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The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles

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Abstract

In vitro experiments typically measure the uptake of nanoparticles by exposing cells at the bottom of a culture plate to a suspension of nanoparticles, which is assumed to be well-dispersed. However, nanoparticles can sediment and this means the concentration of particles on the cell surface and those actually taken up by the cells may be higher than the initial bulk concentration. Here we use upright and inverted cell culture configurations to show that cellular uptake of gold nanoparticles depends on the sedimentation and diffusion velocities of the nanoparticles and is independent of size, shape, density, surface coating and initial concentration of the nanoparticles. Generally more nanoparticles are taken up in the upright configuration than the inverted one and nanoparticles that sediment faster showed greater differences in uptake between the two configurations. Our results suggest that cellular uptake of nanoparticles is sensitive to the way cells are positioned and sedimentation need to be considered when performing *in vitro* studies for large and heavy nanoparticles.

Nanoparticles have been used as carriers to deliver genetic materials into cells and therapeutic agents into tumours through the enhanced permeation and retention (EPR) effect^{1,2}, and to regulate the release of drug molecules according to the physiological states of an organism^{3,4}. Their superior magnetic, optical and/or photothermal properties have also been exploited for disease diagnosis and treatment^{5–11}. At the same time, the potential toxicity of nanoparticles has become a public concern^{12–14}. All these studies of applications and toxicity rely on our ability to quantify the interactions between nanoparticles and cells, including their uptake by cells. Many reports have shown that the cellular uptake of nanoparticles depends on their size^{15–19}, shape^{20,21}, and surface coating^{22–27}. Here we demonstrate that all prior work may need to be re-evaluated due to the effect of sedimentation on nanoparticle dosimetry^{13,14}.

In a typical *in vitro* experiment, cells are immobilized at the bottom of a culture plate or on a substrate placed at the bottom of a culture plate, and incubated with a suspension of nanoparticles. The nanoparticles are assumed to be well-dispersed in the culture medium due to diffusion (or Brownian motion) and thus, the concentration of nanoparticles at the cell surface is the same as the initial bulk concentration. However, some nanoparticles may aggregate and change their physical characteristics when exposed to a culture medium²⁸. Moreover, large and heavy nanoparticles can sediment quickly, causing the dose of

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Author contributions

E.C.C. and Y.X. conceived and designed the experiments. E.C.C. performed the experiments, analyzed the data, and prepared the manuscript. Y.X. revised the manuscript. Q.Z. synthesized the nanocages.

Additional information

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nanoparticles on the cell surface to vary; in such cases, the actual concentration of nanoparticles for cellular uptake could be significantly different from the initial value. Because cellular uptake is directly related to the concentration of nanoparticles²⁹, the results from such studies can be inaccurate and misleading. Although a recent theoretical work considered the effect of sedimentation and diffusion of nanoparticles on their dosimetry³⁰, it is still important to understand how the physical parameters of nanoparticles such as size, shape, density and surface coating influence their transport properties (typically described as diffusion and sedimentation) in a culture medium, and how these properties affect cellular uptake. By culturing cells with gold nanoparticles in both upright and inverted configurations, here we demonstrate that in the absence of particle aggregation, the cellular uptake of nanoparticles depends on the ratio of sedimentation to diffusion velocities regardless of size, shape, density, surface coating and initial concentration.

Upright vs. inverted configuration

We measured the numbers of gold nanoparticles up taken by cells in the upright and inverted configurations (Fig. 1a) and then correlated the disparity in uptake values with sedimentation and diffusion velocities of the nanoparticles. We used a 6-well culture plate with each well containing a glass coverslip on which cells were immobilized. In the upright configuration, the coverslip containing cells was placed at the bottom of a well and incubated with a suspension of particles as is commonly done in the literature. In the inverted configuration, the coverslip was suspended from above (with the cells facing the bottom of a well) by gluing a small block of rubber on the backside of the coverslip and a syringe needle to the inner side of the cover of the culture plate. This way, we could easily position the cells in an inverted configuration right before an uptake experiment by inserting the syringe needle into the rubber block. The length of the needle was cut to make sure the cells would be hanged approximately 1.2 mm above the bottom of the well, thus minimizing the difference in liquid height above the cells between the two configurations. By using the same batch of coverslips for all experiments, we could reduce the difference in cell morphology and uptake activity caused by dissimilar surfaces³¹. The medium in each well was 5.2 mm deep, and the cells were positioned approximately 0.2 mm and 1.2 mm, respectively, from the bottom of the well for the upright and inverted configurations. At the particle concentrations we used, all samples showed cell viability over 90% relative to a control sample that involved no gold nanoparticles (Supplementary Fig. S1). No difference in cell viability was observed when the cells were cultured (medium only) for 24 h in the two configurations (Supplementary Fig. S2).

