Colloidal particles for cellular uptake and delivery

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Numerous colloidal particles have been designed for cellular uptake and intracellular delivery in biological systems. The different kinds of colloidal particle systems and the interactions between the particles and cells are highlighted, with a focus on the parameters governing cellular uptake. A perspective of the research is also proposed.

Colloidal particles are characterized by a length scale ranging from 1 nm to microns (typically 1 μm). They can be isolated as tiny particles, a group of such tiny particles (aggregate, agglomerate), molecular aggregates (e.g. micelles) or even a single macromolecule (e.g. dendrimer). Many of the colloidal particles are known as engineering particles with variable chemical structures and are widely used as fillers, gas sensors and carriers for catalysts in industry. Along with the rapid development of nanotechnology and nanomedicine, these tiny particles are taking more and more important roles in the biological field. They can be used as carriers for controlled drug release, cancer and gene therapy, disease diagnosis and bio-imaging. Compared to the few applications of metallic particles, the inorganic and organic particles have shown great success in the biological field and thereby have dominated bio-applications nowadays. In this context, the particles are usually integrated with other functions such as tailored wettability, specific targeting, imaging and controlled release of desired substances (Fig. 1), which have been summarized previously by several excellent reviews. Results have shown that many of these functions or properties have an important impact on the interaction between the particles and organisms in terms of the delivery efficacy, toxicity and biocompatibility.

Due to their small enough size and/or their functionalities, when the colloidal particles come into contact with the organisms, uptake of the particles by living cells takes place either positively or passively. The uptake of the particles may bring some unexpected side effects, for example toxicity and long term accumulation of the nondegradable particles. On the other hand, many of the biological applications of the particles are dependent on their cellular uptake, for example, bio-imaging, gene delivery and more recently, intracellular drug delivery. In these situations, the particles should be transported through the cell membrane and be able to travel in the cytosol. Therefore, it is urgent that we understand the interaction between the colloidal particles and cells, including the uptake pathway, the distribution of the colloidal particles in intracellular organelles and the mechanism of cellular uptake.

It is known that the colloidal particles can be imported into the cells via pinocytosis for smaller particles (<0.2 μm) and phagocytosis for larger ones (>0.5 μm). Conclusively, the membrane transport process for particles and proteins is completed by both endocytosis and exocytosis which are defined as cytosis. The mechanism of endocytosis includes receptor-mediated and adsorptive endocytosis. In view of cell biology, in the endocytosis process the particles are wrapped by the cell membrane to form membrane-bounded vesicles called endosomes. Via a series of steps, the endosomes...
are finally transported into the lysosomes, where the particles will suffer from a relatively low pH value (pH $\sim 4.5$) and various enzymes. Consequently, some are degraded, and others escape from the lysosomes because of the generated rupture. The escaped colloidal particles can then travel to other intracellular compartments, even nuclei (Fig. 2).

In view of the rapid development of nanomedicine, the present article shall focus on the cellular uptake of colloidal particles with respect to their physical and chemical parameters. It starts with the introduction of those colloidal particles frequently used in the biological field, and is followed by the highlighting of some key issues of the cellular uptake of colloidal particles and the cytotoxicity brought about by the cellular uptake. In our opinion, more special efforts should be made deliberately on the basic research of cellular uptake, which is of great importance to formulating ideal colloidal carriers with desirable physical properties and biological performance.

**Typical colloidal particles used in biological fields**

During the past decades, many colloidal particles have been fabricated and extensively investigated in terms of biological applications. They can be used as carriers for bio-imaging, diagnosis of pathology, and disease treatment etc. via the way of intracellular uptake and delivery. According to the chemical structures of these colloidal particles, they are usually classified into two categories: inorganic and metallic colloidal particles (such as calcium phosphate, gold, quantum dots, carbon materials, silicon oxide and iron oxide), and organic colloidal particles (most of which are polymeric particles such as carriers made of natural and synthetic polymers).

**Inorganic and metallic colloidal particles**

This type of particle has an absolute dominance over the particle family, and has been well developed during the past decades. Some of these inorganic and metallic colloidal particles have been used clinically, or shown great potential to be used clinically. The most successfully applied particles in the clinic are iron oxide nanocrystals with the superparamagnetic property. They are frequently used as a contrast agent for bio-imaging under a magnetic resonance field, which can yield a high diagnostic accuracy for detecting arthritis, atherosclerosis and cancer. For example, the iron oxides of 5–10 nm are successfully used to localize various cancers of the breast, lung, prostate and endometrium under magnetic resonance imaging. Various vast efforts are being made to use the iron oxides to kill the cancer cells by a so-called hyperthermic treatment.

