preferably evaluated quantitatively and may be examined at the level of the single cell for cell-associated NPs ("cell-associated" includes NPs that are intracellular and bound to the cell surface). While several methods based on microscopy (e.g., electron microscopy and EM) have adequate resolution for examination at the level of the cell, they mainly have low throughput, are typically restricted to analyzing intracellular NPs qualitatively using standard preparations, and are time-consuming. Related to fluorescence microscopy, flow cytometry can only give qualitative data regarding cell-associated NPs, but enables greater throughput of samples. While having the ability to gather quantitative data regarding cell-associated NPs, techniques based on analytical mass spectrometry (MS) are not capable of analyzing NPs at the level of the single cell using the standard set-up and are limited to metal-based NPs in utility. Laser ablation ICP-MS (LA-ICP-MS), mass cytometry (MC, which merges time of flight mass spectrometry [ToF-MS] with flow cytometry), nano-secondary ionization mass spectrometry (nanoSIMS), and time-resolved ICP-MS (TR-ICP-MS) are examples of novel MS techniques brought into use lately. Table 1 summarizes these techniques.

Colloidal stability in biological fluids with serum is a preferable characteristic of NPs for conducting nano-cell research. Stabilizing NPs with steric hindrance may be attained by coating NPs with long polymer chains which are biocompatible (e.g., poly(ethylene glycol) [PEG]). Aggregation and disassembly in culture medium and intracellularly do not occur easily for NPs which are densely coated in PEG (with >1 PEG strand per nm^2 NP surface loading density), even though PEG has a surface charge close to neutral [43]. Serum protein adsorption onto the NP surface is remarkably restrained by a dense PEG coating as well, along with a decrease in nonspecific uptake by phagocytes and enhancement in the targeting specificity of cell-surface receptors [44]. NPs coated in PEG frequently serve as a negative control for research into mechanisms, since NPs coated in PEG do not enter the majority of cell types in abundant amounts [45, 46].

Coating NPs with an anionic or cationic substance to prevent them from aggregating by electrostatic repulsion is an additional technique for stabilization [48, 49]. Nanocarriers that are effective at delivering genes include lipid NPs or polycationic liposomes, because nucleic acids (e.g., plasmid DNA and small interfering RNA [siRNA]) can be electrostatically condensed by them into weakly cationic NPs. Compared with uncomplexed nucleic acids, these NPs have the ability to penetrate the anionic cell membrane in a more effective manner [50]. Cell membrane integrity, however, is liable to becoming undermined by cationic NPs, which may be cytotoxic or lead to immune responses that could hinder translation to the clinic [51, 52]. The typically greater biocompatibility and considerably reduced uptake by cells (probably because of electrostatic

Table 1 Features and limitations of some currently available techniques for analyzing nanoparticle-cell interactions [47]

	Nanoscale resolution	Nanoparticle identification	Nanoparticle quantitation	Nanoparticle transformation	Cellular localization of nanoparticles	High throughput	Single cell analysis	Live cell analysis	Sample preservation
Fluorescence microscopy	−/+[a]	+	−	−/+[b]	+	−/+	+	+	+
Dark-field microscopy	+	+/−[c]	−	+/−[c]	+	−	+	+	+
Raman microspectroscopy	−/+[d]	−	−	−	+	−	+	+	+
Electron microscopy	+	+	−	−/+[e]	+	−	+	−/+[f]	+/−
Flow cytometry	−	+	−/+[g]	−/+[b]	+	+	+	+	+
Mass spectrometry (e.g., ICP-MS)[h]	−	−	+	−	−/+[i]	+	−	−	−
Laser ablation ICP-MS[h]	+	−	+	−	−/+[i]	+	+	+	−
Time-resolved ICP-MS[h]	+	+	+	−	−/+[i]	+	+	+	−
Nano-secondary ionization mass spectrometry	+	+	−	−	+	−	+	+	−
Mass cytometry[h]	+	+	+	−	−/+[i]	+	+	+	−
Synchrotron X-ray absorption spectroscopy	+	+	−	+	−/+[i]	−	+	+	+

ICP-MS, inductively coupled plasma mass spectroscopy. +, Information can be acquired using this technique; −, information cannot be acquired using this technique; −/+ or +/−, information can be acquired with some reservations.