The cells in the upright configuration are expected to experience a higher concentration of nanoparticles than the initial value if the nanoparticles sediment due to gravitational force. When this occurs, the concentration of nanoparticles responsible for cellular uptake will be higher than the initial concentration. Conversely, cells in the inverted configuration are expected to show an opposite trend because sedimentation will reduce the concentration of nanoparticles on the cell surface. We anticipate that the disparity in cellular uptake between the two configurations will be negligible when diffusion prevails over sedimentation, and vice versa.

In this study, we examined six different types of gold nanoparticles: nanospheres of three sizes, nanocages of two edge lengths, and nanorods (Supplementary Method S1). Figure 1, b-g, shows their transmission electron microscopy (TEM) images. We call these nanoparticles “as-prepared” samples, and their surfaces were covered by citrate ions for nanospheres, a mixture of poly(vinyl pyrrolidone) and trifluoroacetate ions for nanocages, and hexadecyltrimethylammonium bromide for nanorods. The surfaces of these nanoparticles could change upon exposure to a culture medium due to physical and/or

chemical adsorption^{32,33}. When serum proteins in the culture medium adsorb onto the nanoparticles, the hydrodynamic diameters of the nanoparticles increased and their surfaces became negatively charged although their initial surface charges measured in water were typically different from sample to sample (Table 1). The adsorbed serum proteins may induce and facilitate receptor-mediated endocytosis of the nanoparticles^{32,34}.

We also PEGylated gold nanoparticles with poly(ethylene glycol) to examine the influence of surface coating on cellular uptake in the two configurations (Supplementary Method S1). When the PEGylated nanoparticles were transferred into a culture medium, their surface charges changed the sign in a way similar to the as-prepared samples (Table 1). This change was also likely due to the adsorption of serum proteins. However, only a much smaller amount of the serum proteins could adsorb onto the PEG layer due to its well-known antifouling properties^{35–37}. As a result, the protein layers on PEGylated nanoparticles were much thinner relative to those on the as-prepared samples, and the impact of protein adsorption on cellular uptake should be reduced too.

Cellular uptake of nanoparticles

We determined the number of gold nanoparticles taken up by the cells using a UV-visible (UV-vis) spectroscopic method³⁸. According to the Beer-Lambert law, there is a linear correlation between the concentration of gold nanoparticles and the absorbance of their localized surface plasmon resonance (LSPR) peak. As such, the concentration of gold nanoparticles in a cell culture medium can be directly and quickly obtained without involving extensive sample preparation. We have validated this method by comparing the cellular uptake data with those obtained using the conventional method based on analysis of gold content by inductively-coupled plasma mass spectrometry (ICP-MS, Supplementary Tables S1 and S2). Supplementary Fig. S3 shows UV-vis spectra of the as-prepared gold nanoparticles dispersed in a culture medium, before and after 24-h incubation with cells in the two different configurations. We had to use the Dulbecco's Modified Eagle's Medium (DMEM) free of phenol red because this dye has a strong absorption at 550 nm, which overlaps with the LSPR peaks of both gold nanospheres and nanorods³⁸. After the nanoparticles had been incubated with the cells, the LSPR peaks were observed to drop in intensity. Notably, the drop was greater in the upright configuration than in the inverted configuration for the same sample of nanoparticles. We also observed a similar trend for the PEGylated nanoparticles (Supplementary Fig. S4).

From the UV-vis spectra, the corresponding calibration curves (Supplementary Fig. S5), and the number of cells in each sample, we calculated the number of nanoparticles taken up per cell for the two different configurations (Fig. 2). The uptake values of the as-prepared nanoparticles were much higher than those of the PEGylated nanoparticles. In addition, the uptakes were generally higher for cells in the upright configuration than in the inverted configuration, and the disparity in cellular uptake between these two configurations was dependent on the physical parameters of the nanoparticles.

