Effect of Surface Properties on Nanoparticle–Cell Interactions

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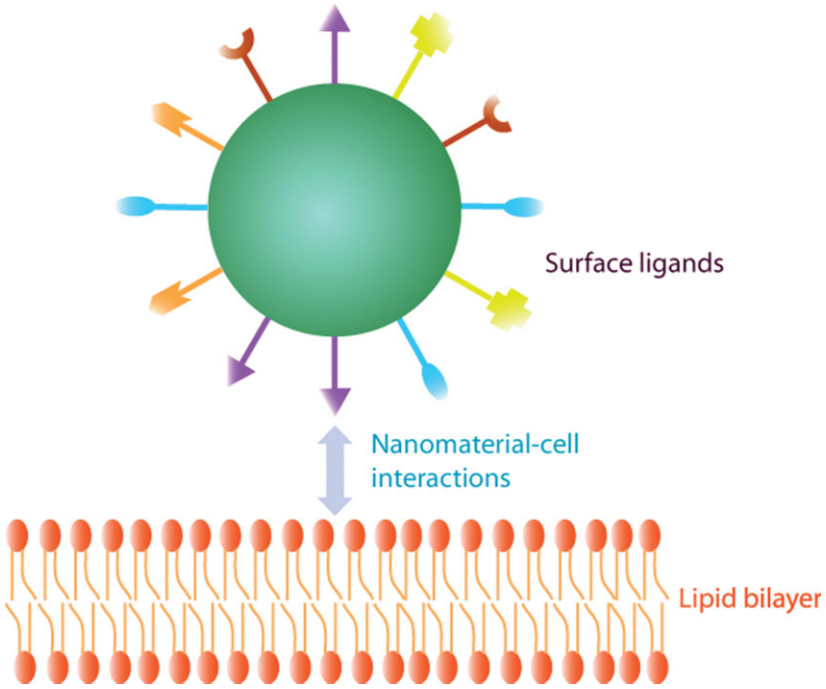
Effect of Surface Properties on Nanoparticle–Cell Interactions

*Ayush Verma and Francesco Stellacci**



From the Contents

The interaction of nanomaterials with cells and lipid bilayers is critical in many applications such as phototherapy, imaging, and drug/gene delivery. These applications require a firm control over nanoparticle–cell interactions, which are mainly dictated by surface properties of nanoparticles. This critical Review presents an understanding of how synthetic and natural chemical moieties on the nanoparticle surface (in addition to nanoparticle shape and size) impact their interaction with lipid bilayers and cells. Challenges for undertaking a systematic study to elucidate nanoparticle–cell interactions are also discussed.

1. Introduction

The plasma membrane is a selectively permeable membrane that defines the boundary and maintains the essential intracellular environment of the cell. Small and nonpolar molecules such as O_2 and CO_2 can readily diffuse across the lipid bilayer, however, polar molecules such as ions and larger nanomaterials are incapable of crossing the plasma membrane on their own. In nature, important ions and nanometer-sized proteins are transported across the lipid bilayer through specialized membrane-transport protein channels.[1] Most other nanoscale macromolecules and molecular assemblies are internalized through endocytosis (the process of uptake of macromolecules into cells by enclosing them in membrane vesicles, see Figure 1 and Table 1) upon contact with the cell membrane. These endocytosed nanomaterials are confined in endolysosomes (membrane-bound vesicles) and are incapable of reaching the cytosol.[2] This endocytic fate of nanomaterials has been shown by various precedents in literature. As an example, 3.4-nm gold nanoparticles have been shown to be taken up into macrophage cells via a mechanism involving pinocytosis as indicated by atomic force microscopy (AFM) measurements.[3] In this study, transmission electron microscopy (TEM) and confocal microscopy images confirmed that 24 h after internalization, the nanoparticles are found in lysosomal bodies arranged in a perinuclear fashion. Endocytic fate is not limited to gold nanoparticles but has also been observed for iron oxide nanoparticles[4–7] and fullerenes.[8] While qualitative observations show that in most cases nanomaterials are contained within vesicular structures and cannot breach cell membrane barriers, the kinetics, amount, and mechanism of uptake vary depending on numerous factors in the study such as purity of the nanomaterial, nanomaterial–cell incubation conditions, cell treatment, type of cell, and the type of nanomaterial.

Many applications that are conceived for synthetic and biological nanomaterials require breaching of the cell-membrane barriers to reach the cytosolic machinery or the nucleus of the cell. Transportation across cell membranes can allow for the delivery of siRNA[9] or other drugs to the cytosol[10] by utilization of a carrier that can circumvent the natural inability of these drugs to penetrate the cell membrane. The safe and efficient localization of nanomaterials into the cytosol may be critical in other applications such as phototherapy[11,12] and intracellular imaging[13] as well. However, crossing cell membranes is inherently challenging due to the nature of the lipid bilayer and, from an evolutionary point of view, to protect the cellular function. Many nanomaterials have nonetheless demonstrated an ability to successfully penetrate cell membranes. Among natural systems, this ability has been observed in cell-penetrating peptides (CPPs) or cell-fusogenic peptides (such as those found on viruses) that are efficient at penetrating cell membranes without forming overt pores in lipid bilayers.[14–17] Among synthetic nanomaterials, with the exception of very small molecules (such as metal clusters)[18] or needle-shaped materials (e.g., chemically functionalized carbon nanotubes), only cationic nanoparticles (metallic, quantum dots (QDs), and dendrimers)[19] are capable of penetrating cell membranes. However, in the process, they create pores in the cell membranes that can lead to cellular toxicity by destroying the delicate concentration balance of intracellular versus extracellular ions, proteins, and other important macromolecules that are required to protect the integrity and the normal function of a cell. Alternatively, different approaches have been utilized to transport nanomaterials into the cytosol of cells: i) disruption of endosomes and entry into cells through the "sponge-effect" mechanism[20] or the usage of chloroquine,[21] ii) direct microinjection of nanomaterials into cells,[22,23] iii) the use of electroporation,[24] and iv) conjugation of natural cell-penetrating/-fusogenic chaperons to nanomaterials.[25]

