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Review Paper

Optimization of Bio-Nano Interface Using Gold Nanostructures as a Model Nanoparticle System

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Abstract: Better knowledge of interface between nanotechnology and biology will lead to advanced biomedical tools for imaging and therapeutics. In this review, recent progress in the understanding of how size, shape, and surface properties of nanoparticles (NPs) affect intracellular uptake, transport, and processing of NPs will be discussed. Gold NPs are used as a model system in this regard since their size, shape, and surface properties can be easily manipulated. Recent experimental and theoretical studies have shown that NP-uptake is dependent upon size and shape of the NPs. Within the size range of 2-100 nm, Gold nanoparticles (GNPs) of diameter 50 nm demonstrate the highest uptake. Cellular uptake studies of rod-shaped gold nanoparticles (GNRs) show that there is a decrease in uptake as the aspect ratio of GNRs increases. The surface ligand and charge of NPs play an important role in their uptake process as well. Different proteins on the surface of the NPs can be coated for effective targeting of NPs into specific organelles. Once in the cell, most of the NPs are trafficked via an endo-lysosomal path followed by a receptor mediated endocytosis process at the cell membrane. Exocytosis of NPs is also dependent on the size and shape of the NPs, however, the trend was different to endocytosis process. These findings provide useful information to tailor nano-scale devices at single cell level for effective applications in diagnosis, therapeutics, and imaging.

Keywords: gold nanoparticles; nano-bio interface; size; shape; surface properties; endocytosis; exocytosis

1. Introduction

Recent progress in the use of nanotechnology for biomedical research has led to the development of novel materials called "nanoparticles (NPs)" for various applications. These new nanoscale biomedical platforms include quantum dots, nanoshells, gold NPs (GNPs), paramagnetic NPs, and carbon nanotubes. The potential to manipulate structures at nanometer scale offers the possibility to explore and better understanding of the interface between NPs and living systems (nano-bio interface), such as cells. As a step forward in this direction, several efforts have been made to optimize this bio-nano interface using GNPs as a model system since their size, shape, and surface properties can be manipulated easily [1]. For example, the size and shape of GNPs can be tailored to range between 2-100 nm and their surface functionalization allows targeting to specific biological structures within the cell [2-8]. In addition, the ability to incorporate GNPs into either polymer- or lipid-based NPs, such as, liposomes, micelles or dendrimers has increased the application scope of these NPs [9-13]. There have been a number of studies investigating the potential cytotoxic effects of these NPs [14, 15]. The biocompatibility of these NPs have motivated interest in employing gold nanostructures in cell imaging, targeted drug and gene delivery, and biosensing [1, 14, 16-25]. In order to promote the use of NPs in the above biomedical applications, it is also important to discuss possible cell uptake mechanisms of NPs.

Figure 1. Schematic explaining the optimization of interface between biology and nanotechnology by tailoring the physiochemical properties (size, shape, and surface properties) of NPs. This would lead to optimized responses between a single cell (fundamental unit in biology) and NPs.

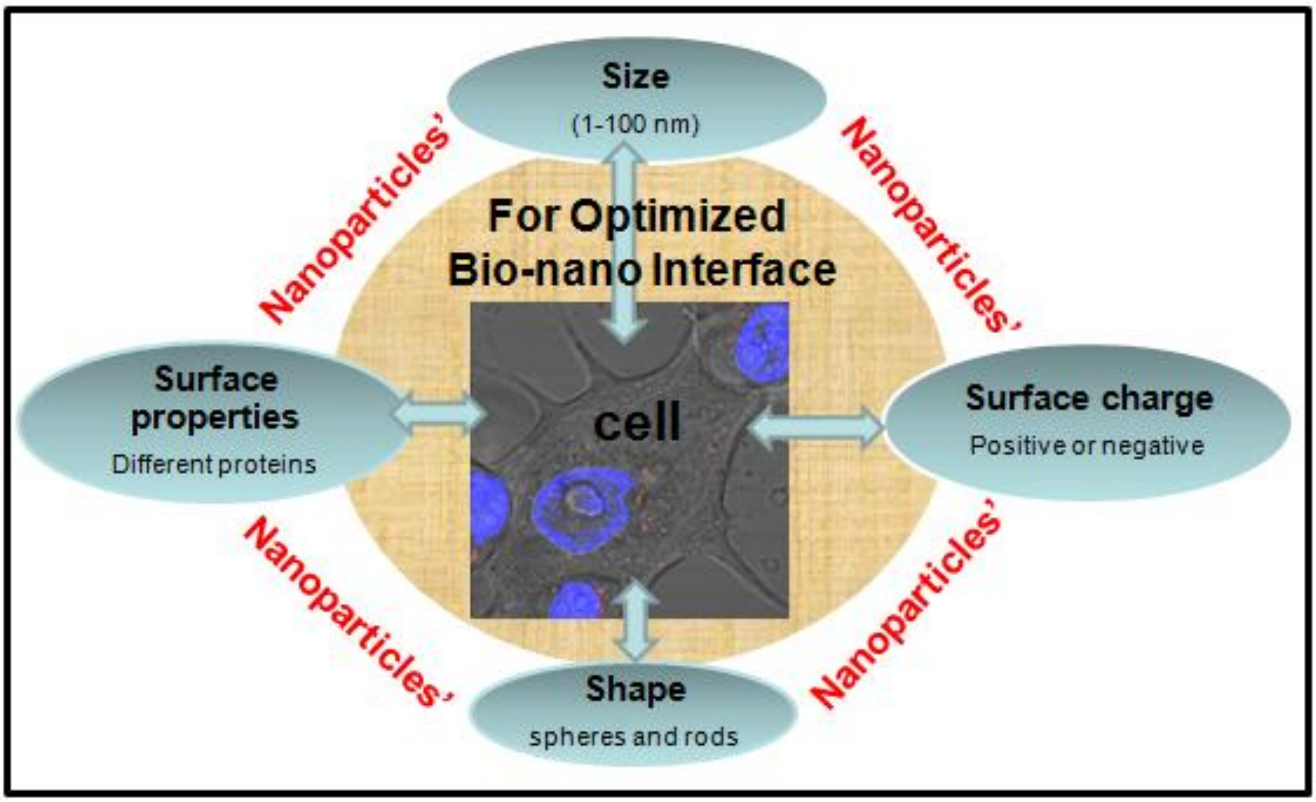
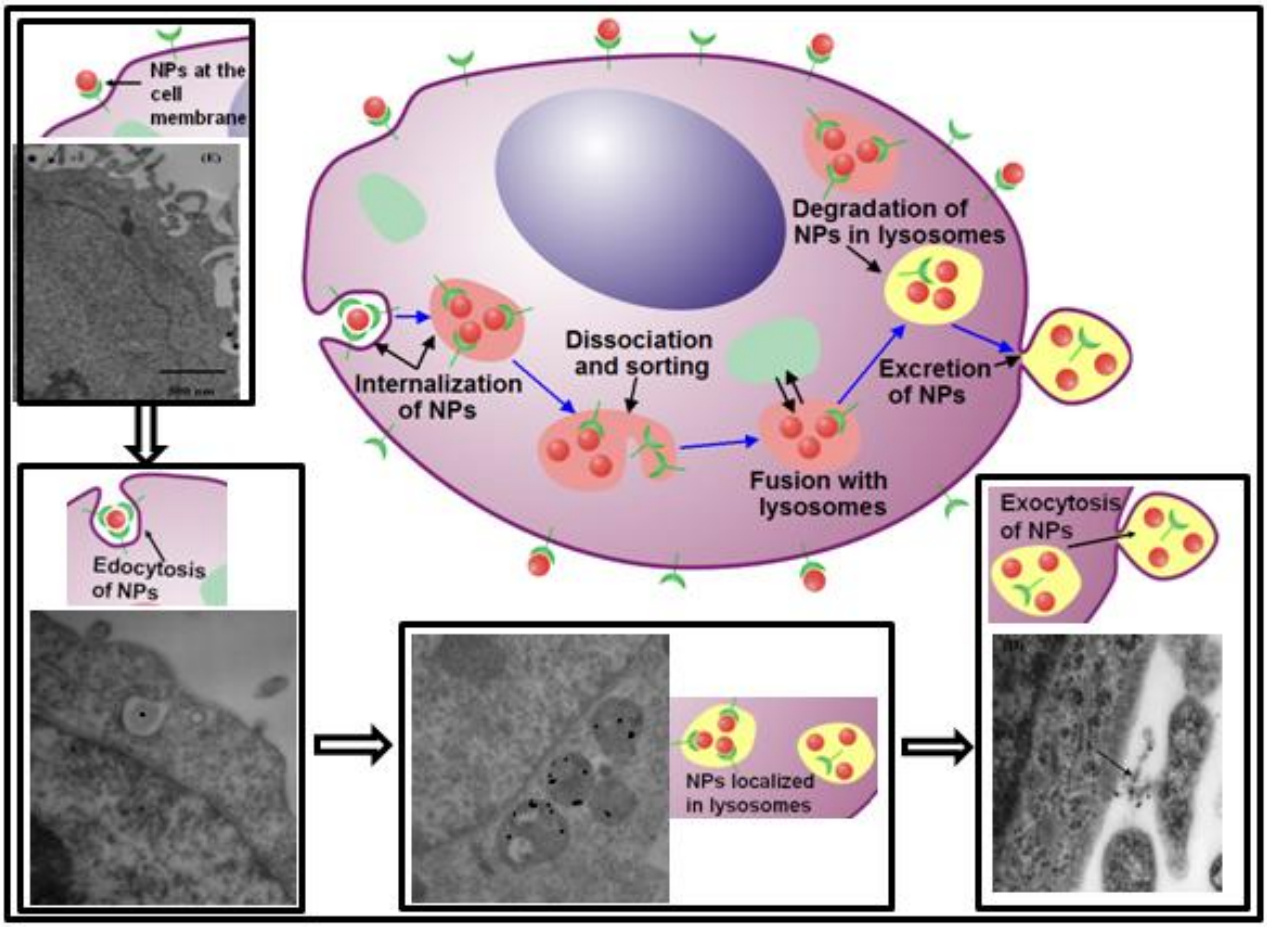