The gold nanoparticles can absorb light over a broad spectral range from visible to the near-infrared light, which is mediated by their size and shape, and are believed to have potential as optically tunable carriers for diagnosis. The inorganic light-emitting nanoparticles, known as semiconductor quantum dots (QDs), can emit fluorescence in the broad range of 400–2000 nm depending on their size and composition. Together with the features of a very high level of brightness and excellent photostability, these QDs can be used as a novel class of fluorescent probes for bio-imaging.

Carbon-based nanomaterials including fullerenes, nanotubes and nanodiamonds are another class of fascinating materials that are seeking diverse applications in...
various fields including biology. They have ultra physical and chemical stability at normal conditions, but their surfaces can be chemically modified in some conditions to meet the demands of applications, such as wettability for dispersion in water. Various attempts have been made to explore their applications including carriers for drugs, genes, and proteins, contrast agents for bioimaging and biosensors. Moreover, making use of the sensitivity of their conductance towards their environment and adsorbed molecules, carbon nanotubes (CNTs) can be potentially used for detecting biological molecules such as cancer markers in fluids. Recently, the application of single-walled CNTs for photothermal cancer therapy has been demonstrated based on their strong optical absorbance in the near infrared (NIR) region. Besides the above mentioned particles, mesoporous silica and calcium phosphate or hydroxyapatite colloidal particles can interact with cells and are usually used as carriers to delivery drugs and DNA to intracellular organelles. Composite colloidal particles turn out to be advantageous since the features of different materials are integrated. Typical examples include silica/gold core shell particles, mesoporous silica loaded with iron oxides, metallic nanoparticles and fluorophores, and other novel heterostructures.

Apart from the applications of the particles only, integration of other functional agents such as drugs, proteins and genes can also be implemented to endow the particles with multifunctions and applications. These molecules can be physically adsorbed or chemically immobilized into the porous structures or onto the surfaces of the colloidal particles. By anchoring hydrophilic polymers such as poly(ethylene glycol) (PEG) onto their surface, a stealth layer can be formed which makes the particles more compatible with the organisms. These particles are also expected to have a longer circulation time in the blood stream.

### Polymeric colloidal particles

Due to the similarities in chemical structures and physical properties, the polymers used in the biological field have good processibility and biocompatibility and desirable biodegradability. Synthetic hydrophobic polymers such as polystyrene (PS), polymethyl methacrylate (PMMA), polylactic acid (PLA) and poly(lactic-co-glycolide) (PLGA) are most frequently used as matrix materials to formulate the polymer colloidal particles by methods of emulsion–polymerization, emulsion–solvent evaporation, and membrane emulsification. On the other hand, the hydrophilic polymers, including PEG, poly(vinyl pyrrolidone) (PVP), poly(ethyleneimine) (PEI), chitosan and dextran, are generally used as the hairy layers to prohibit particle coagulation and provide other functions on both organic and inorganic particles.

Another type of polymer used to form the colloidal particles is based on graft or block copolymers containing hydrophilic and hydrophobic segments, which can spontaneously assemble into micelles by a solvent exchange protocol. Most of the micelles have a desirable stability because of their ultra low critical micelle concentration (CMC), rational particle size range (from tens to hundreds of nanometers) and high drug loading capacity. In 2004, doxorubicin-loaded micelles were advanced into human clinical trials in Japan. More recently, new polymers with hierarchical structures including dendrimers and star-shaped polymers have turned out to be another type of amphiphilic polymer forming colloidal particles. The dendrimers have a highly-branched and tumbleweed-like symmetrical structure and a reactive surface, on which drugs, targeting ligands and imaging agents can be anchored to obtain multifunctional carriers. So far the biological applications of the dendrimers have been extensively investigated both in vitro and in vivo.
Apart from the surface anchorage of the functional moieties, the desired substances also can be loaded into the polymeric colloids during the fabrication process or subsequent encapsulation by diffusion and special attraction. So far many bioactive species such as drug, DNAs, enzymes and cell growth factors have been loaded, which can then be protected, held, delivered and released. Compared with the inorganic colloidal particles, sustained release is easier to control. The release can be realized from the polymer matrix via surface or bulk erosion, diffusion, or swelling followed by diffusion, in a time- or condition-dependent manner at a controlled rate. In addition, surface modification can further make the carriers target the desired sites, leading to less usage of the required substances. More recently, intracellular delivery of drugs and genes has attracted more attention, which may improve the therapeutic efficiency and decrease the toxicity to normal cells and tissues.