An alternate means of membrane translocation of nanoscale-sized materials can rely on intelligent surface-structure design. However, the design principles of synthetic nanoscaffolds for membrane transport are far from being comprehensively understood. Typically, nanomaterial interactions with cell membranes are dictated by the chemical functionalities on the surface in addition to their shape and size. It is also observed that among natural systems, such as CPPs, not only the type of chemical groups but their relative arrangement also plays a key role in their interaction with cell membranes. Such peptides, while ineffective at crossing the cell-membrane barrier in their random-coil conformation, display an ability to penetrate or fuse with membranes after adopting an amphipathic α-helical structure.[26] Clearly, there is much need to understand the impact of surface properties of nanomaterials with respect to their interactions with cells and to also harness that knowledge to create smart nanosystems. In line with this effort, this review will focus on the current nanoparticle systems and effect of their surface-chemical properties on interaction with lipid bilayers and cells. Such interactions can have other important implications (such as cytotoxicity) and readers are pointed to a more in-depth review on this topic elsewhere.[27]

This review is formatted in the following way. First, the effect of size and shape of nanomaterials on their interaction with cells is discussed. Then, the review focuses on the effect of nanoparticle surface coatings on nanoparticle–cell interactions. Among the surface coatings, we first discuss the effect of the nature of the organically modified nanoparticle surface,

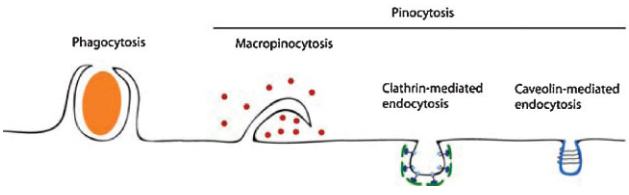


Figure 1. Major endocytic pathways. Adapted with permission from Reference [2]. Copyright 2003, Nature Publishing Group.

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starting with neutral and protein-resistant nanoparticle coatings, followed by negatively and positively charged surfaces. Thereafter, we highlight how the arrangement of such organic ligands on the particle surface impacts nanoparticle interaction with cell membranes. In the last section, the effect of natural biological ligands conjugated to nanoparticles is examined, where first oligonucleotide coatings are discussed, followed by cell-penetrating motifs conjugated to the nanoparticle surface and their utilization for intracellular transportation.

2. Effect of Nanomaterial Size and Shape on Cellular Uptake

Here, we summarize the impact of shape and size on nanomaterial cell internalization. The shape and size of nanoparticles has been found to greatly influence their cellular uptake. In a study by Chithrani et al., the uptake of 14-, 50-, and 74-nm gold nanoparticles was investigated in Hela cells (Figure 2).[28,29] It was found that the kinetics of uptake as well as the saturation concentration varied with the different-sized nanoparticles with 50-nm-size particles being the most efficient in their uptake, indicating that there might be an optimal size for efficient nanomaterial uptake into cells. The effect of nanoparticle shape on its internalization was also examined: spherical particles of similar size were taken up 500% more than rod-shaped particles, which is explained by greater membrane wrapping time required for the elongated particles. In other studies, nanoparticle size has been shown to strongly affect the binding and activation of membrane receptors and subsequent protein expression.[30] Examining the influence of shape and size of nanomaterials on cell interactions is crucial as this can have implications in toxicity.[27] One has to also consider that there is an inherent polydispersity within any given batch of nanoparticles, which needs to be controlled for predictable nanoparticle behavior with cells, otherwise different batches of the same nanomaterial might display different results in cell studies. Another very important factor is that even though nanoparticles display a certain size after synthesis, during the in vitro and in vivo studies they might aggregate into vastly different shapes and sizes that may dictate the outcome and interpretation of results.

Ayush Verma received his B.Sc. (Hons.) in Chemistry at St. Stephen's College, University of Delhi in 2000 and his Ph.D. at the University of Massachusetts at Amherst in 2006, during which his work was focused on the use of nanoparticles for biomolecular recognition and development of novel biomaterials. Currently, he is a postdoctoral researcher at MIT, studying the effects of nanoparticle ligand shell composition and structure on particle–cell interactions with possible applications towards drug and gene delivery.

Francesco Stellacci got his degree in Materials Engineering in 1998 at the Politecnico di Milano with a thesis on photocromic molecules and polymers under the guidance of Profs. Zerbi and Gallazzi. He then moved to the University of Arizona for a post-doctoral experience with Prof. Joe Perry working on two-photon microfabrication techniques for three-dimensional metallic structures. In 2002 he was appointed assistant professor in the Department of Materials Science and Engineering at MIT, where he currently is the Paul M. Cook Career Development Associate Professor.

