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Biophysical Responses upon the Interaction of Nanomaterials with Cellular Interfaces

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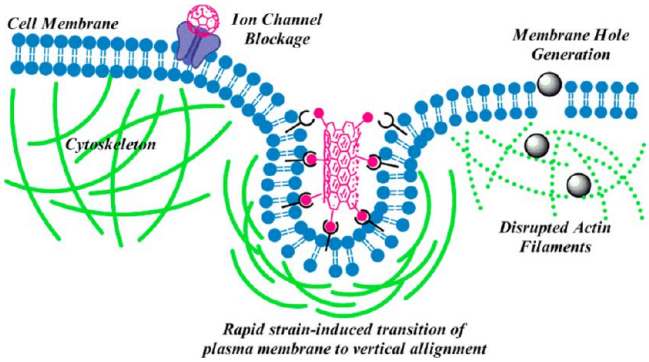

The explosion of study of nanomaterials in biological applications (the nano–bio interface) can be ascribed to nanomaterials' growing importance in diagnostics, therapeutics, theranostics (therapeutic diagnostics), and targeted modulation of cellular processes. However, a growing number of critics have raised concerns over the potential risks of nanomaterials to human health and safety. It is essential to understand nanomaterials' potential toxicity before they are tested in humans. These risks are complicated to unravel, however, because of the complexity of cells and their nanoscale macromolecular components, which enable cells to sense and respond to environmental cues, including nanomaterials.

In this Account, we explore these risks from the perspective of the biophysical interactions between nanomaterials and cells. Biophysical responses to the uptake of nanomaterials can include conformational changes in biomolecules like DNA and proteins, and changes to the cellular membrane and the cytoskeleton. Changes to the latter two, in particular, can induce changes in cell elasticity, morphology, motility, adhesion, and invasion. This Account reviews what is known about cells' biophysical responses to the uptake of the most widely studied and used nanoparticles, such as carbon-based, metal, metal-oxide, and semiconductor nanomaterials.

We postulate that the biophysical structure impairment induced by nanomaterials is one of the key causes of nanotoxicity. The disruption of cellular structures is affected by the size, shape, and chemical composition of nanomaterials, which are also determining factors of nanotoxicity. Currently, popular nanotoxicity characterizations, such as the MTT and lactate dehydrogenase (LDH) assays, only provide end-point results through chemical reactions. Focusing on biophysical structural changes induced by nanomaterials, possibly in real-time, could deepen our understanding of the normal and altered states of subcellular structures and provide useful perspective on the mechanisms of nanotoxicity. We strongly believe that biophysical properties of cells can serve as novel and noninvasive markers to evaluate nanomaterials' effect at the nano–bio interface and their associated toxicity. Better understanding of the effects of nanomaterials on cell structures and functions could help identify the required preconditions for the safe use of nanomaterials in therapeutic applications.

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1. Introduction

Over the past 20 years, the study of nano–bio interfaces has witnessed an explosion of development. One of the reasons for this is that engineered nanomaterials have been widely used in biological assays to detect nucleic acids and protein markers for many diseases, since they provide novel ways of detecting and imaging biomarkers at low concentrations, in complex sample media (such as serum), and with a wide variety of assay read-outs.[1,2] Furthermore, nanomaterials-based therapeutics have been employed for the treatment of cancer, diabetes, neurodegenerative diseases, infections, and inflammation to mention a few.[3] An emerging field of theranostic nanomedicine, in which disease diagnosis and therapy are combined within a single formulation,[4] is rapidly gaining ground. For instance, multifunctional nanoparticles that combine diagnostic and therapeutic applications by triggering gene or drug release at target sites when exposed to external stimuli are drawing much attention, as they can be very powerful for real-time visualization and in understanding drug delivery, release, and efficacy.[5] Finally, the study on how chemical and physical cues modulate the responses of cells in contact with the nanomaterials can produce profound and fundamental new insights into biological processes, such as cell migration, differentiation, metastasis, and immune function.[6,7]