Figure 3 summarizes the disparity in cellular uptake between the two configurations for all the gold nanoparticles we tested. In the plot, we expressed the disparity as $1-(N_{in}/N_{up})$, where N_{up} and N_{in} are the number of nanoparticles taken up per cell in the upright and inverted configurations, respectively. It should be emphasized that the disparity in cellular uptake cannot be attributed to the change in cellular activity that might be caused by the upside-down orientation because the uptake values of 15-nm nanospheres were essentially the same (within experimental errors) for both configurations. Similar to the case of 15-nm nanospheres, the nanorods also showed little disparity. Significantly, we found that the disparity had a strong dependence on the size of the nanoparticles. For both nanospheres and

nanocages, the disparity increased as they became larger. Regarding the effect of surface coating, it seems that the disparity was insensitive to this parameter for all the nanoparticles we tested. In addition, for the as-prepared 54-nm and 100-nm nanospheres, the disparity showed no dependence on the initial concentration of nanoparticles (Supplementary Fig. S6). Lastly, the disparity was found to be dependent on the incubation time, as shown by the time-dependent uptake studies with the as-prepared gold nanospheres (Supplementary Table S2 and Fig. S7). In the present work, we only focused on the data obtained with the same incubation time of 24 h.

Seeking an explanation for the disparity in cellular uptake observed for all different types of gold nanoparticles and at different concentrations, we assume that the uptake proceeded in the following order: transport of nanoparticles to the interaction zone, attachment of nanoparticles to the cell surface through adsorption, and internalization of the nanoparticles (Fig. 4a). For a specific type of nanoparticles with the same surface coating, the adsorption and internalization steps were expected to be essentially the same regardless of the configuration, as confirmed by the results of the 15-nm gold nanospheres. In the interaction zone, the gravitational forces (10^{-19} to 10^{-16} N) are too small, as compared with those of specific and nonspecific bindings (10^{-12} N to 10^{-9} N)^{39–43}, to have an impact on the adsorption and desorption of the nanoparticles. Therefore, the disparity in cellular uptake for the gold nanoparticles between the two configurations should be caused by the difference in particle concentration in the interaction zone. For cells in the upright configuration, the nanoparticles can be transported into the interaction zone through diffusion and sedimentation. In contrast, for cells positioned in the inverted configuration, the nanoparticles can only be transported into the interaction zone via diffusion while sedimentation of nanoparticles in the medium below the cells tends to reduce the concentration of nanoparticles. Although other factors such as convective forces in the culture medium as induced by thermal fluctuations, liquid flow, and vibrations from the incubator motor may influence the concentration profiles of the nanoparticles, they were excluded from our consideration because we did not purposely apply any of these factors. The lack of difference in cellular uptake of 15-nm nanoparticles under these two configurations also suggests that these factors were far less significant than sedimentation.

To quantify the effects of diffusion and sedimentation on cellular uptake, we obtained the diffusion (V_d) and sedimentation (V_s) velocities of the nanoparticles from their hydrodynamic sizes (Fig. 4, b and c; Supplementary Fig. S8 and Table S4)^{13,44}. The V_d and V_s values increased in the following orders: 15-nm nanospheres > nanorods > 54-nm nanospheres > 62-nm nanocages > 100-nm nanospheres > 118-nm nanocages for V_d ; and 100-nm nanospheres > 118-nm nanocages > 54-nm nanospheres > 62-nm nanocages > nanorods > 15-nm nanospheres for V_s . It is worth noting that the values of V_s for the 54-nm and 100-nm nanospheres were higher than those of nanocages with equivalent sizes although the values of V_d for these nanospheres were higher than those of the nanocages. This can be attributed to the difference in elemental composition (the nanocages were made of gold-silver alloys; Supplementary Method S1), shape (spherical vs. cubic), structure (solid vs. hollow) and the thicknesses of surface coating (e.g., serum proteins and PEG).

Putting these transport parameters together with the cellular uptake data, it is clear that the nanoparticles (15-nm nanospheres and nanorods) with high V_d and low V_s could move quickly to the interaction zone before they sediment and be supplied to the cells at a similar dose in both configurations, thereby causing very little disparity. In comparison, other types of nanoparticles showed greater disparity than the 15-nm nanospheres and nanorods. This result implies that in the inverted configuration, large nanoparticles (low V_d and high V_s) tend to sediment quickly before reaching the interaction zone via diffusion, and more

nanoparticles would be accumulated in the interaction zone in the upright configuration due to sedimentation.