3. Neutral Surface Charge on Nanoparticles Minimizes Cellular Interaction

The effect of neutral bioresistant synthetic coatings on nanoparticle–cell interactions is highlighted here. Nanomaterial shape and size contribute significantly to their interaction with cells, however, functional groups on the nanoparticle surface are the primary dictators of many important nanomaterial properties, such as solubility and macromolecule and cell surface interactions. Typically, incubation of nanomaterials with cells in media results in adsorption of serum proteins on their surface[31] that induce the entry of nanoparticles into cells by receptor-mediated endocytosis. However, in many applications, generating nanoparticles that do not interact with cell membranes or other biological macromolecules is also desirable. As an example, for in vivo applications, nonspecific

Table 1. A brief description of the major endocytic pathways.[2]

Type of endocytosis	Brief description	Size of vesicle formed
1) Phagocytosis	Internalization of solid particles such as bacteria and yeast by specialized cells.	Dependent on the particles being engulfed.
2) Pinocystosis	Fluid-phase uptake of extracellular molecules. Multiple pinocytic pathways are possible (below).	(Below)
3) Macropinocytosis	Trapping of large fluid pockets by formation and enclosure of membrane protrusions.	$>1\ \mu m$
4) Clathrin-mediated endocytosis	Concentration of transmembrane receptors and bound ligands in "coated pits" on the plasma membrane formed by the assembly of cytosolic proteins, the main assembly unit being clathrin.	$\approx$120 nm
5) Caveolae-mediated endocytosis	Flask-shaped invaginations in the plasma membrane that mediate uptake of extracellular molecules into the cell by specific receptor binding.	$\approx$50–60 nm

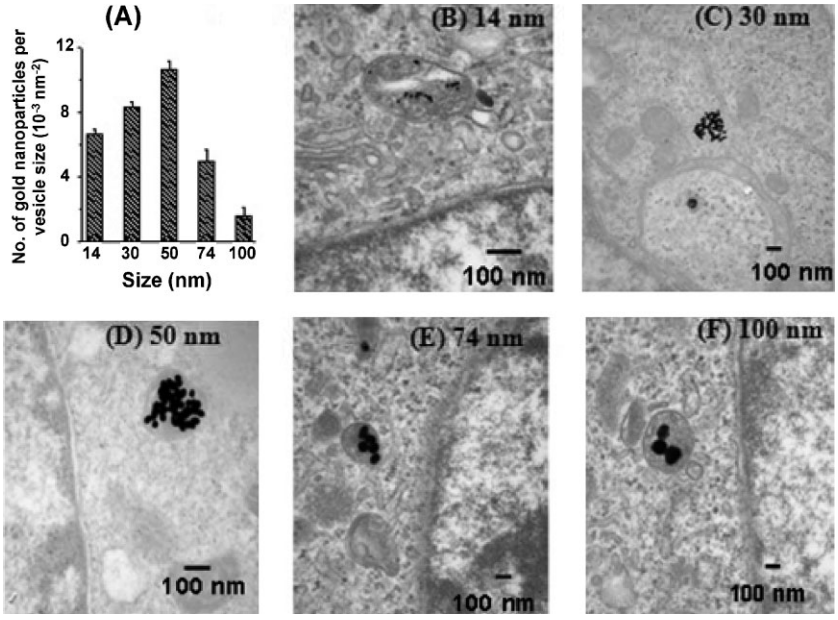

Figure 2. TEM images of nanoparticles entrapped in vesicles within cells. A) Graph showing the number of nanoparticles per vesicle diameter versus nanoparticle size. TEM images of nanoparticles with a diameter of B) 14 nm, C) 30 nm, D) 50 nm, E) 74 nm, and F) 100 nm within vesicles. Reproduced with permission from Reference [29]. Copyright 2006, American Chemical Society.

adsorption of proteins on the nanoparticle surface can occur that may lead to particle agglomeration and clearance from the reticular-endothelial system, which hiders its ability to deliver drugs/genes to the target site. Nonspecific interactions can also lead to nanoparticles binding to cell membranes and the extracellular matrix leading to inefficient tagging and detection. To avoid such issues, nanoparticles can be coated with a neutral ligand such as poly(ethylene glycol) (PEG), which is well-known to resist protein adoption. Xie et al. have shown that coating PEG on monodisperse Fe_3O_4 nanoparticles produced negligible aggregation in cell-culture conditions and reduced nonspecific uptake by macrophage cells (Figure 3).[32] A

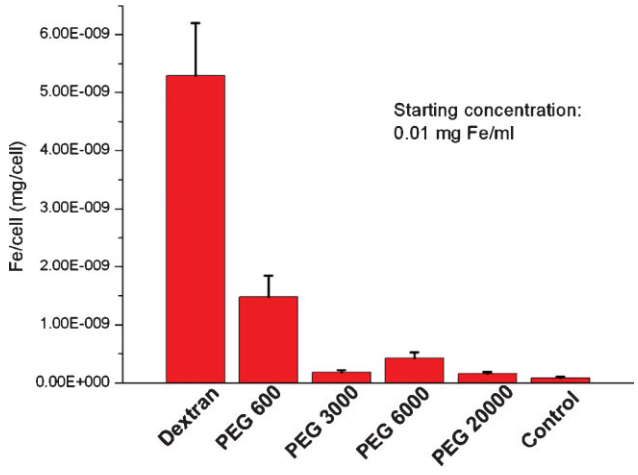

Figure 3. ICP-AES analysis of the result of the uptake of dopamine–PEG-coated Fe_3O_4 nanoparticles.

systematic comparison between non-pegylated QDs and those coated with 700 and 6000 MW PEG showed a fivefold higher endocytosis uptake of the non-pegylated QDs.[33] In other studies, the amounts and polymer conformations of PEG on the nanoparticle surface was varied and its effect on cellular internalization observed. It was shown that a relatively higher concentration of PEG on the nanoparticle surface alone does not lead to a lower nanoparticle uptake, but rather the spatial configurational freedom of PEG chains on the particle surface plays a determinant role.[34] It was also shown that between different molecular architectures of the pegylated polymers present on the nanoparticle surface, the one having most of the PEG moiety exposed to the surface resulted in less protein binding and cell interaction, even with a relatively lesser overall PEG content.[35] Another study has shown that hydroxyl-coated QD surfaces have a significant reduction over PEG-coated analogs in nonspecific cellular binding.[36] Neutral surface charge on nanoparticles can also be obtained through surface modification of nanoparticles with zwitterionic ligands[37] that feature both positive and negative charges with an overall neutral charge. The utilization of such ligands has also ensured relatively low cell interaction and uptake.[38,39] These studies display that neutral-charged ligands can provide a very useful route to minimize nonspecific interactions of nanoparticles with their biological environment if positioned favorably on the nanoparticle surface.