The scrutiny of the nano–bio interface requires a clear understanding of not just functions of the nanomaterials but also about their toxicity. The sizes, shape, surface chemistry, and compositions of nanomaterials can be systematically tailored to produce materials with appropriate intrinsic physicochemical properties, which make these materials ideal for biomedical applications. However, a growing number of critics have raised several concerns on their potential threats or risks (e.g., toxic effects) to environmental health and safety. For example, postinjection, nanomaterials were found to be accumulated in kidney, liver, or spleen and were excreted via kidney or renal elimination, which is heavily dependent on their size as well as surface properties.[8] Surface modification of nanomaterials with polyethylene glycol (PEG), known as PEGylated nanomaterials, is considered a breakthrough in avoiding macrophage recognition and phagocytosis for a prolonged period of circulation and enhanced permeability and retention. However, this promising development still presents some issues such as the accelerated blood clearance (ABC) phenomenon upon repeated injection and consequently PEGylated nanomaterials lose their sustained circulation ability.[9] Furthermore, nanomaterials pose carcinogenic risk, which is triggered by the production of reactive oxygen species (ROS) by macrophages attempting to destroy the foreign material in the inflammation site and in terms of DNA damage as well as induction of inflammatory lesions associated with carcinogenesis.[10] It can be safely said that the sizes, shapes, surface functionality, and compositions of nanomaterials are important issues in nanotoxicity studies,[10−12] where MTT assay, a colorimetric strategy for assessing the viability or the proliferation of cells, and lactate dehydrogenase (LDH) assay, an indicator of cell membrane integrity,[13] are routine methods to determine the cytotoxicity of the nanomaterials. In addition to the current characterization approaches, further understanding of the interactions between nanomaterials and cellular systems and exploring the mechanism of the biological fate (both the adverse and favorable aspects) of nanomaterials are critical for the design and development of safe nanomaterials in biomedical applications.[11]

Many toxicological aspects of nanomaterials have been reported in detail in a number of recent reviews.[12,14,15] Such studies focus on the issues of material composition and dimensions, routes of exposure and administration, translocation and distribution, and clearance from the body. However, perhaps more importantly, detailed mechanisms on nanomaterial–cell interaction as well as its impact on toxicity are still poorly understood. The biophysical properties of cells have been increasingly recognized as key determinants of normal cell function, and their alterations under pathological conditions are well documented.[16,17] For instance, a red blood cell (RBC) becomes stiff when the RBC is affected by sickle cell anemia.[16] Along the same lines, an interesting proposition is whether biophysical responses upon the uptake of nanomaterials into cells could be considered as markers for cellular phenotypic events associated with toxicity assessments. Cell viability is closely related to the effects of nanomaterials on cellular morphological and nuclear shape changes.[18]

In this Account, we would like to explore the biophysical responses upon the interaction of nanomaterials and cellular systems, by discussing general routes of the interaction between nanomaterials and cells, and highlight their emerging opportunities and challenges. The effect of nanomaterials on cell structures and functions, from a nanotoxicology perspective, could help identify the required preconditions on the use of nanomaterials for therapeutic applications. Moreover, we will elaborate on the interaction of nanomaterials with key cellular components, namely, cell membrane, cytoskeleton, and nucleus and their subsequent biophysical response. The biophysical responses include