Key issues of colloidal particles for intracellular uptake and delivery

Fully understanding the correlation between the physicochemical properties of the colloidal particles and intracellular uptake and delivery is one of the most paramount issues at present. So far some experiences have been gained to learn that the size, shape, charge and surface chemistry of the colloidal particles are the main factors governing the cellular uptake and the process of cellular delivery. Due to the complexity of in vivo investigation, most of the following results are obtained by in vitro cell culture in the existence of the colloidal particles. Indeed, the in vitro cell culture is advantageous since the influencing factors can be easily isolated and the structures and properties of the particles can be as far as possible kept unchanged as characterized by the traditional methods.

Size dependence

Cellular uptake can take place when cells are exposed to various kinds of colloidal particles with a diameter ranging from several nanometers to microns by the adsorptive or receptor mediated endocytosis pathway. As recognized previously, internalization of the microparticles is usually through a phagocytosis route with non-specific interactions between the particles and cells. It is known that the efficiency of the intracellular delivery of bioactive substances, especially genes, is critically dependent on the internalization rate and amount of the carriers, and usually follows a positive correlation supposing that the cytotoxicity of the particles is small enough. However, so far there is a lack of universal rules describing accurately the relationship between the amount and rate of the cellular uptake and the particle size. Case reports reveal that the size influence may depend on both the chemical structures of the particles and the cell types. For gene delivery with polycation vectors, better transfection efficiency is generally achieved when the DNA/vector particles have a size within 100–200 nm and an N/P (amine group to phosphate group) ratio of 10–20. Yet the result does not convey exclusively the largest uptake of particles with this size and property since the gene transfection efficiency is influenced by many factors including the cellular uptake, endosomal escape and toxicity of the vectors etc. Luo et al. found that silica particles with an average diameter of 200 nm had the largest uptake dosage to COS-7 (a cell line from the kidney of an African green monkey) cells and thereby the highest gene transfection efficiency compared to the smaller ones. The same size PS particles could be internalized into Caco-2 (the human colon carcinoma cell line) cells with a greatest uptake dosage too. Chithrani et al. reported that among the Au particles below 100 nm the one with a size of 50 nm was more easily endocytosed by HeLa (human epithelial cells from a fatal cervical carcinoma) cells. On the other hand, magnetic iron oxide particles with a size of 20 nm were more quickly internalized with a final larger uptake dosage in comparison with those of 100 nm. Qaddoumi et al. compared the uptake performance of PLGA particles of various sizes (100 nm, 800 nm, and 10 μm) containing 6-coumarin (as a fluorescent marker) on rabbit conjunctival epithelial cell layers. They found that the 100 nm particles had the highest uptake in comparison with other particles. Based on the fact that the uptake was inhibited significantly by lowering the incubation temperature and by the presence of metabolic inhibitors and cytochalasin D, they concluded that the uptake was likely occurring through the adsorptive-type endocytosis. A similar comparison was made by Dawson and Halbert by using PLGA particles with diameters of 155 nm, 200 nm, 375 nm and 600 nm to which bacterial invasion was covalently coupled. By in vitro culture with HEP2 2B (human Caucasian larynx carcinoma) cells the same authors found that the larger particles (375 nm and 600 nm) were internalized by a larger amount than the smaller ones (155 nm and 200 nm) due to the higher surface density of invasion, which endows the particles with a receptor dependent uptake mechanism.

Indeed, uptake of the particles may follow different mechanisms depending on the particle size and surface chemistry and cell types, and thereby may generate controversial results. Moreover, due to their smaller size and high surface/volume ratio, sedimentation and agglomeration of the colloidal particles may become a significant problem in some systems, especially in serum containing media, thus great attention should be paid to this.