4. Interaction of Charged Nanoparticles with Cells

Now, we examine how charged functional groups on nanoparticles affect cellular interactions and also highlight several mechanistic studies undertaken to elucidate interactions of such particles with lipid bilayers. While neutral functional groups are excellent in preventing unwanted nanomaterial–biological interactions, most charged functional groups are responsible for active nanoparticle interaction with cells. There has been evidence of uptake of negatively charged particles despite the unfavorable interaction between the particles and the negatively charged cell membrane. Cho et al. have recently examined the role of surface charge in internalization of gold nanoparticles.[40] Importantly they have shown that by using an I_2/KI etchant (that etches the gold core) they can selectively dissolve the gold core of particles that were attached to the cell surface. This approach, when coupled with inductively coupled plasma mass spectroscopy (ICP-MS), allows a quantitative differentiation of surface adsorbed nanoparticles versus internalized nanoparticles as a function of nanoparticle surface charge. In this study, it was observed that neutral and negatively charged nanoparticles adsorbed

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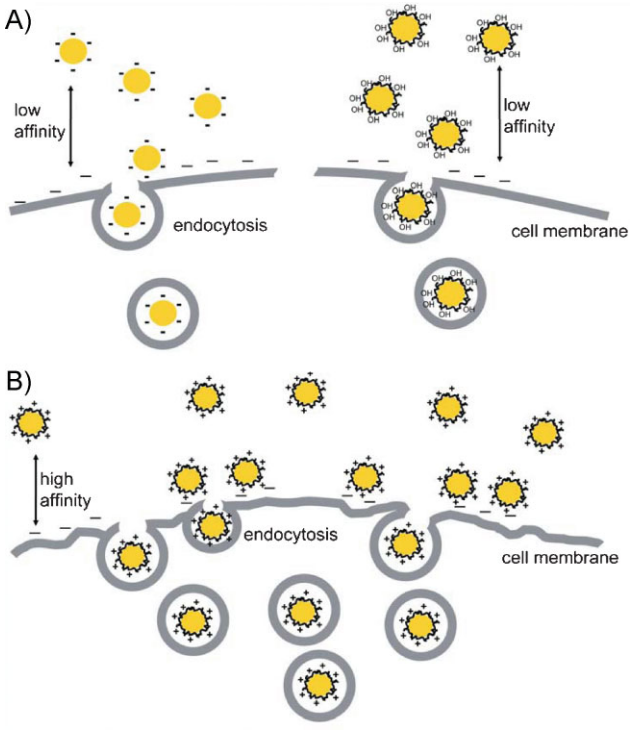

Figure 4. Schematic illustration of gold nanoparticles featuring different surface charges and SK-BR-3 breast cancer cells. A) Citrate-coated and PVA-coated nanoparticles display low affinity of interaction with the cell membrane while a B) poly(allyamine hydrochloride)-coated nanoparticles show high cell-membrane binding affinity. Reproduced with permission from Reference [40]. Copyright 2009, American Chemical Society.

much less on the negatively charged cell-membrane surface and consequently show lower levels of internalization as compared to the positively charged particles (Figure 4). Other studies have examined the cellular uptake of negatively charged nanoparticles with different core materials. Villanueva et al. have studied the uptake of iron oxide nanoparticles (with similar aggregate sizes) functionalized with differently charged carbohydrates in human cervical carcinoma cell lines (HeLa).[41] They did not observe any cellular uptake of neutral nanoparticles, however, the negatively charged nanoparticles showed uptake and toxicity depending on the type of surface coating. Anionic iron oxide nanoparticles without molecular surface coatings have also shown a high level of internalization by interacting strongly and nonspecifically with the plasma membrane as observed through complementary magnetic assays, magnetophoresis (MP), and electron-spin resonance (ESR).[42] The internalization of negatively charged nanoparticles is believed to occur through nonspecific binding and clustering of the particles on cationic sites on the plasma membrane (that are relatively scarcer than negatively charged domains) and their subsequent endocytosis. Iron oxide nanoparticles that are stabilized by carboxyl-functionalized 3rd generation (G3) poly(amidoamine) (PAMAM) dendrimers have also been shown to be taken up into human epithelial carcinoma cells presumably either through pinocytosis or via direct diffusion through the cell membrane.[43] Patil et al. have looked at the cellular uptake of cerium oxide nanoparticles as a function of zeta potential by varying the solution pH.[44] Their results displayed a lesser adsorption of bovine serum albumin (BSA) protein and a more efficient uptake of the negatively charged nanoparticles in adenocarcinoma lung cells as compared to the positively charged particles. In addition to internalization into cells, studies have also shown that negatively charged QDs (≈30 nm in diameter) can penetrate the skin of a mouse, the result being exacerbated with ultraviolet radiation (UVR).[45]