Figure 2. Schematic describing intracellular fate of NPs. NPs are internalized by endocytosis process and trapped in endosomes. These endosomes fuse with lysosomes for processing. Finally they are transported to the cell periphery for excretion. TEM images capturing different stages of NP transport through the cell are also shown for each different process involved. Reproduced with permission [26, 27].



Nanoparticles can be internalized by one or more of the following mechanisms: Phagocytosis, pinicytosis, macropinocytosis, and clathrin- and caveolin-mediated endocytosos [28-33]. The mechanism of uptake can be dependent on many factors, such as, the physiochemical properties of NPs (size and surface properties) and cell type. For example, Rejman *et al*. showed that the particles of sizes between 50 to 200 nm were taken up primarily by clathrin-mediated endocytosis, while particles of size 500 nm and above were taken up in a caveolin-dependent fashion [34]. However, Clathrin-mediated endocytosis is the predominant mechanisms involved in non-macrophage cell uptake of NPs [35]. Clathrin-mediated endocytosis occurs when NPs accumulate on the cell membrane and clathrin-coated pits are formed to transport the NPs into th cell, resulting in the formation of endosomes. The process by which clathrin-coated vesicles are produced involves interactions of multifunctional adaptor proteins with the plasma membrane, as well as with clathrin and several accessory proteins and phosphoinositides. Macropinocytosis is another possible NP-uptake mechanism. For example, macropinocytosis is expected to be responsible for uptake of pegylated poly-lysine-compacted DNA NPs [32]. According to the study by Walsh *et al*., the distribution of the NP-DNA complex did not

overlap with that of receptor-mediated endocytosis (a subset of clathrin-mediated endocytosis) marker or with late endosomal-marker [32]. When cells were incubated with amiloride, an inhibitor of macropinocytosis, intracellular fluorescent rhodamine was significantly reduced indicating that NP uptake could be via macropinocytosis process [32]. In this article, however, optimization of interface between NPs and cell surface is discussed for particles internalized via receptor-mediated endocytosis (RME) (a subset of clathrin-mediated endocytosis).

Optimization of NP-uptake at single cell level would lead to better outcome in previously mentioned applications. In this review, recent progress in NP-based research work towards understanding of how size, shape, and surface properties of NPs affect their intracellular behavior will be discussed. This type of basic research will be a step forward in achieving goals towards optimization of NP-uptake at a single cell level. Recent studies have identified that the size, shape, and surface properties are important factors in cellular uptake of NPs (see Figure 1). Figure 2 is a schematic diagram that highlights some of the important cellular processes involving NPs. The NPs are first internalized by cells through endocytosis process and trapped in small vesicles called 'endosomes'[37]. Endocytosis is one of the major pathways for cellular uptake of NPs [26, 27, 38]. Particularly for GNPs, the internalization mechanism is confirmed to be receptor-mediated endocytosis (RME) [26, 39-42]. Cellular uptake of these NPs is decreased at low temperature (4°C) and in ATP (adenosine try phosphate)-depleted environments (cells pretreated with NaN_3) indicating that Au NPs enter cells via RME [37, 39, 43, 44]. After internalization through RME, these endosomes then fuse with lysosomes for processing before being transported to the cell periphery for excretion. These different stages of NP transport through the cell captured by TEM are shown beside the schematic cell diagram. In the first part of the review the current knowledge about how size, shape, and surface properties affect cellular uptake of NPs is discussed. In the second section, the current understanding of the transport properties of NPs within the cell cytoplasm will be discussed. In the final section, size and shape dependent exocytosis process of NPs will be reviewed. Understanding of the intracellular behavior of NPs will have implications in the engineering of nanostructures for applications in drug delivery, cellular imaging, and nano-modulated therapeutics.