To better understand the effects of V_s and V_d on the disparity in cellular uptake between these two configurations, we plotted $1-(N_{int}/N_{up})$ as a function of $\log(V_s/V_d)$ in Fig. 5. Since all the physical parameters (size, shape and density) of the nanoparticles were reflected in V_s/V_d , we could express the disparity in terms of this dimensionless ratio regardless of other parameters. To make the plot more general, we included the $1-(N_{int}/N_{up})$ values obtained from the as-prepared 54-nm and 100-nm nanospheres at other initial concentrations (Supplementary Fig. S6) and a different sample of as-prepared nanorods (Supplementary Fig. S9). In addition, we included the $1-(N_{int}/N_{up})$ values calculated using the data obtained by ICP-MS analysis of the gold content in cells (Supplementary Table S1). From this plot, one can clearly see how the disparity was affected by the composite effect of sedimentation and diffusion. In general, the disparity increased with increasing $\log(V_s/V_d)$. Somehow, the experimental data can be grouped into two linear regions with the two regression lines crossing at a value of 3 for V_s/V_d , which corresponds to a disparity of 0.3. This point lies between those for the nanorods and the 54-nm nanospheres (or the 62-nm nanocages), suggesting that special caution must be taken when one designs an experiment to study cellular uptake with gold nanoparticles >40 nm in hydrodynamic diameter, especially if one wants to elucidate how the size of nanoparticles affects cellular uptake.

Discussion

The model shown in Fig. 4a suggests that the disparity in cellular uptake between the two configurations was a result of difference in particle concentration in the interaction zone caused by sedimentation. This argument is based on the assumption that the accessible volume was identical in both two configurations so transport of nanoparticles by diffusion would be the same. Based on the uptake values for 15-nm nanospheres, we can conclude that the accessible volume was more or less identical between the two configurations. If there was a major difference in accessible volume, we expected to see a clear difference in cellular uptake between the two configurations due to the absence of sedimentation for such small particles. Since the cellular uptake values were essentially the same, the cells in the two different configurations were supplied with a similar dose of 15-nm nanospheres in the interaction zone. Experimentally, the separation (1.2 mm) between the cells and the bottom of the well in the inverted configuration was sufficiently wide to allow culture medium to move through easily without being entrapped. No back flow was observed for the culture medium in the inverted configuration when we pipetted the medium. In addition, the cell viability was almost identical for the two configurations, indicating that the separation was wide enough to allow for free bulk diffusion of all components in the culture medium.

We also compared diffusion (V_d) and sedimentation (V_s) velocities of the nanoparticles to see if back diffusion due to concentration gradient was a problem (Supplementary Method S8). For the 15-nm nanospheres and nanorods, V_d was larger than V_s ($V_s/V_d < 1$), indicating that diffusion was the prevailing means for transporting nanoparticles to the cell surface and back diffusion should not be involved due to the lack of concentration gradient. For the 100-nm nanospheres and 118-nm nanocages, V_s was much higher than V_d ($V_s/V_d \gg 1$), and the concentration decayed exponentially with increasing distance from the bottom of the culture plate due to sedimentation. In these cases, back diffusion was not significant either^{45,46}. In contrast, the V_s/V_d ratios were 7.03 and 6.38, respectively, for 54-nm nanospheres and 62-nm nanocages. As such, it is necessary to include back diffusion of these nanoparticles in estimating their concentrations as a function of height. As shown in Supplementary Fig. S10, the concentration still decayed with height even though back diffusion was involved.

When the amount of nanoparticles taken up by cells reached a level comparable to the initial concentration, the uptake itself could also alter the concentration profile of nanoparticles in the culture medium. For the 54-nm nanospheres and the 62-nm nanocages in the upright configuration, the particles depleted by cellular uptake over a period of 24 h corresponded to a concentration of 1 pM, which was much lower than the initial particle concentration of 20 pM. As such, the change in concentration induced by cellular uptake should be much smaller relative to the change induced by sedimentation. A similar argument also holds for the 100-nm nanospheres and 112-nm nanocages.