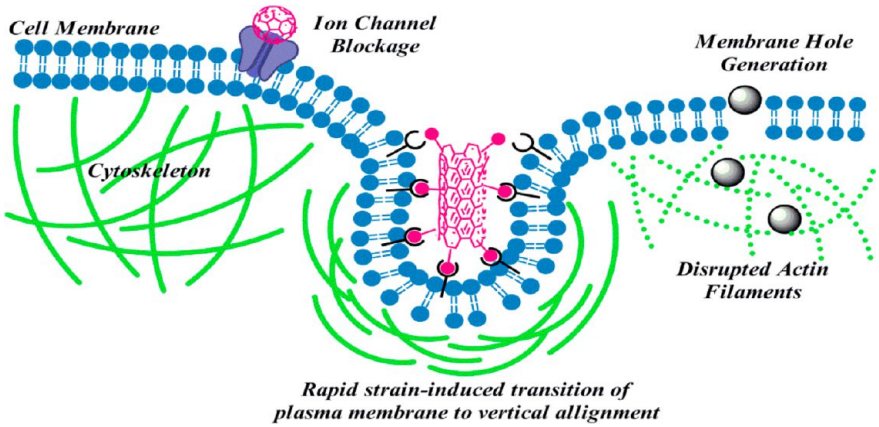

FIGURE 1. Deformation of cellular membrane and reconstruction and disruption of cytoskeleton upon the interaction between nanomaterials and cells.

conformational changes of biochemical molecules like DNA and proteins, deformation of cellular membrane, as well as reorganization of the cytoskeleton at a subcellular level inducing the changes in cell elasticity, morphology, adhesion, motility, and invasion.

2. Deformation of Cellular Membrane

As the primary defense barrier of the cell, the cell membrane forms the interface at which cells and nanomaterials first interact. The phospholipid bilayer structure of the cell membrane, a prime example of a nanomaterial−cell interface, is a 3D assembled layer with a rich variety of physical features designed to modulate cell signaling and protein function, as well as to maintain integrity and stability of the internal environment. When the nanomaterials interact with the cellular membrane, it may induce the disturbance of the phospholipids bilayer and blockage of membrane proteins, to name a few (Figure 1).

Disturbance of Phospholipid Bilayer. The lipid packing (solid gel phase or fluid phase) within a bilayer is closely related to the mechanical properties of the cell, including resistance of the cell to external stimuli such as stretching and bending.[19] It was found that the change in the cell membrane's local phase is closely related to the nanomaterials' surface charge.[20,21] Negatively charged nanoparticles bound to a fluid area of the membrane induced gelation, whereas positively charged nanoparticles turned gelled areas into a fluid state for easier penetration. This may therefore explain why cationic particles are more toxic than net neutral analogues or anionic analogues of the same size.[11] In addition, polymeric nanomaterials were reported to induce "holes" in the living cell membrane (Figure 2), which corresponded to regions of reduced lipid or protein levels and are associated with cytotoxicity.[22,23] This structural change of permeabilized cell membranes could lead to the leakage of cytosolic enzymes and result in toxicity. The formation of "holes" may be induced by the surface positive charges of nanomaterials that could in turn cause the fluid phase change of lipid bilayer while the neutral or negative control does not show any cytosolic enzyme leakage. Besides the surface charge, size of nanomaterials played a role in the cellular membrane disruption.[22,24] Nanoparticles of 1.2−22 nm could induce "holes" in lipid membranes, a process closely associated with nanotoxicity, while those nanoparticles with sizes less than 1.2 nm or more than 22 nm had no such effect.

Moreover, the surface chemistry of nanomaterials plays an important role in cell membrane disruption. Gold nanomaterials (about 6 nm in diameter) with the same chemical composition but different surface ligand organization (subnanometer striations of alternating anionic and hydrophobic groups or same moieties but in random distribution) showed dramatic differences in cell membrane response.[23] Nanomaterials with ordered ligand patterns could penetrate the cell membrane without bilayer disruption, while those with random ligand patterns were mostly trapped in endosomes. However, nanomaterials with suitable surface modification could serve as a good example for safe cell uptake. For example, conjugates between gold nanoparticles (about 13 nm in diameter) and oligonucleotides showcased attractive biological properties including high stability in biological environments, the ability to enter living cells without the use of transfection agents, enhanced target binding, and were nontoxic and nonimmunogenic.[25]