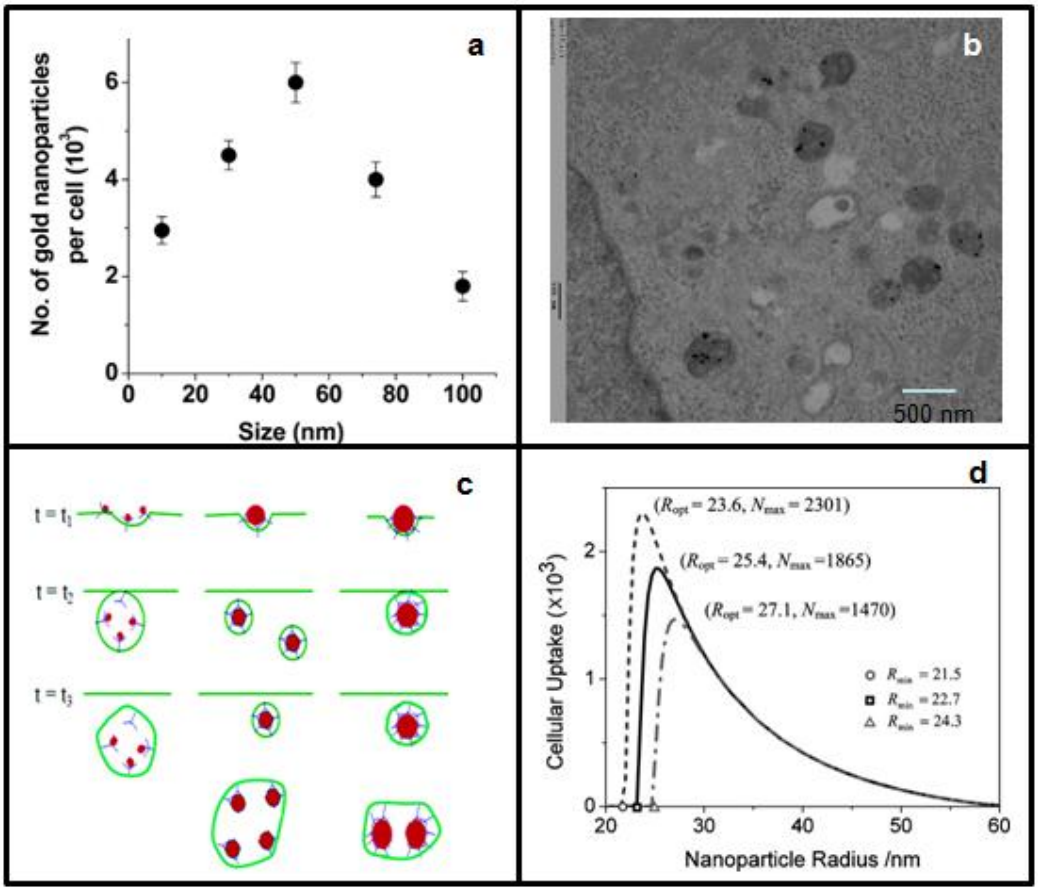
2. Cellular uptake of nanoparticles

2.1. Size dependent nanoparticle cell uptake

Recent research work shows that size of the NP can play an important role in their uptake process. For example, it was shown that NPs with diameter ~ 50 nm exhibited significantly higher uptake compared to smaller (14-30 nm) or larger NPs (74-100 nm) (see Figure 3a) [26, 27, 36, 37, 45-48]. This size dependent uptake is supported by few theoretical models using arguments based on thermodynamics and kinetics as discussed in the next section. Once internalized, these NPs can be visualized using optical microscopy techniques and fixed cell microscopy, such as transmission electron microscopy (TEM). A transmission electron microscopy (TEM) image of a small section of a cell with internalized NPs is shown in Figure 3b. Similar size-dependent NP-uptake was seen for silver NPs as well [49]. These GNPs were internalized via RME as discussed in the previous section.

Aoyama and coworkers have also demonstrated that the RME is strongly size-dependent and the optimum NP-diameter for uptake is ~ 50 nm [47, 48, 50].

Figure 3. Size-dependent cell uptake. **(a)** Variation of cellular uptake of NPs as a function of size. **(b)** TEM image of a fixed cell showing NPs trapped in small vesicles of size ~ 500 nm. **(c)** Model diagram explaining the size-dependent uptake of NPs. **(d)** Suresh and co-workers model explaining how many NPs can be internalized based on their size. Reproduced with permission [26, 36].



Several theoretical models have been established to provide insights into the size dependent uptake of NPs [45, 46, 51]. According to Gao *et al*., optimal particle size is a result of competition between thermodynamic driving forces and receptor diffusion kinetics [46]. For particles smaller than the optimal size, increased elastic energy associated with bending of the membrane results in decreased driving force for membrane wrapping of the particle. Hence, smaller particles need to flock together to create enough driving for uptake as illustrated in Figure 3c. Recently, Strano and co-workers put forward a theoretical model to address this issue [42]. According to the model, it was confirmed that a surface clustering on the external cellular membrane facilitates RME by lowering the otherwise prohibitive thermodynamic barrier for smaller NPs [37, 42]. According to Gao's model, larger particles accommodate many receptors leaving areas with less receptors, which requires diffusion of receptors over a longer distance and this has led to lower uptake of larger NPs (see Figure 3c). Gao's