[a]Nanoscale resolution is possible with super resolution mode.

[b]Nanoparticle (NP) transformation is possible to determine if NP fluorescence is influenced by the transformation.

[c]NP identification is possible by parallel application of hyperspectral imaging.

[d]Nanoscale resolution is possible by surface-enhanced Raman spectroscopy.

[e]NP transformation is possible to determine if NP morphology is influenced by the transformation.

[f]Liquid scanning transmission electron microscopy.

[g]Possible after calibrating the technique (see "Flow cytometry").

[h]Analysis possible only for NPs containing metal.

[i]Possible after prior treatment, such as chemical etching techniques.

repulsion from the cell membrane) of noncationic NPs (anionic, near-neutral, or zwitterionic) differ from cationic NPs [53]. Two kinds of noncationic NPs not needing the help of cationic or lipophilic transfection agents can successfully enter mammalian cells, which is an unusual feature in nanomedicine. NPs having noncationic ligands that interact with known cell-surface receptors are the first kind. For instance, macrophages phagocytose polymeric NPs with galactose and mannose receptor targeting (neutral charge) 10–20 times greater than polymeric NPs without sugar components (close to neutral as well) [54]. The binding of negatively charged ribonucleoprotein NPs with 2-[3-(1,3-dicarboxy propyl)ureido]pentanedioic acid (DUPA) (a ligand for prostate-specific membrane antigen (PSMA)) to PSMA-positive prostate cancer cells has two orders of magnitude higher efficiency than that of DUPA-free ribonucleoprotein NPs [55]. Noncationic NPs that do not have ligands with known targeting abilities when they are first used intracellularly belong to the second group. For example, the receptor regulating NPs coated with DNA entering cells was not thoroughly examined until Choi and colleagues in 2013 demonstrated that they were bound to Class A scavenger receptors (SR-A), even though these NPs were employed for gene regulation [56] and molecular diagnostics [57] over 10 years ago [58]. Similarly, in the early 2010s, NPs coated with polydopamine (PDA) were employed for in vitro cancer cell imaging [59] and in vivo image-guided photothermal cancer therapy [60], but it was not until 2017 when the mechanism that governed these NPs entering cells was found [61]. The receptor responsible for their endocytosis is still unknown.

Many biomacromolecules interface with NPs when NPs come into contact with physiological fluids [32, 62]. Previously, exploring the interaction between constituents of the physiological environment and intrinsic attributes of the NPs, along with the ensuing in vitro effect of the cells in response to the NPs, was at the center of numerous studies [63–66]. The colloidal stability of NPs in biological media is a characteristic which remains underemphasized. A prevalent phenomenon in this intricate environment is the aggregation of NPs [67, 68]. Typically an irreversible adherence between particles, an aggregation may cause sizable, unevenly shaped clusters to form [69, 70]. The toxicity and cellular uptake of NPs can be altered by NP aggregation, which may culminate in misleading results and inability of the experiment to be replicated [26, 71, 72]. The potential transformation of the colloidal properties of NPs after they are suspended in physiological fluids is seldomly considered in spite of the growing application of in vitro systems to assess the cell's response to NPs [62, 73, 74]. Since the in vitro cellular response is impacted by NP aggregation in an indirect (i.e., due to changes in the velocity of diffusion and sedimentation) and direct (i.e., due to greater size) manner, this finding is especially astounding.

From a larger perspective, creating efficacious and safe nanoscale therapeutics for clinical applications depends in part on learning about the characteristics which affect colloidal stability and aggregation of NPs when they enter biological media. The in vivo

biodistribution, pharmacokinetics, and systemic toxicity of NPs are controlled by colloidal stability [75, 76]. For instance, while investigating in vivo toxicity via high-dose intravenous administration of nano-hydroxyapatite in Wistar rats, Aoki and colleagues [77] discovered that the aggregation of NPs and ensuing pulmonary capillary obstruction probably caused morbidity. NP aggregation and capillary obstruction in the lungs may cause morbidity in vivo according to other similar investigations [78, 79]. In addition, NPs with stability in blood had longer circulation half-life and lower probability of fast clearance from the body's reticuloendothelial system (RES) according to some studies [80, 81].