Blockage of Membrane Proteins. Besides the lipid bilayer of the cell membrane, nanomaterials could also induce

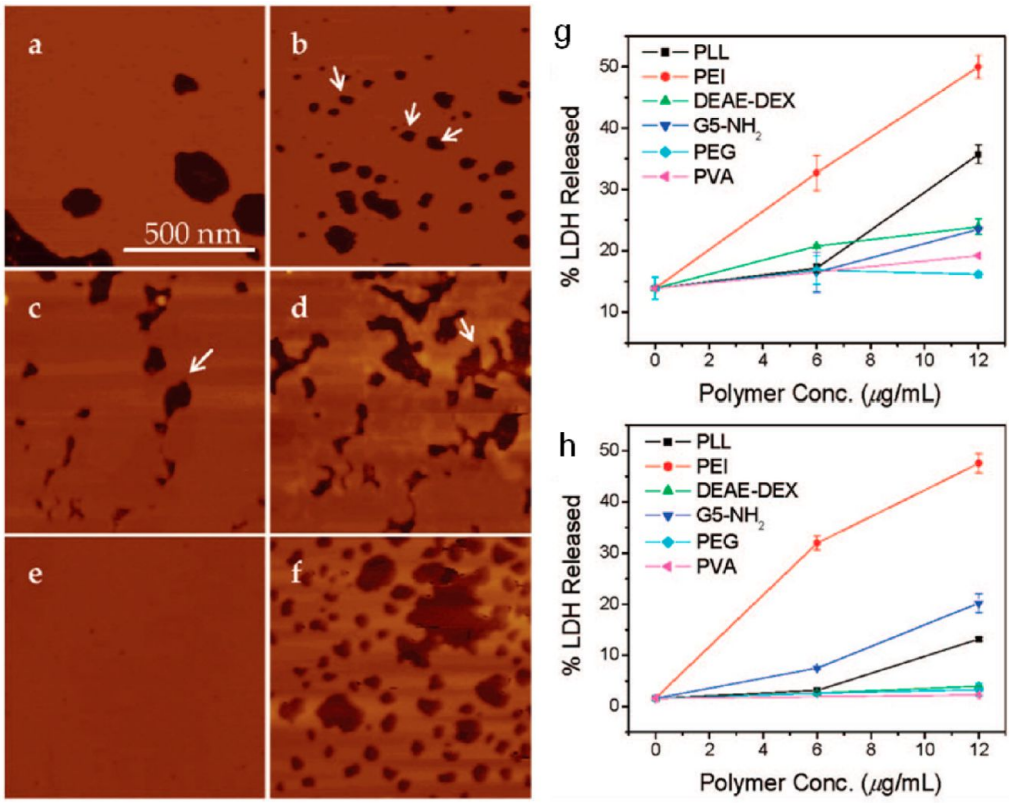

FIGURE 2. Interactions of other polymeric nanoparticles with biological membranes. (a–f) AFM observation of DMPC-supported lipid bilayers (a,c,e) before and after incubation with (b) poly(L-lysine), (d) polyethyleneimine, and (f) diethylaminoethyl-dextran, respectively. (g,h) Cytosolic enzyme LDH leakage out of (a) KB and (b) Rat2 cells as a result of exposure to the various polymeric nanoparticles at 37 °C for 3 h.[22] Reprinted with permission from ref 22. Copyright 2007 American Chemical Society.