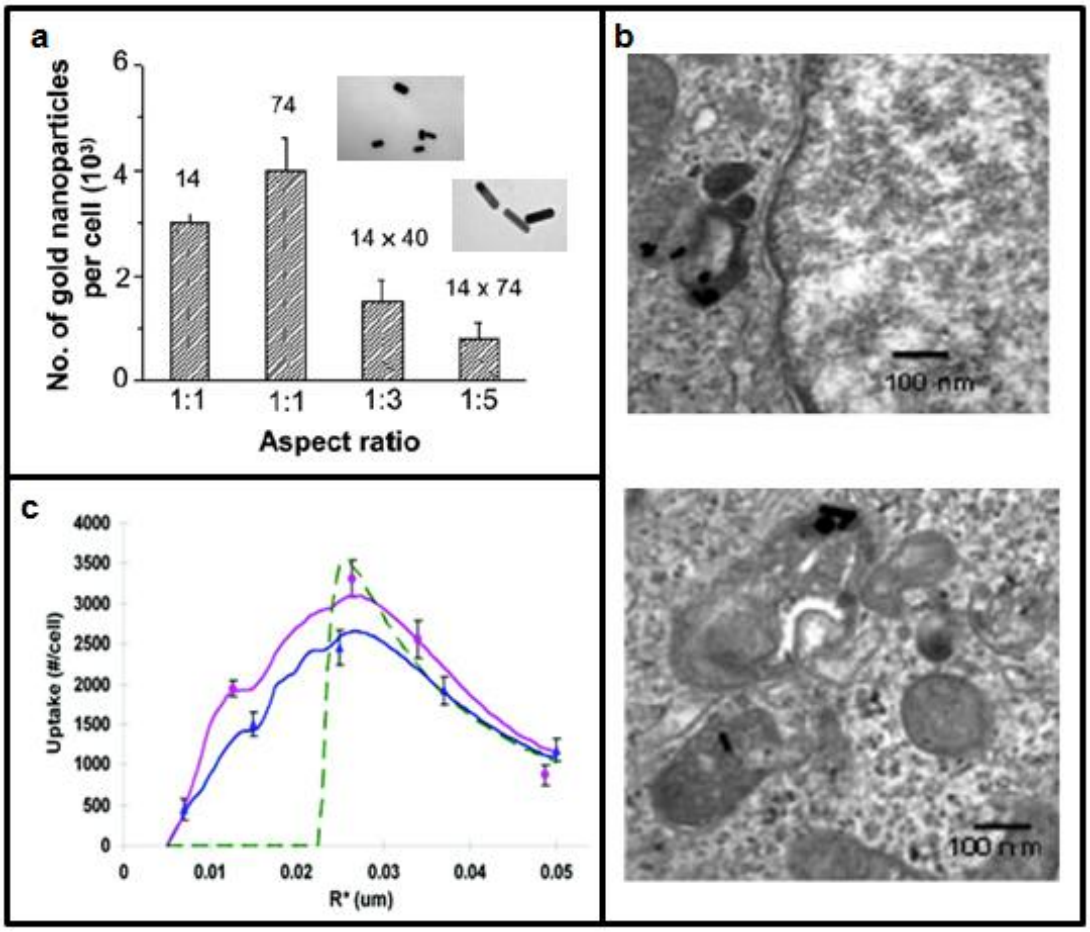
model elucidates the mechanism of size-dependent cellular uptake of NPs from a kinetic point of view. However, Zhang *et al*. went one step further and have addressed the question of "how many" NPs can be endocytosed in a sufficiently long period of time using thermodynamic arguments (see Figure 3d) [51]. The extracellular NPs can reversibly adsorb onto the membrane. Adsorbed NPs diffuse on the cell membrane surface and form clusters with a radius of sufficient size that eventually satisfy the thermodynamic requirement for endocytosis. This model is a very plausible explanation of experimentally observed size-dependent uptake of NPs (see Figure 3d) [42].

2.2. Shape dependent nanoparticle cell uptake

The cellular uptake of NPs is dependent upon shape as well [26]. The uptake of shorter nanorods (NRs) is higher than longer NRs (see Figure 4a) [26, 42]. In addition, the uptake of rod-shaped NPs is lower than their spherical counterparts. One reason could be the difference in the curvature of the different-shaped NPs. For example, the rod-shaped NPs can have larger contact area with the cell membrane receptors than the spherical NPs when the longitudinal axis of the rods interacts with the receptors. This could reduce the number of available receptor sites for binding. In the case of GNRs, it could be due to the amount of CTAB (Cetyl trimethylammonium bromide) surfactant molecules adsorbed onto the surface during synthesis. If the surfactant is still on the surface, the receptor targeting protein molecules may not be able to bind onto the GNR surface efficiently. Also, the protein coating on the surface of the GNRs may not be homogeneous. In such a case, the proteins on the surface of the GNRs may not bind to receptors on the cell surface as strongly (due to a lack of multivalent binding). TEM images of fixed cells with internalized GNRs of dimension 14 nm x 40 nm and 14 nm x 74 nm are shown in Figure 4b.

Gao *et al.* have put forward a model to explain uptake of cylindrical shaped NPs [46]. However, it was difficult to explain endocytic uptake of NRs or carbon nanotubes using this model since the radius of NRs and single walled nanotubes (SWNTs) is much smaller than the critical radius needed for energetically favorable uptake. Recently, Strano and co-workers have used Gao's model, but introduced an effective scaling metric called "capture radius, $R^* = a / \ln(2a/b)$", where a and b are the major and minor axes of the cylinder) to explain the uptake of cylindrical shaped NPs [42, 52, 53]. This model can be used to explain the trend in uptake of not only GNRs, but also nanotubes (see Figure 4c). According to the experimental data showed in Figure 4a, there is higher uptake for shorter nanorods (14 nm x 40 nm) than for longer nanorods (14 nm x 74 nm). In these two cases, the capture radius (R^*) for the shorter and longer NRs are 23 and 31 nm, respectively. According to Figure 4d, cylindrical-shaped NPs with a capture radius of 23 nm have a higher uptake compared to those with a capture radius of 31 nm. Hence, the results are consistent with the theoretical model put forward by Strano and co-workers. In addition, they have developed a quantitative model capable of relating the RME rate of spherical and rod-shaped NPs to their geometry and predict important aspects of their trafficking dynamics [42]. The dependence of cell uptake properties of rod-shaped NPs has been explored using other NP-systems such as carbon nanotubes and the results are consistent with the gold nanorod studies (see Figure 5D) [26, 37, 39, 54].

Figure 4. Shape-dependent cell uptake. **(a)** Variation of cellular uptake of NPs as a function of shape. **(b)** TEM image of a fixed cell showing gold nanorods trapped in vesicles of size ~ 500 nm. **(c)** Strano and co-workers' model explaining the size and shape dependent cell uptake of NPs (spherical shaped NPs- blue; cylindrical shaped NPs- pink; comparison with the model put forward by Freund and co-workers (green)). Reproduced with permission [26, 42].