For the inverted configuration, sedimentation of nanoparticles on the upper side of coverslip may also influence the cellular uptake data, but the trend should be consistent with the observation and argument we want to make in the present work. It does not matter if the nanoparticles were deposited on the upper side of the coverslip or the bottom of the well due to sedimentation, the end result should be the same: a reduction in concentration of nanoparticles on the cell surface relative to the initial solution. As such, the cellular uptake would be reduced relative to the case without sedimentation.

Conclusion

We have demonstrated that the uptake of gold nanoparticles by cells is sensitive to the configurations in which the cells are positioned. Generally, nanoparticles sediment faster showed greater differences in cellular uptake between the two configurations. To date, essentially all studies regarding cellular uptakes of nanoparticles have been conducted with cells in the upright configuration that may give wrong and misleading data depending on the V_s/V_d ratio. The fitting result to our experimental data indicate that one must consider the sedimentation (or configuration) issue when performing studies of cellular uptake with large and/or heavy nanoparticles.

Our study also has important implications for those who conduct permeation tests with nanoparticles and biological tissues (e.g., skin), which are also typically carried out with the tissue placed at the bottom of a suspension of nanoparticles^{47,48}. Taken together, we envision this study will greatly improve our understanding and modeling of the interactions between nanoparticles and cells (or tissues), and thus offer a better and more efficient way to apply nanoparticles to various biomedical applications.

Methods

Methods for the preparation and surface modification of gold nanoparticles can be found in Supplementary Method S1. Human breast cancer cells (SK-BR-3, ATCC HTB-30™) were cultured on coverslips (22x22 mm², Corning Life Sciences) in DMEM (no phenol red, Hyclone® Laboratories) supplemented with 10% fetal bovine serum (FBS, ATCC) and 1% antibiotics (penicillin and streptomycin, Invitrogen). The medium was changed every other day, and the cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂. Please refer to the main text for a detailed discussion of the experimental setup.

The cells were used for uptake studies once they had reached ~90% confluence. Typically, the cells were positioned in an upright or inverted configuration and incubated with 5.0 mL of the culture medium containing gold nanoparticles at 37 °C in a 6-well culture plate. After 24 h, we removed the medium from each well and added 0.8 mL of pristine medium (containing no gold nanoparticles) to wash off the loosely-bound nanoparticles on the cell surface. The washing procedure was repeated two more times. A UV-vis spectrophotometer (CARY50, Varian) was used to record extinction spectra of the culture medium containing nanoparticles. We first obtained the background from 300 nm to 1100 nm with DMEM

containing FBS and antibiotics. We then recorded a spectrum of the culture medium (containing FBS, antibiotics and nanoparticles) before it was used for cell culture (denoted “before”). After incubation with cells, we recorded a spectrum of the medium that also included the solutions from the three washing steps (denoted “upright” or “inverted” in Supplementary Figs. S3 and S4). When the removed medium was mixed with the three washing solutions, the concentration of nanoparticles was reduced as a result of dilution. For this reason, we adjusted the spectrum by accounting for the total volume (2.4 mL) of the added washing solutions. In addition, from control experiments, we found that the LSPR peak intensity tended to drop with time for the as-prepared 112-nm nanocages (this sample only) in the absence of cells, probably due to adsorption onto the surface of culture well (for both configurations) and the upper side of the coverslip (for the inverted configuration). For this type of nanoparticles, we corrected the uptake data by taking into account the reduction caused by surface adsorption.