Shape dependence

The influence of the shape of the colloidal particles is more complicated than that of the size, and thus is rarely discussed. Nevertheless, the present results have demonstrated that the cells can take up colloidal particles with various shapes including spheres, rods, tubes and sheets. For example, Jin et al. observed the endocytosis and exocytosis of DNA wrapped single-walled carbon nanotubes in NIH-3T3 (mouse embryonic fibroblast cell line) cells, whose rates closely matched with each other. Chithrani et al. compared the cellular uptake of spherical and rod-like Au particles, and found that the spherical Au particles were more easily endocytosed by HeLa cells. Geng et al. found that spherical polymer micelles and short filomicelles could be similarly internalized by cells and tissues of mouse. Trewn et al. observed that the cellular uptake dosage of tubular mesoporous silica nanomaterials (TMSN, width 80–150 nm, length 400–1000 nm) was larger than that of spherical ones (S-MSN, 80–150 nm) in both cancerous...
In the culture medium so as to inhibit the promotion of the aggregation of the particles with a high positive charge could copolymer micelles gave the reverse result, in which the rod-like complexes were taken up in amounts 12 times greater than their spherical counterparts.77

In addition, different cell lines may also respond differently to the particle shape. The uptake rate was the same for both the T-MSN and S-MSN in the CHO cells, whilst a faster uptake of the S-MSN was recorded in fibroblasts. This difference might be caused by some variable of the particles such as their sizes and aggregation ability, leading to the difference in uptake mechanism.76 More efforts should be paid to verify the influence of particle shape and to clarify the controversy.

Surface chemistry dependence

The surface properties of the colloidal particles are the most important and most easily changeable parameter for cellular uptake and delivery. Indeed, it is the surface that in most cases provides the driving forces (electrostatic, hydrophobic and hydrophilic (polar) forces) for the uptake and decides the uptake pathway. Decoration of the particle surface with specific ligands can alter the uptake mechanism as well as the uptake rate and amount, which shall be discussed in the next session.

Many other factors can also be altered, for example, the wettability and surface charge. When the particle surface is modified with hydrophilic polymers such as PEG, the cellular uptake is dropped to 1/3 of the initial value.78 By contrast, a coating of poly (N-vinyl caprolactam) promotes the cellular uptake of PS particles.79

The surface charge largely influences the cellular uptake by electrostatic interactions since the cell surface is slightly negatively charged. When the surface zeta potential ranges from tens of $-\text{mV}$ to $+\text{mV}$ the particles can be internalized. Generally, a positive surface provides a stronger interaction with the cells, leading to a faster cellular uptake compared to neutral and negative surfaces.78 Yet Lorenz et al. observed that particles with a high positive charge could promote the aggregation of the particles in the culture medium so as to inhibit the cellular uptake.62 Moreover, the uptake of negative particles by HeLa cells was not monotonously decreased with the charge density, but fluctuation existed.80 In addition, a surface with a high positive charge promotes the non-specific adhesion of the particles to cells, but the neutral particles may minimize the un-specific adsorption of proteins and thereby favor a specific interaction, for example, ligand-mediated uptake.8

Not only the endocytosed dosage and rate of uptake of the colloidal particles but also the cellular endocytosis mechanism is governed by the surface charge. Harush-Frenkel et al. found that positively charged PEGylated PLA nanoparticles were internalized rapidly via the clathrin-mediated pathway, whereas the negatively charged PEGylated PLGA nanoparticles were internalized at an inferior rate without the same pathway.81

Ligand-mediated cellular uptake

Various ligands for targeting the particles to specific cells (e.g. cancer cells),82 intracellular organelles and even the nucleus can be conjugated to the colloidal particles, most frequently on their surfaces. The use of the targeting moieties can not only facilitate cellular uptake by the receptor-mediated endocytosis, but also decrease adverse effects by directly delivering the particles to the desired sites.