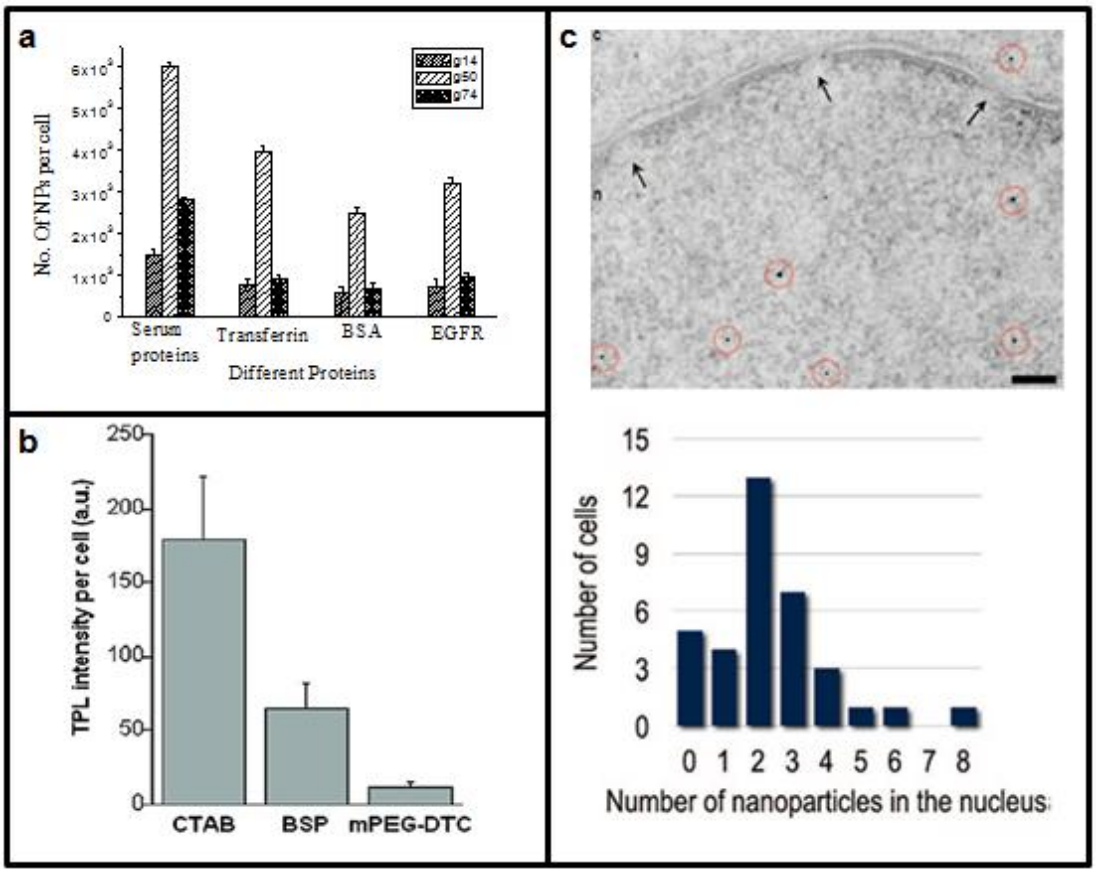
2.3. Surface properties dependent nanoparticle cell uptake

The nature of this outer protein layer is considered to be one of the most important features in determining the NP-cell interaction. The effect of surface properties on cell uptake has been explored and the uptake of NPs coated with proteins such as transferrin, bovine serum albumin (BSA), and epidermal growth factor (EGF) was found to have a similar trend to those coated with serum protein, with a maximum uptake for NPs with diameter 50 nm (see Figure 5a) [27, 37]. However, the uptake of protein-coated NPs is less than for serum protein-coated ones. The result is consistent since, for example, transferrin represents only one kind of protein in cell media and cells display multiple receptor types, diminishing the surface density of transferrin-specific receptors. As a result, transferrin-receptors are quickly saturated by the transferrin-coated NPs while for serum-coated NPs, the surface density of usable receptors is much larger, since many kinds of receptors are available. For untreated GNPs, the uptake mechanism arises from the adhesion of media proteins to the surfaces of NPs during a typical cell culture incubation experiment, as originally discussed by Lynch and co-workers [55].

The dependence of rod-shaped NP uptake on surface properties is similar to that of spherical-shaped NPs. Surface coated NRs showed lower uptake in comparison to untreated NRs as illustrated in Figure 5b [54].

Surface of the NPs can be designed for targeted nuclear delivery for specific applications, such as drug and gene delivery. It is necessary to bypass or escape endosomal/ lysosomal pathways for nuclear delivery. One common approach to targeted nuclear delivery is the conjugation of drug molecules and NPs to nuclear targeting peptides [4, 7, 56, 57]. GNPs are being used in this regard due to their small size, ease of preparation, strong absorbing and scattering properties, as well as their biocompatibility [7, 15, 58-60]. Tkachenko *et al.* have conjugated synthetic cellular targeting peptides to nanometer-sized spherical GNPs through bovine serum albumin (BSA) protein and demonstrated the nuclear delivery of NPs by using video-enhanced color differential interference contrast microscopy. El-sayed and coworkers have conjugated peptides directly onto GNRs for nuclear targeting [7].

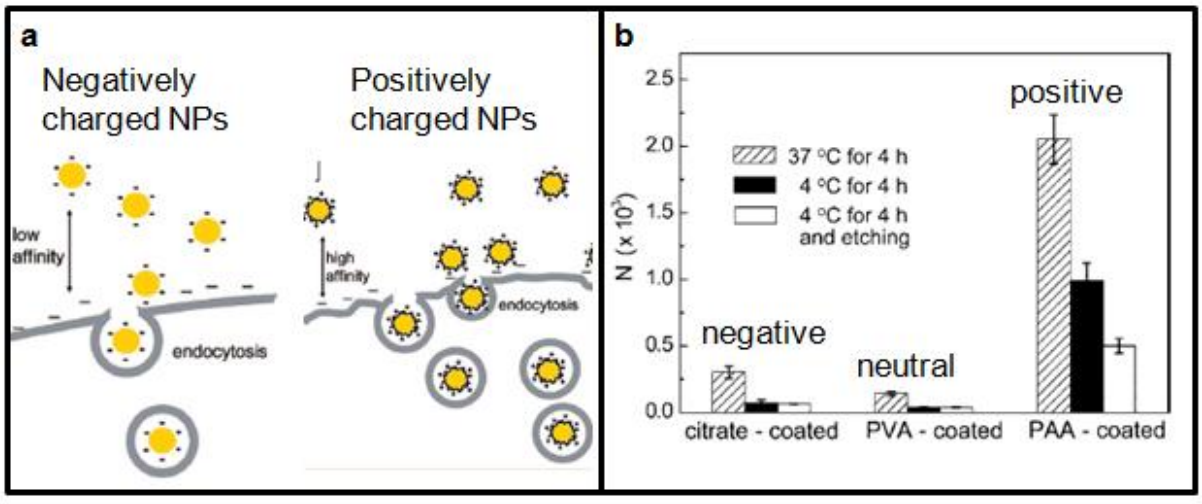
Figure 5. Dependence of cellular uptake of NPs as a function of surface properties. **(a-b)** Variation of cell uptake for spherical NPs and rod-shaped NPs with different surface ligands, respectively. **(c)** Nuclear targeting of NPs by conjugating specific nuclear targeting peptides. Reproduced with permission [5, 27, 54].



Most of the NPs discussed so far were internalized through an endocytosis process. Brust and co-workers have shown that the endosomal pathway of these peptide- GNP complexes can be avoided significantly by appropriate modification of the particles with so-called cell penetrating peptides (CPPs) to cross the barriers of intact cells [5]. TEM images of cells with GNPs localized in the nucleus as well as in the cytoplasm are shown in Figure 5c. The size of the NPs plays an important role in nuclear targeting as well and the diameter of the NPs has to be less than 30 nm for import through the nuclear pore complex [6]. However, more studies are needed to fully understand the mechanisms of cell uptake and intracellular fate of NPs conjugated for nuclear delivery.

Recent studies have shown that the cellular uptake of GNPs was significantly affected by the surface charge of the NPs as well [61, 62]. The uptake efficiency of the positively charged NPs was greater than that of the neutral and negatively charged NPs (Figure 6). According to Cho *et al*., the uptake process occurs in two steps: adsorption onto the membrane of the cell and internalization by the cell [62]. As explained in inset Figure 6a, positively charged NPs should adhere to the negatively charged cell membranes and facilitate the higher uptake into cells. Interactions with some surface molecules on cell membranes may be responsible for the facilitated uptake of negatively charged NPs. Consistent with the previous discussion, several groups have found that positively charged GNRs exhibited higher cellular uptake than negatively charged ones [63, 64].