We calculated the number of nanoparticles taken up by the cells based on the extinction spectra (Supplementary Figs. S3 and S4) and the calibration curves (Supplementary Fig. S5). At the end of each experiment, we counted the number of cells in each well using a hemocytometer. The cell number was then used to calculate the uptake value of gold nanoparticles per cell. For the inverted configuration, we found that ~3% of the cells would have migrated from the glass slide to the bottom of the well during the 24-h incubation. Therefore, for this configuration, our calculation was corrected by considering the uptake of nanoparticles by cells that migrated to the bottom of the well. For the upright configuration, the number of cells migrating from the coverslip to the culture well was negligible when compared with the inverted configuration. Each data point was obtained from four parallel samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold NPs in delivery applications. *Adv. Drug Deliv. Rev.* 2008; 60:1307–1315. [PubMed: 18555555]
2. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Controlled Release.* 2000; 65:271–284.
3. Sawant RM, et al. “SMART” drug delivery systems: Double-targeted pH-responsive pharmaceutical nanocarriers. *Bioconjugate Chem.* 2006; 17:943–949.
4. Rosler A, Vandermeulen GWM, Klok H-A. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. *Adv. Drug Deliv. Rev.* 2001; 53:95–108. [PubMed: 11733119]
5. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: Optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Acc. Chem. Res.* 2008; 41:1578–1586. [PubMed: 18447366]
6. Skrabalak SE, et al. Gold nanocages: Synthesis, properties, and applications. *Acc. Chem. Res.* 2008; 41:1587–1595. [PubMed: 18570442]

7. Lal S, Clare SE, Halas NJ. Nanoshell-enabled photothermal cancer therapy: Impending clinical impact. *Acc. Chem. Res.* 2008; 41:1842–1851. [PubMed: 19053240]
8. Murphy CJ, et al. Gold nanoparticles in biology: Beyond toxicity to cellular imaging. *Acc. Chem. Res.* 2008; 41:1721–1730. [PubMed: 18712884]
9. Zerda ADL, et al. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nature Nanotech.* 2008; 3:557–562.
10. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nature Biotech.* 2004; 22:969–976.
11. Sun C, Lee JSH, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. *Adv. Drug Deliv. Rev.* 2008; 60:1252–1265. [PubMed: 18558452]
12. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. *Science.* 2006; 311:622–627. [PubMed: 16456071]
13. Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG. Particokinetics in vitro: Dosimetry considerations for in vitro nanoparticle toxicity assessments. *Toxicol. Sci.* 2007; 95:300–312. [PubMed: 17098817]
14. Lison D, et al. Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. *Toxicol. Sci.* 2008; 104:155–162. [PubMed: 18400775]
15. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. *Nature Nanotech.* 2008; 3:145–150.
16. Alivisatos AP, Gu W, Larabell C. Quantum dots as cellular probes. *Annu. Rev. Biomed. Eng.* 2005; 7:55–76. [PubMed: 16004566]
17. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin and caveolae-mediated endocytosis. *Biochem. J.* 2004; 377:159–169. [PubMed: 14505488]
18. Panyam J, Labhasetwar V. Biodegradable NPs for drug and gene delivery to cells and tissue. *Adv. Drug Deliv. Rev.* 2003; 55:329–347. [PubMed: 12628320]
19. Prabha S, Zhou W-Z, Panyam J, Labhasetwar V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *Int. J. Pharm.* 2002; 244:105–115. [PubMed: 12204570]
20. Cho EC, Au L, Zhang Q, Xia Y. The effects of size, shape, and surface functional group of gold nanoparticles on their adsorption and internalization by cells. *Small.* 2010; 6:517–522. [PubMed: 20029850]
21. Chithrani BD, Chan WCW. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Lett.* 2007; 7:1542–1550. [PubMed: 17465586]
22. Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. *Small.* 2010; 6:12–21. [PubMed: 19844908]
23. Verma A, et al. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nature Mater.* 2008; 7:588–595. [PubMed: 18500347]
24. Leroueil PR, et al. Nanoparticle interaction with biological membranes: Does nanotechnology present a Janus face? *Acc. Chem. Res.* 2007; 40:335–342. [PubMed: 17474708]
25. Cho EC, Xie J, Wurm PA, Xia Y. Understanding the role of surface charges in cellular adsorption versus internalization by selectively removing gold nanoparticles on the cell surface with a I_2/KI etchant. *Nano Lett.* 2009; 9:1080–1084. [PubMed: 19199477]
26. Zorko M, Langel U. Cell-penetrating peptides: Mechanism and kinetics of cargo delivery. *Adv. Drug Deliv. Rev.* 2005; 57:529–545. [PubMed: 15722162]
27. Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. *Adv. Drug Deliv. Rev.* 2000; 41:147–162. [PubMed: 10699311]
28. Sager TM, et al. Improved method to disperse NPs for in vitro and in vivo investigation of toxicity. *Nanotoxicology.* 2007; 1:118–129.
29. Xu C, Tung GA, Sun S. Size and concentration effect of gold nanoparticles on X-ray attenuation as measured on computed tomography. *Chem. Mater.* 2008; 20:4167–4169. [PubMed: 19079760]