In contrast to the low level of cell interaction and internalization by neutral and negatively charged particles, cationic particles have been well-known to bind to negatively charged groups on the cell surface (e.g., sialic acid)[1] and translocate across the plasma membrane. As positively charged particles have the greatest efficiency in cell-membrane penetration and cellular internalization, they form the primary platform as synthetic carriers for drug and gene delivery. To exploit positively charged nanoparticles for cellular internalization, Martin et al. decorated the surface of superparamagenetic iron oxide nanoparticles with dendritic guanidines that resulted in a similar cell-penetration capability as human immunodeficiency virus-1 transactivator (HIV-TAT) peptide (Figure 5).[46] These nanoparticles exhibited greater cell penetration than amine-coated particles, albeit a greater level of cellular toxicity was also observed. Harush-Frenkel et al. have examined the endocytosis mechanisms of charged particles.[47] Their results showed that while negatively charged nanoparticles displayed a less efficient rate of endocytosis, positively charged nanoparticles internalized rapidly via a clathrin-mediated pathway. Additionally, upon inhibition of this endocytic pathway, compensatory endocytosis resulted in a higher internalization of the cationic particles. Pegylated amine-coated QDs have also been utilized as robust intracellular labeling agents.[48] A strong correlation between the amount of positive charge and internalization into cells has

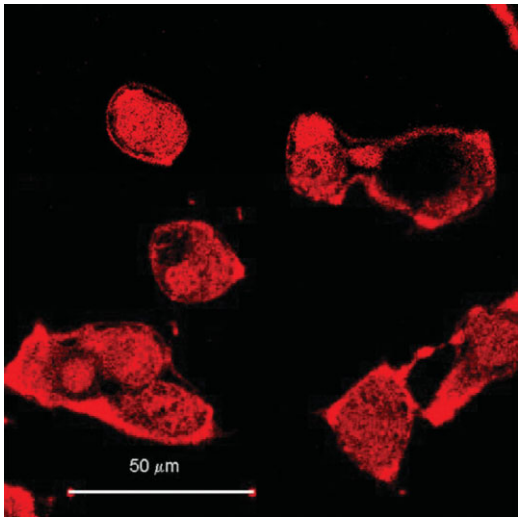

Figure 5. Confocal microscopy displaying GL261 mouse glioma cells after a 2 h incubation with guanidine-functionalized cationic iron oxide nanoparticles. Reproduced with permission from Reference [46]. Copyright 2008, American Chemical Society.

been observed. While at lower charge density a clathrin- and an actin- dependent endocytic mechanism is indicated for silica nanoparticles, a strongly charged surface coating allows bypassing of these mechanisms in certain cells.[49] Positively charged silica nanoparticles have also been shown to escape endosomes and enter the cytoplasm and nucleus.[50] In another study that compared the internalization of QD ellipsoids with different surface coatings, it was observed that the amount of nanoparticle uptake into skin cells was in the following order: QD–COOH > QD–NH_2 > QD–PEG.[51] Even among nanoparticles bearing cationic surface groups it has been observed that slight changes in surface functionalities (such as hydrophobic amount or structure) can lead to varying amounts of cellular internalization,[52] which indicates that the effect of nanoparticle surface properties on their interaction with cells is far more complicated than currently understood.

To better understand the mechanisms of nanoparticle–cell interactions, several studies have examined the interactions of nanoparticles with synthetic and natural lipid bilayers. Holl et al. have been investigating the effect of positively charged dendrimers on lipid bilayers.[53] Their study examined the interaction of PAMAM dendrimers of different generations with supported 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid bilayers and KB and Rat2 cells.[54] It was revealed that the positively charged dendrimers destabilized the cell membranes and were able to either form holes (15–40 nm in diameter by G7 dendrimer) or expand holes at preexisting defects as observed through AFM studies (Figure 6). It was also observed that these holes led to dendrimer internalization into cells, diffusion of cytosolic proteins out of cells, and both intracellular and extracellular diffusion of dye molecules. As a control, acetamide-terminated G5 PAMAM dendrimers failed to form holes at similar concentrations in addition to G3 PAMAM dendrimers that did not show evidence of hole formation. Later studies displayed that while the gel-phase regions of the lipid bilayer were unaffected by cationic dendrimers, the liquid-phase was found to be disrupted by their presence.[55] A model was subsequently developed that accounted for a dependence of size and surface chemistry of the dendrimer on the propensity of pore formation.[56] The model proposed that small lipid-bilayer patches can wrap around dendrimers forming dendrimer-filled vesicles and such assemblies were found to be more stable with larger dendrimers with a high number of polar functional groups. These results have been shown to be consistent across a variety of cationic particles such as poly(L-lysine) (PLL), poly(ethylenimine) (PEI), and diethylaminoethyl-dextran (DEAE-DEX), with PEI bearing the greatest density of charged groups on its surface and displaying the greatest increase in membrane permeability.[57] This was in contrast to PEG or poly(vinyl alcohol) (PVA) polymers that show no such effects. Further studies also demonstrate that hole formation in lipid bilayers by cationic particles is a common property regardless of shape (spherical versus irregular), chemical composition (organic versus inorganic such as gold or silica nanoparticles), deformability (flexible versus rigid), charge density, or size.[19] Pore-forming propensity in membranes by cationic particles has also been confirmed by other groups as well.[58–60]

Efforts have also been pursued to closely examine other aspects of charged particle interactions with lipid bilayers. Roiter et al. have shown in separate studies that l-α-dimyristoyl phosphatidylcholine membranes are capable of forming pores around spherical silica nanoparticles that are between 1.2–22 nm in size, outside which the bilayers tend to envelop the particles.[61] Interestingly, Wang et al. have shown that nanoparticles cause surface reconstruction at the points where they absorb on the lipid bilayers; binding of anionic nanoparticles to a lipid bilayer in the fluid-phase induces a local gelation, while positively charged nanoparticles induce fluidity in otherwise gelled regions of bilayers.[62] Studies focusing on non-endocytotic bulk transport of nanoparticles across lipid bilayers using gold nanoparticles of various sizes (7, 10, and 15 nm in diameter) have indicated that a simple diffusion across lipid bilayers might not be permissible.[63] Vasir et al. have quantified the force between nanoparticles (poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles functionalized with PLL) and cell membranes using AFM.[64] They have shown that this force determines the adhesive interaction of nanoparticles with the cell membrane and consequently the extent of cellular uptake of nanoparticles. These studies collectively indicate further investigation of nanoparticle–cell interactions under controlled conditions with a synergistic combination of characterization tools and relating these studies with in vitro studies might uncover many of the general principles of nanomaterial–cell interactions that are presently unknown.