Figure 6. Dependence of cellular uptake of NPs as a function of their charge. Reproduced with permission [62].



It is also important to mention that surface charge and size of the NP can also play a major role in protein conjugation onto NP surfaces [65-67]. According to these studies, denaturation of proteins occurs when NPs have either positively or negatively charged ligands, whereas they are not denatured at all when linked to NPs with neutral ligands [66]. NP size can also affect the protein structure and activity. For larger NPs, the effective surface area it can access is larger increasing the likelihood of denaturing the protein. For smaller NPs, less denaturation occurs due to less surface area and fewer number of ligands that can interact with the protein [66]. Size and surface characteristics of NPs could

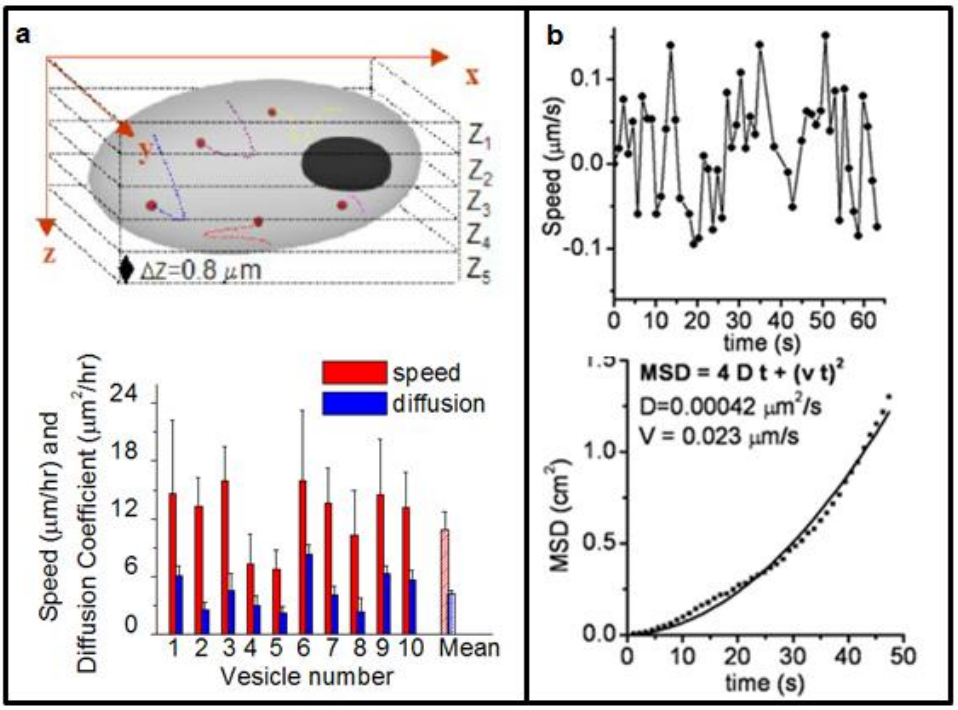
also affect the way proteins bind to these particles, and this in turn influences the way in which NPs interact with cells and tissues. For example, NPs bound with specific proteins can result in activation of certain signaling pathways. A recent study by Deng *et al.* showed that negatively charged poly(acrylic acid)- conjugated GNPs bind to and induce unfolding of fibrinogen, which promotes interaction with the integrin receptor, Mac-1 [68]. Activation of this receptor increases the NF-kB signaling pathway, resulting in the release of inflammatory cytokines. However, not all NPs that bind to fibrinogen demonstrated this effect. Their results show that the binding of certain NPs to fibrinogen in plasma offers an alternative mechanism to the more commonly described role of oxidative stress in the inflammatory response to nanomaterials. According to Deng *et al.*, size of the NPs plays a big role in their interactions with the cell surface receptors. For example, smaller NPs (5 nm) bound to protein interacted better with the Mac-1 receptor while the larger NPs (20 nm) did not promote cell interaction to the same extent when adjusted for similar protein binding. Based on these findings, it is very important to consider size, shape, and surface properties of NPs when designing NP-vectors for their applications in biomedical field.

3. Intracellular Transport of NPs

Transport modes of all intracellular complexes can be divided into diffusive, subdiffusive, and active transport [69]. Most of the studies have so far focused on studying transport of viral and non-viral gene carriers. It has been revealed that these gene carriers are actively transported by motor proteins along microtubules [69-72]. The average velocity of actively transported gene vectors was 0.2 μm/s, which is on the same order of magnitude as movement along microtubules involving motor proteins such as dyneins and kinesins [70, 72, 73]. However, 85% of the viral and non-viral gene vectors were transported slowly and nonactively through either diffusive or subdiffusive transport modes. Recently, the transport of gold, dendrimer, polymer, carbon nanotubes and liposome NPs have been investigated providing new insight into the NP behavior in complex biological environments [27, 74-78].

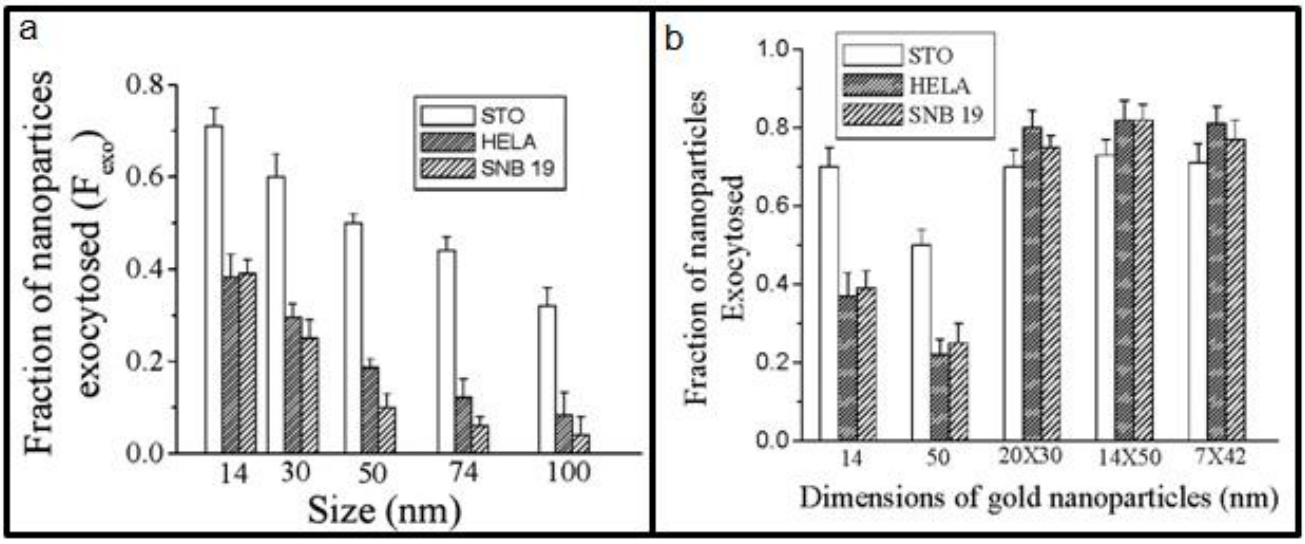
Using fluorescence confocal microscopy, transport of colloidal gold NPs was studied by Chithrani *et al.* [27]. The transport of GNPs of size 50 nm within a single cell is illustrated in Figure 7. Schematic in Figure 7a (top panel) is a model diagram showing a single cell placed inside a 3D box with positive X, Y, and Z directions labeled with reference to the image frame. Paths of NP-vesicles were tracked by imaging the same field of view (XY plane) across several Z-planes along the body of the cell as a function of time. Figure 6a (bottom panel) shows the average speed and diffusion coefficients of the vesicles containing NPs. The ensemble average speed and diffusion coefficient of the NPs were 10.83 (± 1.98) μm/hr and 0.0012 (± 0.0003) $\mu m^2/s$, respectively. The slower transport of the vesicles containing NPs may be attributed to the following: a) the crowded nature of the cytoplasm, b) lower temperature, and most importantly c) the presence of multiple particles within the vesicles instead of a single NP. Similar transport properties have been reported for polymer and liposome NPs with diameters 43 and 80 nm, respectively [74, 77].