physical response in membrane proteins, which play an important role in the molecular transport and cell surface transmembrane signaling. As one of the most important membrane protein complexes, ion channels exhibit unique structures, especially the pore complex, that provide the physical pathway for ion movements across the plasma membrane and several charged domains that attract and/or repel ions. These characteristics make ion channels easy targets for nanomaterials that react with and block these channels as evidenced by the physical blocking of potassium ion channel by spherical fullerenes (diameter 0.72 nm) and carbon nanotubes (diameter 1–15 nm) (Figure 3).[26] Spherical fullerenes showed the highest blocking ability due to their similar diameter to the size of potassium channels, while the multiwalled carbon nanotube with bigger diameter did not show any blocking effects. Another related example is the carboxyl-modified multiwalled carbon nanotubes of 40–50 nm diameter which could block potassium ion channels on the cell membrane, suppressing the current densities of transient outward current, delayed rectifier current and inward rectifier current in undifferentiated rat pheochromocytoma cell lines (PC12 cells).[27] Moreover, silver nanoparticles could cause conformational changes of the ion channel and alter the probability of channel opening, which may lead to nanotoxicity associated neuronal dysfunction.[28]

Unlike carbon-based nanomaterials' mechanism of physical obstruction, semiconductor nanomaterials were reported to induce oxidative stress damage, leading to impairment of the ion channel structure and function.[29,30] For example, oxidative stress induced by CdSe quantum dots (QDs) could activate N-type calcium channels and lead to the influx of extracellular calcium as well as rapid increase of intracellular calcium concentration, which is regarded as a possible mechanism of QD toxicity. The result reveals that both physical and chemical events in the membrane need to be considered during the nanomaterials' interaction with cells.

In short, the biophysical features of the cell membrane are increasingly being recognized as important control elements in cell signaling and membrane protein function. Nearly any chemical change in the membrane caused by

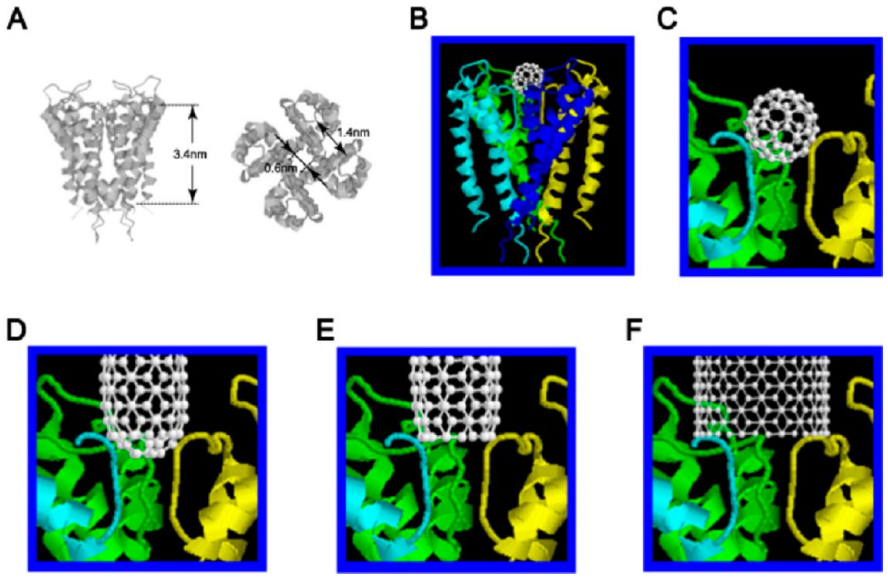

FIGURE 3. Carbon nanotubes block K^+ channels through a pore occlusion mechanism. (A) Crystal structure of the KscA K^+ channel. (B, C) Docking simulation of a fullerene with an average diameter of 0.7 nm. (D) Docking simulation of a capped carbon nanotube with an average diameter of 0.9 nm showing that, because of its spherical end, it can fit into the selectivity filter like a fullerene. (E, F) Docking simulation of an open-ended carbon nanotube with average diameters of 0.9 and 1.3 nm, respectively. Reprinted with permission from ref 26. Copyright 2003 American Society for Biochemistry and Molecular Biology.

lipid hydrolysis, trafficking, or sequestration has a physical consequence. Likewise, the binding and uptake of nanomaterials will cause redistribution of the hundreds of distinct lipid species that form the bilayer, which indicates the biophysical response of cellular membrane to nanomaterials. The investigation of these biophysical responses could have board implications for understanding nanomaterial–cell interaction as well as their corresponding safety issues.