The ligands used so far include antibodies, peptides, proteins, cell growth factors, nucleic acid aptamers, carbohydrates and other small molecules.7 For example, a kind of monoclonal antibody (mAb), vascular endothelial growth factor (VEGF) modified polyelectrolyte complex micelles were specifically targeted to colorectal cancer cells.84 Decoration of paclitaxel-loaded PLGA nanoparticles with human epidermal growth factor receptor-2 (HER2) antibody Trastuzumab made multifunctional carriers targeting cancer cells, such as Caco-2 cells and SK-BR-3 (human Caucasian breast adenocarcinoma) cells.85 A lectin-conjugated isopropyl myristate (IPM)-incorporated PLGA nanoparticle system could locally deliver paclitaxel to the lungs.86 Some proteins such as transferrin can be used to target nanoparticles to those cells that over express the transferrin receptor on the cell membranes, e.g. HeLa K44A (expression of K44A-dynamin in the HeLa cells).11,87

A pH-sensitive polyacrylamide microparticle decorated with polyarginine, a cell-penetrating peptide (CPP), has the capability to penetrate the non-phagocytic cell membrane and release the encapsulated payload in sub-cellular organelles.88 Tat peptide derived from HIV-1 Tat protein is a typical nuclear translocation peptide, which is commonly anchored onto the surface of colloidal particles for aiding the uptake of colloidal particles into the cell nucleus.86 A cyclic arginine-glycine-aspartic acid (cRGD) peptide can bind to $\alpha_\text{v}\beta_3$ integrins, which are abundant on the membranes of lung cancer cells and pancreatic cancer cells, and thus can be used to specifically deliver the colloidal particles.87

Folic acid (folate) is a well known targeting molecule for many kinds of cancer cells in which folate receptors are frequently over expressed. Therefore, folate decoration can significantly promote the targeted delivery of drugs into cancer cells such as MCF-7 breast cancer cells and C6 glioma cells to enhance their therapeutic effect.16

Recently, the anchorage of two or more targeting ligands, to obtain multifunctional particles, has attracted much attention. Feldheim et al.83 decorated gold colloidal particles with a cell-targeting peptide (from an adenoviral receptor mediated endocytosis peptide) and a nucleus-targeting peptide (from an adenoviral nuclear localization signal peptide). Results showed that the gold colloidal particles bearing the two peptides had a greater propensity for nuclear targeting than any other single peptide explored (the gold colloidal particles could not enter the cells with only one kind of peptide, and could enter the cells but not the nucleus with another peptide). Therefore, many attempts at targeting the delivery of carrier particles into the desired cells and cell organs are underway.

Cytotoxicity of the colloidal particles

Internalization of the colloidal particles may cause various effects. One of the most concerning is their cytotoxicity, which is decisive for any application.19,79
The cellular uptake of the colloidal particles may bring about changes in cell morphology, viability, proliferation, function and some other cell events.

One reason for the adverse effects is simply the small size of the particles, since the same materials in bulk are traditionally bioinert and even biocompatible. For example, during the internalization of gelatin nanoparticles the membrane of human fibroblasts is disrupted and the cytoskeleton is disorganized, although the cell viability and morphology are not apparently altered.\(^8\) When H596 (the human non-small cell lung carcinoma cell line) cells are exposed to carbon-based nanomaterials, several morphological alterations are observed with loose attachment or even detachment from the culture dishes, retracted cytoplasms and more condensed nuclei. Consequently, the cell viability and morphology are not apparently altered.\(^8\)

Moreover, results show that the CNTs with a smaller aspect ratio have less cytotoxicity, indicating that the particle shape contributes to the adverse effect too.\(^8\) It should be further noted that the level of cytotoxicity is dependent on the cell types too. Xia et al. found that 60 nm NH\(_2\)-labeled PS nanospheres were highly toxic in macrophage and epithelial cells, but human microvascular endothelial, hepatoma, and pheochromocytoma (PC-12) cells were relatively resistant to particle injury.\(^9\)

Not only the viability and morphology but also cell functions are influenced by the cellular uptake. In vitro culture of MC3T3-E1 (mouse osteoblast-like cells) in PMMA particle-treated culture medium could significantly reduce their mineralization ability.\(^5\) Pisanic et al. found that the influence of the anionic iron oxide on PC-12 cells was dose dependent. The uptake of the particles could decrease the cell viability and capacity of neurites in response to nerve growth factor.\(^9\) Kostura et al. observed that uptake of SPIO (superparamagnetic iron oxide) particles would inhibit the adiagenic and osteogenic differentiation of human mesenchymal stem cells.\(^9\) In addition, since nanoparticles have been shown to penetrate subcellular structures such as the mitochondria and nucleus,\(^9\) their potential genotoxicity and mutagenicity should be noted as well.