It is generally not preferable to have aggregates form in situ from the intravenous administration of nano-therapeutics, because quick hepatic and RES clearance will follow, thus reducing the likelihood that the therapeutic targets will be reached by the NPs. Rational design of NPs based on their behavior at the systemic, cellular, and molecular levels in biological systems, therefore, requires a basic comprehension of NP aggregation.

In vitro studies

Chemical synthesis is used to construct a prototype of the NP. Drugs, fluorophores, oligonucleotides, peptides, polymers, or proteins are next applied to coat the NP, followed by its administration into animal models or cell cultures. Affecting gene expression, toxicity, and uptake by cells, the design of NPs dictates their interaction with serum proteins and cell membrane receptors according to numerous investigations, which contradict the earlier belief that NPs were harmless carriers. Fig. 1 illustrates that, in various situations, interactions may occur between the NPs and cell-surface membrane [82].

Once NPs bind to the receptor target, they enter the cell in many instances. At the NP-biological interface, the behavior of nanomaterials is determined by a few factors after binding (Fig. 1). Cellular uptake is directly affected by the shape of the NPs, for example. The greatest uptake is seen with rods, then spheres, cylinders, and cubes. This order was established by Gratton and colleagues, who created NPs >100 nm [83]. There is a substantial advantage of spheres over rods in investigations using sub-100 nm NPs [84, 85]. Overall cellular uptake was reduced when the aspect ratio of nanorods was elevated in this size range.

A nanomaterial's ligand density and engineered geometry influence how ligands bound to NPs interact with cellular receptors [86]. How many ligands interact with the receptor of interest is determined by the design of the NP scaffold. The interaction of various NP ligands with various cellular receptors leads to a multivalent effect [87]. With greater binding strength than the individual affinities added together, the ligand complex may be measured by their avidity. Antibodies with at least two sites that bind antigens reveal this phenomenon. Total avidity of the NP ligands for accessible receptors on cells depends on the density of ligands on NP surfaces over a particular curvature.