3. Reconstruction and Disruption of Cytoskeleton

After passing the cell membrane, nanomaterials within the cell interact with the cytoskeleton, an interconnected network of filamentous proteins (microtubules, actin filaments, and intermediate filaments), and regulatory proteins which possess the ability to resist deformation. The cytoskeleton provides the mechanical stability and integrity of biological cells, transports intracellular cargo, and plays a key role during eukaryotic cell movement.

Reconstruction of Cytoskeleton. In particular, cellular uptake of nanomaterials is closely related to the deformation of cytoskeletal networks as well as changes in plasma-membrane tension and displacement of fluid in the cytoplasm (Figure 1). For example, significant increase in plasma-membrane tension was observed as neutrophils engulfed antibody-coated beads through phagocytosis, an actin-driven process.[31] This process starts with the interaction between nanomaterials coated with suitable ligands and cellular membrane receptor at the nano–bio interface.[11] This specific binding of nanomaterials to membrane receptors was driven by initial extension of cell membrane around the particle in a process that does not require actin polymerization. Active signaling from the receptor leads to the recruitment of numerous cytoskeletal proteins, including the Arp2/3 complex, which nucleates actin filaments beneath the particle. The formation of an actin network pushes the plasma membrane further around the target based on myosin-actin contractile activity, which leads to engulfment of the particle within the cell.

While most nanomaterials have been speculated to enter the cell via caveolin- and clathrin-mediated endocytosis, a subset of carbon nanomaterials enter the cell by adhesive interactions and are found free in the cytoplasm.[32,33] This subset of nanomaterials thus has the potential to interact directly with the cytoskeleton to influence mechanotransduction. As a typical example, the cytotoxicity of single-wall carbon nanotubes that resulted from high amounts of cellular uptake is likely due to the changes induced in cytoskeleton and cell morphology.[34] Human fibroblasts exposed to such nanotubes showed a random and irregular actin network compared with untreated cells with an organized radially distributed actin network.

Disruption of Cytoskeleton. Besides the reconstruction of cytoskeleton during the uptake of nanomaterials, the

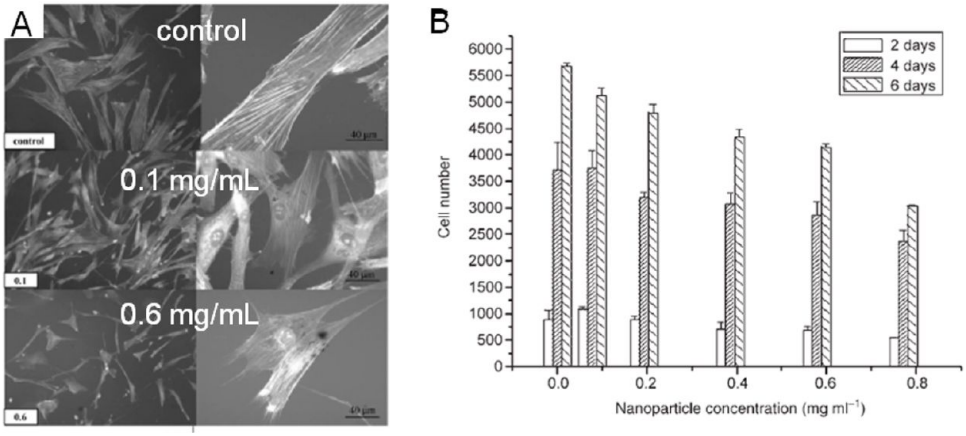

FIGURE 4. (A) Dermal fibroblasts imaged with an Hg lamp after 6 days for the control and for cells with 13 nm gold nanoparticle concentrations of 0.1 and 0.6 mg/mL. (B) Viability of cells incubated at nanoparticle concentrations of 0, 0.2, 0.4, 0.6, and 0.8 mg/mL for 2, 4, and 6 days. Reprinted with permission from ref 37. Copyright 2006 John Wiley and Sons.