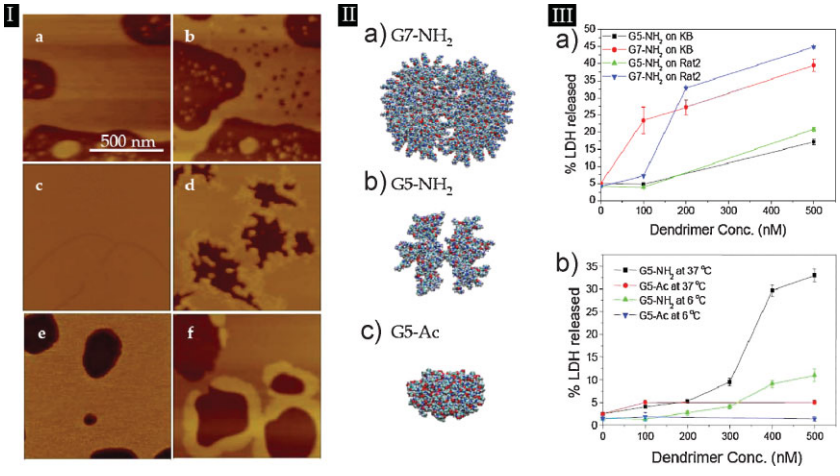

Figure 6. I) Interaction of dendrimers with dimyristoylphosphatidyl choline-supported lipid bilayers. AFM images of lipid bilayers (a,c,e) before and after incubation with b) G7–NH_2, d) G5–NH_2, and f) G5-acetyl (Ac) PAMAM dendrimer. II) Space-fill models of the various dendrimers. III) Graphs displaying leakage of lactate dehydrogenase (LDH) upon incubation with a) G5– and G7–NH_2 dendrimers at 37 °C and b) G5–NH_2 and G5–Ac dendrimers at different temperatures. Reproduced with permission from Reference [53]. Copyright 2007, American Chemical Society.

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5. Effect of Nanoparticle-Surface-Ligand Arrangement on Cell-Membrane Penetration

Next, we examine the role of synthetic ligand arrangement on the nanoparticle surface on particle–cell-membrane interactions. While most studies have reported the effect of surface functional groups/ligands/penetration motifs on the nanoparticle cellular uptake, the impact of ligand arrangement on the nanoparticle surface is seldom examined. Inspired by the natural amphiphilic design of CPPs, our group has examined the cell-penetrating ability of organically coated amphiphilic gold nanoparticles that feature the same size, zeta potential, ligand packing density, and hydrophobic content. The only difference resides in the arrangement of the ligands that coat the gold core.[65] The studies revealed that while nanoparticles coated with these amphiphilic molecules in an ordered ribbon-like alternating arrangement penetrated the cell membrane (at 4 °C and in the presence of an endocytic inhibitor), nanoparticles that were coated with the same molecules but in a random arrangement on the surface were inefficient in breaching cell membrane barriers and were instead trapped in vesicular bodies such as endosomes (Figure 7). Further, the "striped" particles displayed no overt pore formation upon crossing membrane barriers, in a manner reminiscent of some of the CPPs.[14]