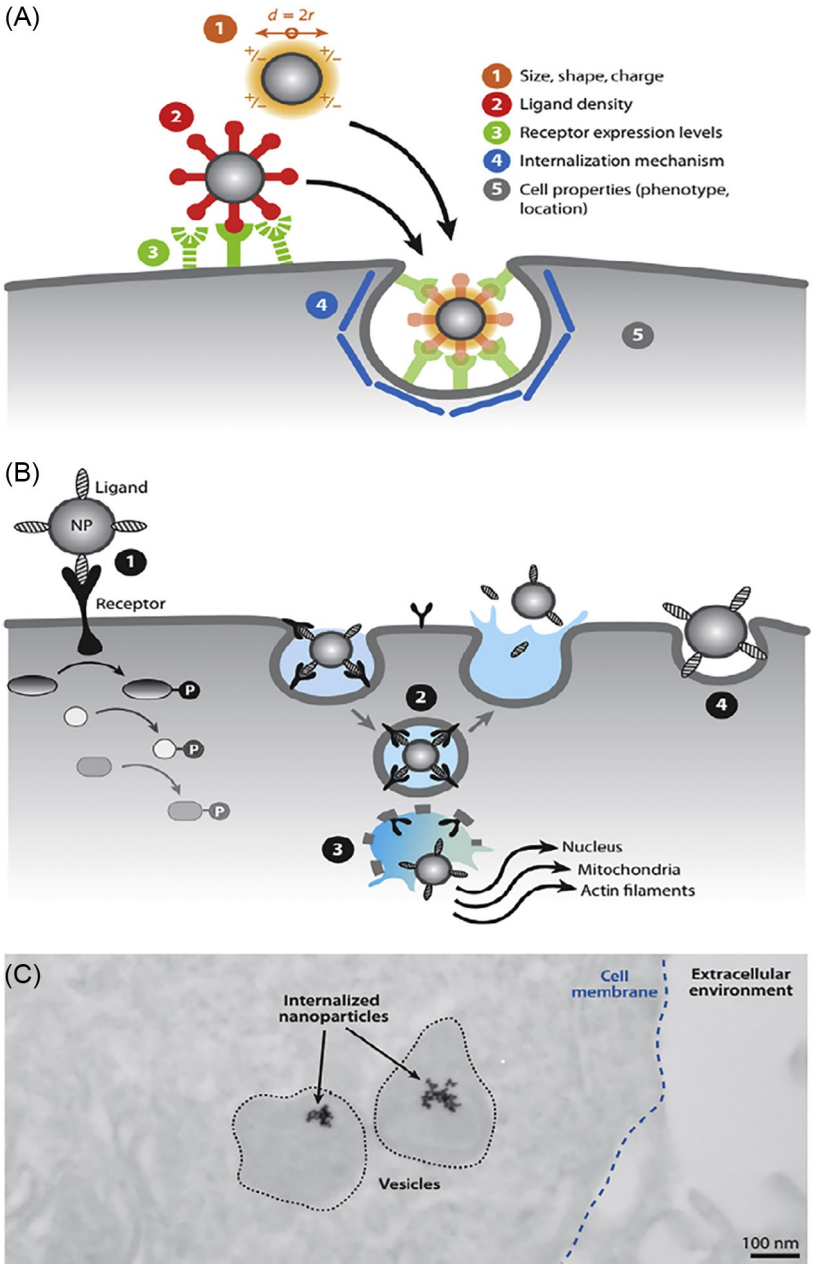

Fig. 1 Interactions between nanoparticles (NPs) and cells. (A) NP-cell interactions are affected by multiple factors at the nano-bio interface. (B) NPs coated in ligands interact with cells. ❶ Membrane receptors are bound by ligand-coated NPs that do not enter the cell. This leads to a signaling cascade. ❷ Cells may endocytose and exocytose ligand-coated NPs as well. The NPs do not leave the vesicle. After binding to the membrane receptor and entering the cell, they leave the cell. ❸ NPs taken up by the cell can escape the vesicle and interact with different organelles. After binding to membrane receptors and entering the cell, NPs target subcellular structures. ❹ There may be nonspecific interactions between the NPs and cell-surface membrane. The NPs are then taken up by the cell. (C) The uptake of various transferrin-coated 15-nm gold NPs by HeLa cells into vesicles inside the cells [82].

For example, Herceptin has a binding affinity to the ErbB2 receptor of 1.5×10^{-13} M on a 70 nm NP, 5.5×10^{-12} M on a 10 nm NP, and 10^{-10} M in solution [88]. Due to a greater density of proteins on the surface of the NP, as the NP size increases, the binding affinity of the ligand proportionally increases as well.

Characteristics aside from binding affinity, such as the ErbB2 receptor's downstream signaling, should be taken into account as well, because gold NPs in the 40–50 nm size range gave rise to the largest effect. In relation to free ligands in solution, cell signaling may be influenced by the design of NPs according to a few studies. For instance, a change in cellular apoptosis due to effects on caspase enzyme activation was seen in the previously noted 40–50 nm gold NPs which were coated in Herceptin [88]. The capacity to induce angiogenesis was likewise enhanced by the conjugation of receptor-specific peptides to the surface of NPs [89]. Based on receptor-mediated signaling, angiogenesis was augmented by peptide presentation on a structured scaffold. These discoveries underscore the benefits of ligand binding to NPs compared with free ligands in solution. The avidity may increase and cell signaling may change due to a dense area of ligands on the NP surface.