The other main reason is related to the chemical composition and intrinsic toxicological properties of the chemicals. Among TiO\(_2\), SiO\(_2\), Co and Ni nanoscale particles, only the Co particles induce toxicity in endothelial cells, which is accompanied by the production of the pro-inflammatory cytokine IL-8.\(^8\) The QDs can release Cd\(^{2+}\) ions and produce reactive oxygen species (ROS) to induce cytotoxicity in a biological environment.\(^8\) The cytotoxicity of silver nanoparticles is attributed to the emergence of ROS,\(^9\) while silica-induced toxicity may be attributed to the particles or cell-derived free radical species and the lysosomal permeability.\(^9\) In addition, chemicals adsorbed on their surfaces may affect the reactivity of nanoparticles. Fractions isolated from particulate air pollutants (diesel exhaust particles) are demonstrated to exert toxic effects on cells in vitro.\(^9\) Nanoparticles in ambient air can have a very complex composition. For example, metallic iron is able to potentiate the effect of carbon black nanoparticles, resulting in enhanced reactivity including oxidative stress.\(^9\)

Nonetheless, more effort should be made to clarify the various effects.

The toxicity of the particles can be mediated by surface modification. For example, uptake of bare gold particles causes disorganization of the cytoskeleton and high cytotoxicity, whereas uptake of poly (e-caprolactone) (PCL) coated gold particles with the same dosage does not bring any apparent influence on cells.\(^17\) Moreover, silane-modified silica nanoparticles (16–50 nm) have a lower cytotoxicity for COS-1 cells,\(^101,102\) while the poly(L-lysine)-modification increases the cytotoxicity for HNE I (human nasopharyngeal carcinoma cell line) cells.\(^103\) The magnetite colloidal particles modified with lactoferrin, ceruloplasmin, pullulan, dextran and albumin\(^104–106\) show higher cell viability. Moreover, PEGylation and specific targeting can decrease unwanted adsorption and thereby increase the viability of cells and tissues.

**Perspective of the cellular uptake**

As described above, cellular uptake and intracellular delivery provide a lot of fundamental information for studying the interaction between colloidal particles and biological systems. Although many results have been obtained, some important issues still need to be further clarified.

The widely used labeling and detecting methods such as confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) are suitable for studying the subcellular distribution of colloidal particles, but they cannot be used to observe online the transportation process of the colloidal particles in the intracellular organelles. Novel labeling and detecting methods need to be used.\(^73,107\) For example, the gene encoding green fluorescent protein (GFP) can be linked to an endosome specific marker gene. After gene transfection, the cells express fluorescent endosome protein without disruption of the cell functions.\(^108\)

Together with online cell culture monitoring and CLSM observation techniques, it is possible to follow the intracellular processes of the colloidal particles.

The fate of colloidal particles after cellular uptake is another important issue. Generally, colloidal particles have three possible fates after cellular uptake. The first one is degradation by enzymes in the lysosomes, which is most applicable for biodegradable particles. Another one is exocytosis, the comparative process of endocytosis. The colloidal particles could be partially degraded to make them smaller, and then expelled out of cells via exocytosis. Compared with endocytosis, little attention has been paid to this issue.\(^66,109,110\) Chan’s group has reported the recent progress for cellular exocytosis and tried to elucidate the mechanism for both endocytosis and exocytosis.\(^71\)

However, most inorganic colloidal particles and some colloidal particles made of nondegradable polymers cannot be degraded or expelled easily due to their excellent stability and relatively large sizes. Consequently, accumulation of the particles inside the cells occurs, leading to side effects on the original cell functions. For example, the accumulation of gold nanoparticles inhibits the proliferation of multiple myeloma cells through cell-cycle arrest in the G1 phase.\(^111\) Therefore, long term evaluation of the cytotoxicity is necessary to elucidate the comprehensive effects of the colloidal particles. Another interesting question is where the particles are accumulated when the cells proliferate. Whether they will divide equally into the two daughter cells as other subcellular organelles do or just remain in one daughter cell is still a mystery.
Lastly, the alteration of cell functions should be paid more attention. Actually, the cellular uptake of colloidal particles may induce oxidative stress, affect the cell cycle, reorganize the cell skeleton, influence cytokine synthesis and even show oxidative damage to DNA. Yet these issues are rarely mentioned and discussed. Moreover, cells of different types may respond differently to the same particles (for example, macrophages have a preference for nanosurfaces and are activated by them but osteoblasts move away from the nanosurfaces), thus comparison studies are required too.

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References