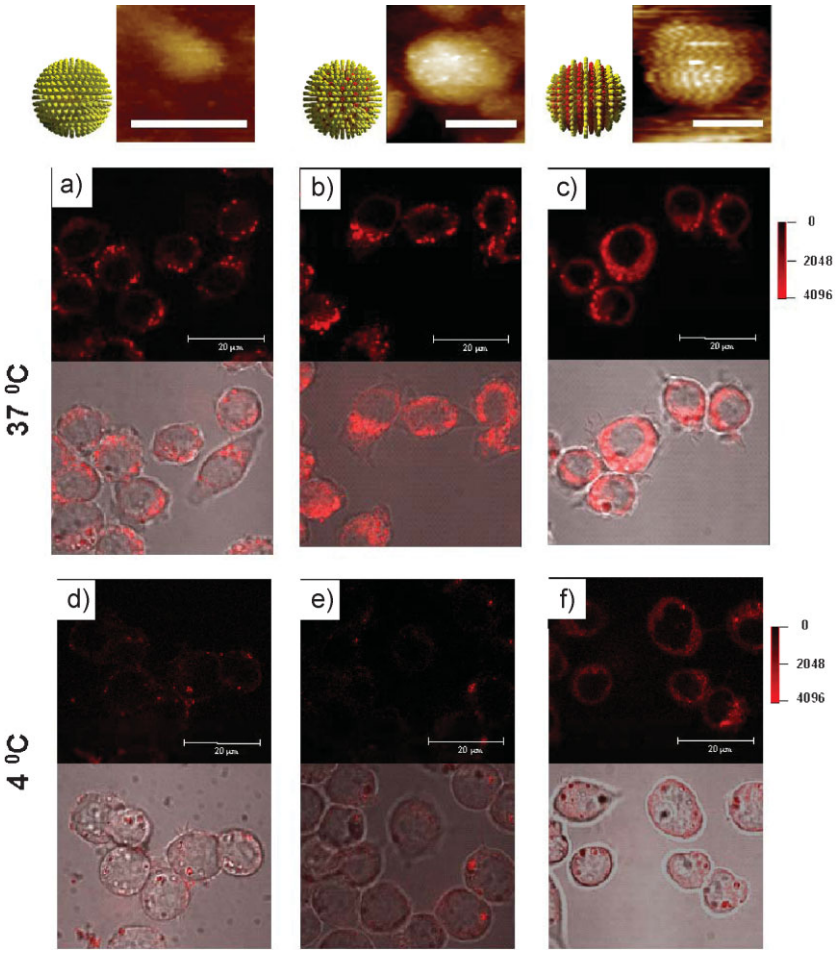

Figure 7. The arrangement of surface ligands on nanoparticles plays a key role in cell membrane penetration. Scanning tunneling microscopy (STM) images of particles with homoligand and unstructured and structured ligand shells (with scale bar 5 nm) are shown. Confocal images of mouse dendritic cells incubated with the nanoparticles at a–c) 37 °C and d–f) 4 °C in serum-free condition.

6. Oligonucleotide-Coated Nanoparticles

The interaction of oligonucleotide-modified nanoparticles with cells is briefly discussed here. In many instances, nanoparticles have been coated with natural biological moieties such as oligonucleotides, peptides, and proteins (as opposed to synthetic organic molecules) and their interaction with cells observed. Recently, the cellular internalization of oligonucleotide-modified gold nanoparticles was examined.[66] It was observed that despite their negative surface coating, the nanoparticles were readily taken up by mouse endothelial cells and were efficient in gene regulation. Initially, this behavior seemed surprising as negatively charged particles are typically poorly internalized in cells. However, further analysis through fluorescence-based assays for protein quantification revealed that the nanoparticles adsorbed serum proteins on the surface through electrostatic and hydrophobic complementarity, which allowed the nanoparticle to interface with the cell membrane (Figure 8).[67] Nanoparticle uptake was found to be dependent on the density of oligonucleotide loading on the nanoparticle

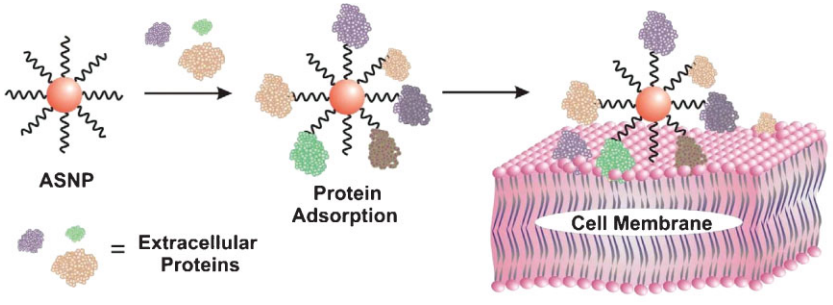

Figure 8. Proteins in the media bind to the antisense oligonucleotide-functionalized gold nanoparticle (ASPN), which allows interfacing of the nanoparticle with the cell membrane and its subsequent internalization. Reproduced with permission from Reference [67]. Copyright 2007, American Chemical Society.

surface, with higher densities providing greater uptake. These studies indicate an important observation that during a typical cell-culture-incubation experiment, the outermost layer of serum adsorbed proteins will be a significant factor in determining the interaction of nanoparticles with cell membranes.

7. Nanoparticle Internalization Assisted by Natural Membrane-Penetrating/-Fusogenic Motifs

The utilization of cell-penetrating or cell-fusogenic biological ligands conjugated to particles in assisting nanomaterials to reach the cytosol or nucleus of cells is discussed here. In addition to oligonucleotides, synthetic nanoparticles have been conjugated to natural cell-membrane-penetrating motifs that can chaperon the cargo inside the cell into the cytosol or nucleus. CPPs, which are short polycationic or amphiphilic peptides, form the most popular cellular transporters of nanoparticles among natural systems.[68] In many studies, CPPs have been conjugated to QDs for intracellular imaging. Medintz et al. functionalized QDs with CPPs to examine intracellular delivery of two fluorescence proteins conjugated to the QDs.[69] However, their results showed that the QD complexes were localized within endolysosomal compartments. To bypass the endosomes, cellular microinjection of QD–fluorescent-protein assemblies was performed. Other studies have utilized CPP derived from the HIV-TAT protein for delivering QDs into cells.[70,71] It is believed that TAT-functionalized QDs enter the cell through macropinocytosis, a fluid-phase endocytosis process triggered by Tat–QD binding to the negatively charged cell membrane.[72] Examining the mechanism of internalization, Ruan et al. have shown the internalized TAT–QDs are retained and tethered to the inner surface of the vesicle and thereafter transported by molecular machines (such as dyneins) along microtubule tracks to an asymmetric perinuclear region known as the microtubule organizing center (MTOC) (Figure 9).[73] In other studies, nuclear-localization-signal (NLS) peptides, which assist the delivery of cargo to the nucleus, have also been harnessed in combination with CPPs for cellular delivery. Nativo et al. have reported a detailed TEM study on the uptake of 16-nm gold nanoparticles into HeLa cells.[74] They have shown that CPP conjugation to the nanoparticles enables a cytosolic delivery of the particles by either passing through the cell membrane or via endosomal escape. Further, it has been shown that an appropriate combination of CPP and NLS can mediate the nuclear entry of nanoparticles. TAT and NLS have also been utilized to deliver antisense oligonucleotides (conjugated to gold nanoparticles) and pegylated nanoparticles into cells.[75] It has also been shown that the shape of nanoparticles that are conjugated to TAT also dictate their cellular uptake. In this study, cellular internalization studies of spherical (≈10 nm) and cylindrical amphiphilic block-copolymer micelles (20–30 nm in diameter and >200 nm in length) functionalized with TAT displayed that the smaller spherical particles were more rapidly internalized than the larger cylindrical particles and also that the rate of release of the smaller particles from the cell was