Figure 7. Transport of NPs within a single cell. **(a)** Transport properties of spherical GNPs. Top panel shows a model diagram of a single cell placed inside a 3D box with positive X, Y, and Z directions labeled with reference to the image frame. Bottom panel shows average speed and diffusion coefficients colloidal GNPs. **(b)** Transport properties of GNRs. Top panel shows NR velocity over a 60-s period. Positive values indicate motion in the direction of the cell nucleus; negative values indicate motion toward the cell membrane. Bottom panel shows mean-squared displacement of NR signal. Reproduced with permission [27, 54].



Recently, the transport of cylindrical shaped GNRs was studied by Wei and co-workers using a scanning two photon laser microscope (see Figure 7b) [54]. The GNRs clearly exhibited bidirectional motion over a 60-s interval, traveling alternately in the direction of the nucleus or towards the cell membrane. The mean-squared displacement of the GNRs contains a quadratic time-dependent term consistent with directed motion (Figure 7b) leading to the belief that these NPs are transported actively through the cell cytoplasm [69]. However, the average velocity and diffusion rate was low in contrast to actively transported viral and non viral gene vectors [71]. NRs displayed a velocity of ~ 80 μm/hr towards the nucleus and a diffusion rate of 0.0004 $\mu m^2/s$. These GNRs took the endo-lyso path and transport of the GNRs through vesicles is likely (similar to the transport of colloidal GNPs) [79]. In addition, it has also been reported that the transport of NRs is directed along microtubules similar to the transport of other organelles such as endosomes and lysosomes in the cytoplasm [54, 80-82].

Figure 8. Size and shape dependent exocytosis process of gold NPs. (a-b) Size dependence of exocytosis of colloidal GNPs and GNRs, respectively. Reproduced with permission [37].



The distinct values obtained for the velocity and diffusion coefficients for the GNRs, in comparison to the spherical gold colloids, may be attributed to the differences in size, shape and surface properties [37, 83]. These NPs are internalized through endocytosis process and localized in either endosomes or lysosomes. It is also known that these organelles move along microtubules and hence, we believe that endosomes and lysosomes carrying GNPs also travel along microtubules [81, 82]. Recent studies on transport of lysosomes show diffusion coefficient values comparable to the ones observed for colloidal GNPs and GNRs [81]. In addition, transport of dendrimer NPs, polymer NPs, liposomes, cellular components such as secretory granules, and 80% of viral and non-viral gene carriers displayed diffusion coefficients comparable to that of GNPs. So far we have reviewed the recent progress made towards understanding of cellular uptake and transport of Au NPs. However, it is interesting to look into their excretion or removal process.

The basic biological processing of the vesicles containing NPs is outlined in Figure 2. The NPs are first taken up by cells through RME and trapped in endosomes [84]. These endosomes then fuse with lysosomes for processing before being transported to the cell periphery for excretion. Intuitively, it is not surprising that the GNPs travel to lysosomes as they are the final degrading organelles of the endocytic pathway [85, 86]. Exocytosis of NPs was studied quantitatively and qualitatively by several research groups. The NP uptake increased with incubation time in the presence of NPs in the medium; however, once the extracellular NP concentration gradient was removed, exocytosis of NPs occurred with about 65% of the internalized fraction undergoing exocytosis in 30 to 40 minutes [37, 87, 88]. The exocytosis process was dependent on the size and shape of the NPs; however, it yielded a different trend as compared to the endocytosis process (see Figure 8) [37, 87]. Smaller colloidal GNPs appeared to exocytose at a faster rate and at a higher percentage than large NPs. Less number of proteins and receptors on smaller NPs could facilitate faster processing leading to higher rate of exocytosis compared to larger NPs where number of proteins and receptors attached could be higher. According to these results, both uptake and removal of NPs were highly dependent upon the size of the NPs but

the trend is different compared to endocytosis process. The exocytosis process is dependent on shape as well. There is a remarkable difference between the percentages of exocytosis of nanorods versus spherical-shaped NPs. Rod-shaped NPs are exocytosed faster than colloidal Au NPs. Fast processing and excretion of GNRs can be attributed to the fewer number of proteins and receptors on their surface. Based on these studies, it can be concluded that the exocytosis of NPs is also dependent on the size and shape of the NPs. However, in comparison to endocytosis process, the trend is different. The exocytosis process is fairly active following the removal of gold NPs from the external media and these results are consistent with the other NP systems as well [37, 87-89]. Recent studies suggest that the proteins on the surface of the NPs would probably interact with the exocytic pathway leading to their exocytosis [26, 37, 87]. So far, we have discussed NP-cell interaction when NPs are directly exposed to the cells and NPs don't face any barriers in reaching cell membrane for their intracellular uptake process. However, it is not the case in real biological environments and is important to consider behavior of NPs through these barriers in order to further optimize the bio-nano interface between NPs and cells as discussed in the next section.