30. Hinderliter PM, et al. ISDD: A computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. *Particle Fibre Toxicology*. 2010; 7:36–54. [PubMed: 21118529]
31. Kong HJ, et al. Non-viral gene delivery regulated by stiffness of cell adhesion substrates. *Nature Mater*. 2005; 4:460–464. [PubMed: 15895097]
32. Alkilany AM, et al. Cellular uptake and cytotoxicity of gold nanorods: Molecular origin of cytotoxicity and surface effects. *Small*. 2009; 5:701–708. [PubMed: 19226599]
33. Lynch I, et al. The nanoparticle-protein complex as a biological entity: A complex fluids and surface science challenge for the 21st century. *Adv. Colloid Interface Sci*. 2007; 134–135:167–174.
34. Conner SD, Schmid SL. Regulated portals of entry into the cell. *Nature*. 2003; 422:37–44. [PubMed: 12621426]
35. Feldman K, Halhner G, Spencer ND, Harder P, Grunze M. Probing resistance to protein adsorption of oligo(ethylene glycol)-terminated self-assembled monolayers by scanning force microscopy. *J. Am. Chem. Soc*. 1999; 121:10134–10141.
36. Sigal GB, Mrksich M, Whitesides GM. Effect of surface wettability on the adsorption of proteins and detergents. *J. Am. Chem. Soc*. 1998; 120:3464–3473.
37. Horbett, TA.; Brash, JL., editors. *Proteins at Interfaces II*. Washington DC: American Chemical Society; 1995. p. 396
38. Cho EC, Liu Y, Xia Y. A simple spectroscopic method for differentiating cellular uptakes of gold nanospheres and nanorods from their mixtures. *Angew. Chem. Int. Ed*. 2010; 49:1976–1980.
39. Leckband D. Nanomechanics of adhesion proteins. *Curr. Opin. Struct. Biol*. 2004; 14:524–530. [PubMed: 15465311]
40. Dammer U, et al. Specific antigen/antibody interactions measured by force microscopy. *Biophys. J*. 1996; 70:2437–2441.
41. Allen S, et al. Detection of antigen-antibody binding events with the atomic force microscope. *Biochemistry*. 1997; 36:7457–7463. [PubMed: 9200694]
42. Tha SP, Shuster J, Goldsmith HL. Interaction forces between red cells agglutinated by antibody. II. Measurement of hydrodynamic force of breakup. *Biophys. J*. 1986; 50:1117–1126. [PubMed: 3801572]
43. Vasir JK, Labhasetwar V. Quantification of the force of nanoparticle-cell membrane interactions and its influence on intracellular trafficking of nanoparticles. *Biomaterials*. 2008; 29:4244–4252. [PubMed: 18692238]
44. Hiemenz, PC.; Rajagopalan, R. *Principles of Colloid and Surface Chemistry*. New York: Marcel Dekker, Inc; 1997. p. 94
45. Brown PH, Schuck P. Macromolecular size-and-shape distributions by sedimentation velocity analytical ultracentrifugation. *Biophys. J*. 2006; 90:4651–4661. [PubMed: 16565040]
46. Sperling RA, et al. Size determination of (bio)conjugated water-soluble colloidal nanoparticles: A comparison of different techniques. *J. Phys. Chem*. 2007; 111:11552–11559.
47. Shim J, et al. Transdermal delivery of minoxidil with block copolymer nanoparticles. *J. Controlled Release*. 2004; 97:477–484.
48. Sonavane G, et al. In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. *Colloids Sur. B*. 2008; 65:1–10.

Figure 1. Experimental setups and the gold nanoparticles used in this study

a, Schematic of the upright (left) and inverted (right) configurations for measuring cellular uptake of nanoparticles. Cells are not drawn to scale and each well of a 6-well culture plate contained only one glass coverslip on which cells are immobilized. **b-g**, TEM images showing the six types of gold nanoparticles used to examine the effects of size, shape and density on the disparity in cellular uptake between the two configurations: 15-nm (in diameter) nanospheres (**b**), 54-nm nanospheres (**c**), 100-nm nanospheres (**d**), 62-nm (in outer edge length) nanocages (**e**), 118-nm nanocages (**f**), nanorods (**g**, 16 nm×40 nm in diameter by length). The 50-nm scale bar applies to all images.