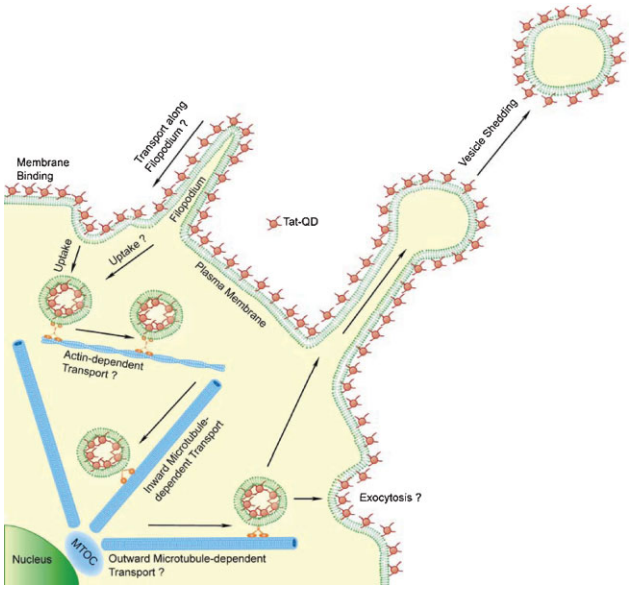

Figure 9. Schematic illustration of internalization and transport of TAT-functionalized QDs. TAT–QD internalizes through macropinocytosis after which it is actively transported to the MTOC. Reproduced with permission from Reference [73]. Copyright 2007, American Chemical Society.

proportional to the TAT loading.[76] In addition to TAT, several other peptide motifs such as the Arg–Gly–Asp (RGD) motif,[77] allatostatin 1 (an insect neuropeptide),[78] PLL,[79] and arginine-rich peptides[80] have been shown as effective mediators of nanomaterial internalization into various cell types. In another study, Deniger et al. have mimicked one of the steps in a viral infection that is mediated by interactions between specific virus-envelope proteins with their cell-bound receptors.[81] Here, fluorescent nanoparticles were coated with membranes derived from moloney murine leukemia virus (Mo-MLV) and virus proteins on these membranes were harnessed to specifically target the nanoparticles to receptor-bearing cells and a subsequent internalization into the cell cytosol was also observed. The cellular uptake of nanoparticles coated with model proteins such as transferrin into various cell lines is also reported.[28] It was seen that the transferrin-coated nanoparticles enter cells via a clathrin-mediated endocytosis pathway; their entry and exocytosis are dependent on nanoparticle size. These studies display that cell-penetrating/-fusogenic biomolecules retain their properties upon conjugation with nanoparticles and are effective intracellular transporters of nanomaterials. Many such biological motifs seem to have positively charged residues (assisted by hydrophobic residues) for efficient cellular internalization that correlates very well with the behavior of positively charged organically functionalized nanomaterials.[82] However, some biological cell-penetrating motifs also have an amphipathic surface structure (hydrophilic residues alternating with hydrophobic ones) that assists them in penetrating or fusing with cell membranes in a ''stealthy'' manner. This special structural arrangement of organically functionalized molecules on a nanoparticle, however, is very difficult to achieve and control.

NANO MICRO small

8. Summary and Outlook

The surface-chemical properties of nanoparticles play a crucial role in their interaction with cells. Given the numerous opportunities nanoparticles hold for bioapplications, a systematic understanding of predictive nanoparticle–cell interaction has become increasingly important. This report is aimed at examining our current understanding of how various chemical modalities (synthetic and natural) on nanoparticle surfaces impact their behavior with cells. Most nanoparticles are internalized through endocytosis and remain trapped in endolysosomal vesicles unless co-internalized with a membrane-disrupting agent. Nanoparticles with neutral surface coatings, such as PEG, resist interaction with cells and consequently display minimal internalization, if none at all. In pegylated nanoparticles, the molecular architecture of PEG on the nanoparticle surface is a key determinant of nanoparticle–cell interactions. Studies have also reported the uptake of negatively charged nanoparticle into cells, despite their interaction with the negatively charged membrane. Positively charged nanoparticles, however, are most effective in crossing cell-membrane barriers and localizing in the cytosol or nucleus. Studies examining the interaction of positively charged nanoparticles have shown that regardless of the type of particle, cationic particles penetrate cell membranes, which may contribute to the observed cytotoxicity with such particles. Other efficient nanoparticle–cell transporters include nanoparticles featuring natural cell-penetrating motifs conjugated to the nanoparticle surface. Among these, HIV-TAT, NLS peptides, arginine-rich peptides, and virus proteins have been effectively utilized to breach cell-membrane barriers. Nanoparticle interaction with cells is not only dependent on the subtle structural changes in the surface ligands but also on how the ligands are spatially arranged on the nanoparticle surface.

While numerous studies have examined the nanoparticle–cell interactions, we have just begun our understanding of the role of surface properties of nanomaterials in such interactions. One of the primary challenges in this area is the reproducible control over nanoparticle-surface properties. Another important issue is that results across various studies cannot be normalized as differences in the media solubility of nanomaterials and in the experimental conditions are never considered. In the cell-incubating conditions, nanoparticles can aggregate to different sizes, which may influence results and efficacy of the nanomaterial. Additionally, the varying response of different cell types, the nonspecific serum-protein adsorption on the nanoparticle surface, and the varying experimental conditions utilized also make such a systematic study a challenging one. However, given the advent and the potential benefits of nanobiotechnology, this would be a worthwhile goal.

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Keywords:

cell penetration · drug delivery · nanobiotechnology · nanomaterials · nanoparticles

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