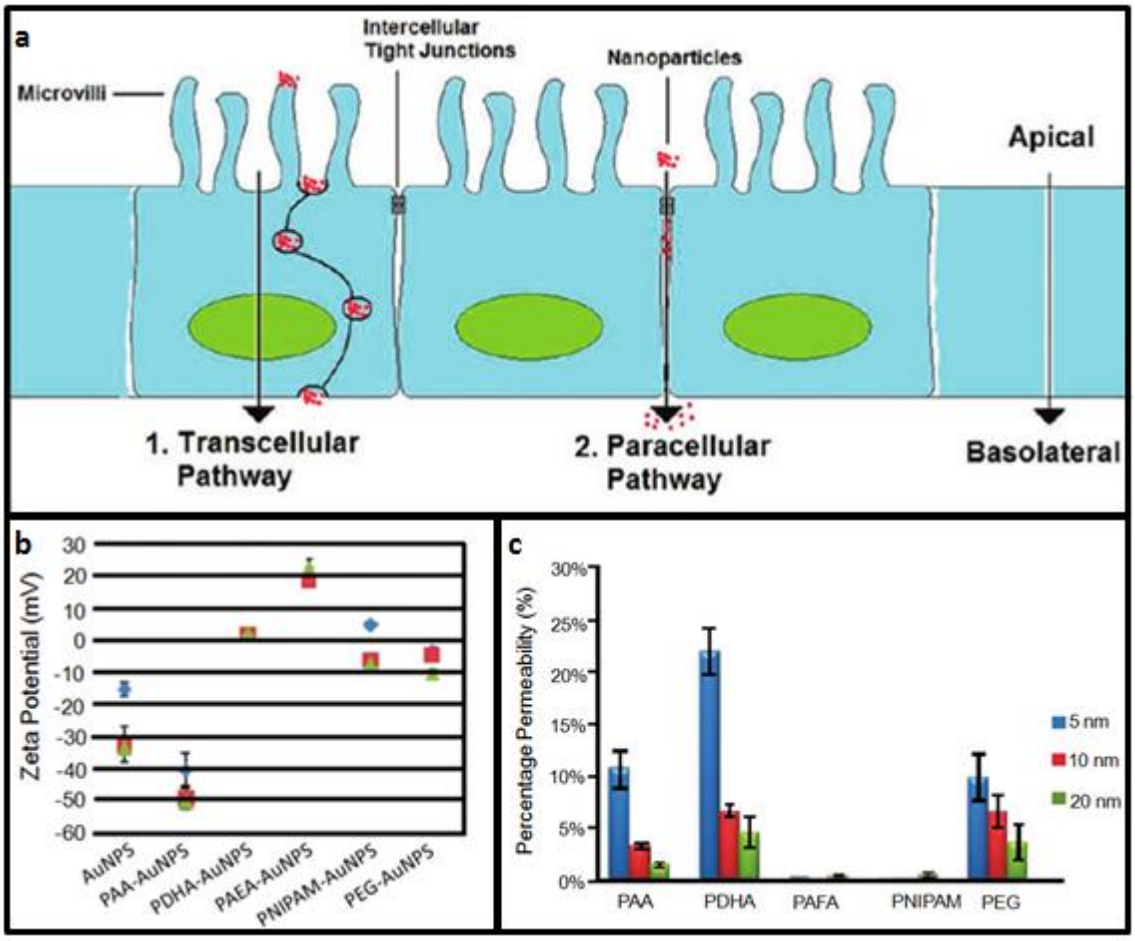
5. Translocation of nanoparticles across cell barriers

Epithelial and endothelial cell barriers serve as the frontline to protect the body from uptake of foreign substances, making delivery of NPs inefficient and difficult. For example, when NPs are used as noninvasive devices for delivery of drugs via inhalation or ingestion, NPs must first cross the alveolar epithelium of the lung or the intestinal epithelial cell barrier and enter the bloodstream or even translocate to other organs of the body to be effective. However, when NPs are injected intravenously, the vascular endothelial monolayer forms a semi-selective permeability barrier between blood and the interstitial space to control their movement across the vessel wall. Alteration of permeability barrier integrity plays a major role in drug-based therapies [90-92]. Several studies have shown that intravenously administrated NPs can translocate from the blood circulation into various targeted tissues and organs [93, 94]. Translocation of NPs across these barriers can occur by two possible pathways (see Figure 9A). The transcellular pathway allows molecules to pass through the epithelial cell membrane either via passive diffusion or active processes such as transcytosis (involving both endocytosis and exocytosis). Due to the size limit for passive diffusion, NPs could enter the cells by endocytosis. The paracellular pathway allows molecules to pass through the tight junctions between the individual epithelial or endothelial cells.

However, it is not still fully understood how NPs cross the endothelium from the blood stream into the targeted sites. Similarly, there is little information on the translocation of NPs across epithelial barriers and the effect of size and surface properties of NPs on these cell barriers. As a step forward in this direction, a recent study by Toth and co-workers shows how size and surface charge of NPs affect their translocation through an epithelial cell barrier [95]. Nanoparticles were coated with different polymers to change the surface charge as shown in Figure 9B. The importance of size and charge of NPs to cross epithelial monolayer is illustrated in Figure 9C. The neutral 5 nm NPs showed the greatest translocation across the monolayer, with a permeability twice that compared to the neutral NPs and the negatively charged NPs of the same size. Little or no translocation was observed for either the positively charged NPs or the hydrophobic NPs. The larger the NP size the lower amount of

translocation across the monolayer observed. This study provides insights into the translocation of NPs across epithelial barriers, and all the surface-coated NPs were found to transiently disrupt the tight junctions. This could potentially enhance the adsorption of poorly absorbed substances via a paracellular pathway. It was also found that the damage of monolayer was found to be reversible, and its barrier function slowly recovered once the NPs were removed. Based on these studies, it is important to further investigate the effect of size, shape, and surface properties of NPs on their crossing against epithelial or endothelial cell barriers in order for their optimum delivery of cancer therapeutics to targeted locations.

Figure 9. Effect of size and surface properties of NPs on their translocation through cell barriers. (a) Translocation of NPs across the epithelial barriers can occur by two possible routes: 1) The transcellular pathway allows molecules to pass through the epithelial cell membrane either via passive diffusion or active processes such as transcytosis (involving both endocytosis and exocytosis). 2) The paracellular pathway allows molecules to pass through the tight junctions between the individual epithelial cells. **(b)** Zeta-potential measured for both "naked" and polymer-coated GNPs. **(c)** Accumulative percentage of polymer-coated GNPs passing through the epithelial cell monolayer into basolateral chamber over the 3 h period. Reproduced with permission [95].



5. Summary

Many important applications of nanotechnology in the field of medicine would not be possible without the proper design of NPs. These detailed studies on the interface of nanostructures with biological systems provide guidance for proper design of NPs for applications in the field of nanomedicine [96]. Specifically, engineering of multifunctional NPs requires proper understanding of how size and shape and surface will affect their interactions with cells. In this review, Gold NPs were chosen as a model system to discuss how physiochemical characteristics affect the uptake, transport, and excretion of NPs at cellular level and most of the results were in agreement with other NP systems as well. However, further studies are needed to fully understand the how physiochemical properties of NPs affect their biological fate both *in vitro* and *in vivo* for proper designing of NP-vectors for biomedical applications. As new tools and probes emerge from biomedical research, these fundamental studies will be important for further optimization of the bio-nano interface. The clinical perspectives of NPs are promising. However, NP-based platforms are still at the initial stage of development and much more research is required before they can be applied in clinical applications.

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