However, there are also disadvantages. Unanticipated cell signaling changes may result from nanomaterials. Intercellular adhesion molecule I (ICAM-I) protein-coated NPs may be taken up by cells, for instance. Since endocytosis is not known to be induced by ICAM-I, this is an atypical phenomenon. However, unforeseen internalization occurs when numerous ICAM-I proteins are added onto the surface of a nanomaterial [90]. A different investigation reported the interaction of 14 nm carbon NPs with β1-integrins and epidermal growth receptors on alveolar II epithelial cells of rats. The Akt signaling pathway became activated due to this trigger, leading to cell proliferation [91]. Proteins may become denatured when they bind to the engineered surface of NP-ligand complexes, causing another problem. Greater nonspecific interactions, issues with receptor binding, or inflammation may arise from protein denaturation. For example, the denaturation of lysozymes and their interaction with other lysozyme molecules to form protein-NP aggregates may occur when they bind to gold NPs [92]. Once it binds to the surface of gold NPs coated with polyacrylic acid, fibrinogen unfolds as well. After the fibrinogen denatures, it may bind to the Mac-1 integrin receptor and cause inflammation [93].

In vitro systems in which interactions take place between NPs and constituents of the cell culture medium before contacting any cells can regularly determine cellular interactions due to the impact of NP physico-chemical properties [94, 95]. Fig. 2 shows that, upon suspension in a cell culture medium (CCM) containing electrolytes and proteins, NPs enter a complex environment.

Illustrated in the figure is a 40 nm gold sphere NP in CCM. Various biomolecules such as ionic salts *(red dots)* and amino acids *(blue spheres)*, along with serum albumin

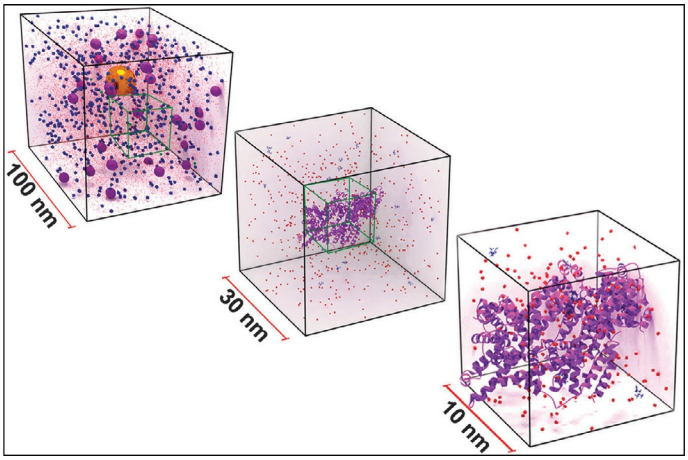

Fig. 2 Model displaying the suspension of a gold nanoparticle (NP) in a cell culture medium supplemented with 10% serum. The medium volume at various size scales is depicted by the boxes. At 100 nm^3, the gold NP is visible and surrounded by serum proteins *(violet spheres)* [96].

or globulins *(violet spheres)*, are examples of the main proteins in CCM, which is a buffered solution. A NP's hydrodynamic behavior is affected by these components. Instability in the stabilized NPs may arise due to loss of surface functionality or molecule/protein adsorption, and aggregates may form in effect. NP mobility and in vitro behavior may be additionally affected by these processes.

Although research has demonstrated that cellular interactions and effects are influenced by alterations in the NP dispersion state, there has been infrequent and inadequate consideration of the CCM's influence on NP colloidal behavior [65, 71, 73, 97]. In the biological environment, it is difficult to experimentally evaluate colloidal stability. Another factor adding to the complexity is the variety of CCM which has various components. Aside from proteins, the large amount of ions may affect the chemical and colloidal properties of NPs once they enter the CCM (Fig. 2). While examining how colloidal stability was impacted by electrolyte ionic strength, investigators observed that electrostatic interaction screening may undermine stability. Aggregation may develop from the screening. The electric double layer's (EDL) effect on stabilization was inhibited at higher efficiency by multivalent electrolytes than by monovalent ions. In addition, the morphology and rate in which the aggregate formed were determined by the nature of electrolytes and ionic strength [98]. Important for colloidal stability was a change in conformation driven by pH with regard to the adsorption of polyelectrolytes onto NPs [99]. The chemical equilibrium between deprotonated functional groups (NH_2 or COO^-) and protonated functional groups (NH_3^+ or COOH) makes the surface charge of NPs ending in amine and carboxyl groups pH-dependent. Ions may be released by

NPs (e.g., quantum dots, Ag, or ZnO). Particle functionalization and size, along with the local environment, may affect the rate that ions are released. Furthermore, a correlation has been found between their breakdown and cytotoxic effects (e.g., producing reactive oxygen species) [100–105].