Figure 2. Uptake values of various types of gold nanoparticles for cells positioned in the upright and inverted configurations

The number (N) of particles taken up per cell was measured for both the as-prepared and PEGylated samples using the UV-vis method. The nanoparticles and their concentrations (based on particle number) used to incubate with the cells were: **a**, 15-nm nanospheres (120 pM); **b**, 54-nm nanospheres (20 pM); **c**, 100-nm nanospheres (2.8 pM); **d**, 62-nm nanocages (20 pM); **e**, 118-nm nanocages (2.6 pM); and **f**, nanorods (20 pM). Error bars shown in the plots are standard errors with $n=4$.

Figure 3. Comparison of the disparity in cellular uptake between the upright and inverted configurations for different types of gold nanoparticles

We express the disparity in uptakes between the two configurations as $1 - (N_{in}/N_{up})$, where N_{up} and N_{in} are the number of nanoparticles taken up per cell in the upright and inverted configurations, respectively. The disparity increased with the particle size for both the nanospheres and nanocages. In addition, the disparity was insensitive to the surface coating for all types of nanoparticles. Error bars shown in the plots are standard errors with $n=4$.

Figure 4. Different zones that are involved in cellular uptake of gold nanoparticles and the two factors affecting the uptake process

a, Schematic showing the transport, interaction and uptake zones when the cell is positioned in an upright (left) or inverted (right) configuration. The concentration of nanoparticles in the interaction zone for both configurations may differ depending on the sedimentation (S) and diffusion (D) characteristics of the nanoparticles. **b**, Diffusion (V_d) and sedimentation (V_s) velocities of the as-prepared nanoparticles. **c**, The ratios of sedimentation to diffusion velocities (V_s/V_d) for various types of nanoparticles. The ratios determine which factor is dominant in transporting the nanoparticles to the cell surface, thereby affecting the uptake process.

Figure 5. Disparity in uptake between the upright and inverted configurations as a function of the ratio of sedimentation to diffusion velocities

The disparity in uptake is expressed as $1 - (N_{in} / N_{up})$ and the plot includes all the $1 - (N_{in} / N_{up})$ values measured using both the UV-vis (Fig. 3 and Supplementary Figs. S6 and S9) and ICP-MS methods with the cells (Supplementary Table S1). The disparity increased with increasing V_s / V_d ratio regardless of particle size, shape, surface coating and initial concentration. The data can be grouped into two linear regions; the two dashed lines cross at $V_s / V_d = 3$, corresponding to a value of 0.3 for disparity. In general, the effect of sedimentation must be taken into consideration for nanoparticles with V_s / V_d greater than 3.

Hydrodynamic diameters (d_h) and surface charges of the gold nanoparticles before and after incubation in the cell culture medium^a.

Table 1

Gold nanoparticles	$d_{h, \text{ water}}(\text{nm})^b$				Zeta-potentials (mV) ^c				
	As-prepared		PEGylated		As-prepared		PEGylated		
	before	after	before	after	before	after	before	after	
Nano-spheres	15-nm ^d	18	21	21	22	-13.7	-14.2	0.0	-9.2
	54-nm	55	67	67	76	-16.1	-12.7	-1.0	-9.3
	100-nm	97	109	109	124	-17.3	-15.1	-1.3	-12.7
Nanocages	62-nm ^d	101	110	110	115	-6.6	-12.5	+0.3	-12.0
	118-nm	145	164	164	165	-25.5	-15.4	+1.4	-6.6
Nanorods	16 nm x40 nm ^d	23	24	24	26	+20.2	-15.9	+2.8	-12.7

^aSee Supplementary Methods S2 and S3 for the experimental details.

^bThe d_h , water values were measured in de-ionized water. Here “before” and “after” refer to samples before and after incubation in the culture medium, respectively. After incubation for 24 h, the nanoparticles were centrifuged, collected, and re-dispersed in de-ionized water. For d_h values measured for nanoparticles dispersed in the culture medium, see Supplementary Tables S6 and S7.

^cThe surface charges were measured while the nanoparticles were dispersed in deionized water.

^dThe sizes were measured from TEM images.