The influence of the intracellular environment needs to be considered as well. As NPs are internalized by cells via endocytosis, the environment around them changes. The pH is 7.4 in the extracellular medium, then decreases to 5.5 in late endosomes, and further drops to 4.5 in lysosomes [106, 107]. Multiple ramifications may arise under the new conditions, such as the fast degradation of adsorbed or bound proteins. Among the most extensively investigated engineered NPs are silver NPs, and their dissolution in endosomes and lysosomes has been reported. The NP coating or whole NPs may degrade due to large amounts of hydrolytic enzymes, which are also in the acidic lysosomes. Changing conditions inside cells should be taken into consideration, including the impact of electrolytes, enzymes, and other proteins on the stability and degradation of NPs [108–110].

The complexity of interactions between nonspherical NPs and cells appears to be much greater, even though only a small number of investigations have examined them. Two distinct orientations of interactions between rod-shaped NPs coated with ligands and cells are possible. Cell-surface receptors have many more interactions with the long axis than the short axis [111]. The presentation of the ligand to target cell receptors for spiky nanostructures (e.g., gold nanourchins) is influenced by its position on or between the spikes [112]. Greater control over presenting ligands to target receptors may be possible with asymmetrical NPs in terms of engineering.

For a certain geometric shape, overall cellular internalization is dependent on the dimensions of the nanomaterial. To achieve the highest cellular uptake rate and concentration inside cells, the ideal diameter is 50 nm for quantum dots, silica NPs, single-walled carbon nanotubes, and spherical gold NPs in some mammalian cells [111, 113, 114]. Aside from size and shape, internalization is influenced by nanomaterial composition. For nanomaterials with diameter of 50 nm, the rate of endocytosis for gold NPs is 10^{-6} min^{-1} and for single-walled carbon nanotubes is 10^{-3} min^{-1}. The dissimilar intrinsic properties between gold and carbon may account for this difference of three orders of magnitude. Biological downstream effects are impacted by the composition of ligands coating the nanomaterial as well. When two different proteins that target an identical receptor were used to coat the NPs, they exhibited different cytotoxic and internalization effects [115].

NPs usually gain entry into the cell after binding to their receptor through receptor-mediated endocytosis [85, 88, 116]. The Gibbs free energy decreases locally when the NP-ligand conjugate binds to the receptor. In effect, the NP is surrounded by the membrane, leading to a closed-vesicle structure [116]. An endosome forms with the budding off of the vesicle from the membrane and fusion with other vesicles. The endosome then fuses with lysosomes which degrade materials. The process involved in the membrane

wrapping around the NP is probably associated with the dependence on size for NP uptake. The interaction of ligands with receptors is lower for smaller NPs. Just one or two receptors of the cell may interact with a 50-kDa protein-coated 5 nm NP. On the other hand, the interactions between ligands and receptors for each NP are much higher for a 100 nm NP. For sufficient free energy to be generated to induce membrane wrapping, receptors need to be bound by some small NPs (coated in ligands) which are in close proximity. Uptake can be stimulated by larger NPs which cross-link and cause receptors to cluster. In terms of thermodynamics, membrane wrapping may be driven by a 40–50 nm NP, which can recruit and bind sufficient receptors. NPs larger than 50 nm bind too many receptors, which actually restricts uptake. To offset the reduction locally, receptors are redistributed by diffusion on the cell membrane. NPs above 50 nm have high affinity for many receptors and may restrict more NPs from binding. No shortage of NP ligands and localized cell-surface receptors lead to optimal endocytosis, according to a mathematical model of this process [117, 118].

NPs with diameters of 30–50 nm have optimal ligand density, allowing the ideal conditions to be met. However, investigations showing that uptake is influenced by the diameter of NPs mainly used immortalized cell lines. The examined cells (e.g., immortalized HeLa cells as opposed to primary macrophages) may affect the optimal size of NPs for internalization, since every kind of cell has a distinct phenotype. Different mechanisms of uptake may be used and varying target receptor levels may be expressed by different kinds of cells. Primary and immortalized cells in a variety of cell culture configurations (monolayer and 3D) should, therefore, be used to further assess the phenomenon. The optimization of cellular internalization and intracellular accumulation through wide-ranging improvements in engineered parameters can then be achieved.

An ongoing enigma is NP behavior inside endolysosomal vesicles. The protease Cathepsin L may cleave NP ligands in these vesicles, according to some reports [119]. Enzymes gradually degrade the core component of quantum dots in intracellular vesicles of macrophages [120]. The type of cell and nanomaterial size may affect the localization of CdTe quantum dots in various organelles. The nucleus favors entry of sub-2.1 nm quantum dots, while the cytoplasm contains 4.4 nm quantum dots [121]. Peptides (e.g., mitochondrial localization sequence) may control the location of NP compartmentalization inside the cell. NPs can go into the cytosol if they are designed to evade the endolysosomal system. Many organelles may interact with the NPs in the cytosol and cell behavior may be influenced by the NPs. Generating reactive oxygen species, interfering with mitochondrial function, and initiating the oxidative stress-mediated signaling cascade are examples of biological effects induced by NPs. Oxidative damage to DNA, harmful effects on the mitochondrial genome, and formation of micronuclei are all consequences of generating reactive oxygen species [122–124]. In addition, detrimental effects on nuclear DNA, which may result in gene mutations, development of cancer, arrest of the cell cycle, or cell death may be driven by some NPs. Numerous experimental designs

have demonstrated the oncogenic effect of hydrophilic titanium oxide NPs [125]. The return of NPs to the endolysosomal system enables their exocytosis. Otherwise, they will stay in the cytoplasm. Daughter cells arising from mitosis will continue to retain NPs in the cytosol [126]. The effect of NPs on successive generations of cells is not yet known. Regarding NP toxicity and NP properties within the cytoplasm, no general agreement currently exists. There was greater production of reactive oxygen species by 30 nm amorphous TiO_2 and 15 nm silver NPs than by NPs of other sizes [127, 128]. Some studies, however, have not shown the size of NPs to have a major effect. For example, the NP size did not influence the expression of inflammatory cytokines (e.g., IL-1β, MIP-2, and TNF-α) which were generated when macrophages internalized quantum dots and silver NPs [120, 127]. To better comprehend the safety and outcomes of NPs that are taken up by cells, more research is needed.

For determining the fate of cells, the importance of surface charge should not be overlooked, in addition to the ligand density, shape, and size of nanomaterials mentioned earlier. More rapid uptake rate is observed in NPs with a positive charge relative to those having a negative or neutral charge [129, 130]. The small negative charge of the cell membrane and the induction of cellular internalization due to electrostatic attractions may explain this phenomenon [114, 115]. In one study, internalization resulted from adhesion of NPs onto the surface of the cell, which was promoted by the electrostatic attraction between positively charged NPs and the cell membrane. Ca^{2+} may enter cells and cellular proliferation may be impeded due to disturbance of the cell membrane potential by positive charges for tiny NPs (2 nm) [53]. Lipid bilayers may be reconstructed in response to the surface charge for larger NPs (4–20 nm) [131]. Fluidity results from the binding of NPs with positive charges to a lipid bilayer, while local gelation results from the binding of NPs with negative charges. The importance of surface charge in biological effects downstream to NPs has been shown by some studies. A corona composed of various proteins swiftly surrounds the nanomaterial's surface charge in serum or other biological conditions. The impact of corona composition may have been reported by investigations examining NPs with positive and negative charges since composition of the corona is influenced by the surface charge. The incubation of citrate-capped gold NPs having negative charges with cell cultures led to this observation [85, 111]. The interaction of DNA-coated NPs having negative charges with serum proteins led to cellular uptake in a different study [132].

It is important to evaluate colloidal stability in physiological fluids. The accurate assessment of the NP dispersion state in biological settings is challenging due to the complicated analytical techniques, along with the involvement of many chemical and physical forces. The variety of complicated physiological fluids that interact with NPs increases the difficulty. The dispersion state of NPs and their physico-chemical properties are often analyzed using several different methods, including novel innovative techniques.

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