nanomaterials could also bind directly and impair the ordered subcellular structures, consequently bearing a negative impact on cellular function. Nanomaterials with different shapes could lead to different effects on the cytoskeleton of the same cell lines.[35] For instance, mesoporous silica nanoparticles (diameter of 100 nm) did not disturb the actin filaments in the cytoskeleton, which remained well organized in thick bundles forming stress fibers that stretched between the cell surface and the cytoplasm. However, mesoporous silica nanorods with large aspect ratio of length to width (4:1) could disrupt and disorganize the actin filaments with poorly formed filament bundles in the region near the cell membrane and at the edges of lamellipodia and filopodia. Such shape-dependent cytoskeleton damage might rely on the cellular penetration ability of differently shaped nanomaterials, which was evident in the higher uptake amount of rod-shaped mesoporous silica nanoparticles leading to more serious damage to the cytoskeleton.

Moreover, gold nanomaterials have also been reported to induce cytoskeletal defects as well as profound effects on the morphology of several cell types, such as A549 human lung carcinoma cells.[36] Gold nanomaterials have also been described to have a concentration-dependent effect on the actin fibrils of human dermal fibroblasts (Figure 4).[37] Furthermore, disruption of cytoskeletal filaments is a function of gold nanomaterials exposure time, concentration and size, although actin or β-tubulin protein expression levels are not affected.[37,38] As a consequence, cell viability, spreading, adhesion, as well as synthesis of protein to form an extracellular matrix are impaired. These results indicate that the change in biophysical structure may induce major adverse effects on normal cellular functions.

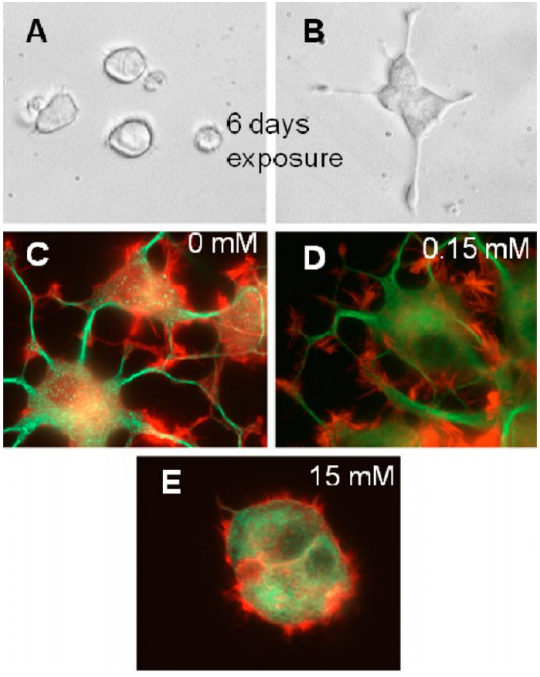

FIGURE 5. Phase contrast images of PC12 cells, 48 h in culture (A) without NGF and (B) with NGF. PC12 immunofluorescence for tubulin (green) and actin (red) at 6 days post Fe_2O_3 (5−12 nm) exposure and 5 days post NGF exposure at Fe concentration of (C, control cells) 0 mM, (D) 0.15 mM, and (E) 15 mM. Control cells form more actin microfilaments throughout the entire cell and produce more mature neurites than cells exposed to Fe_2O_3 nanomaterials. Reprinted with permission from ref 39. Copyright 2007 Elsevier.

Exposure to metal-oxide nanomaterials, such as ZnO or Fe_2O_3, leads to alterations in the cellular cytoskeletal network. For instance, the exposure of Fe_2O_3 nanoparticles with diameters ranging between 5 and 12 nm to PC12 pheochromocytoma neuronal cells could result in decreased number of actin filaments (Figure 5).[39] By increasing exposure

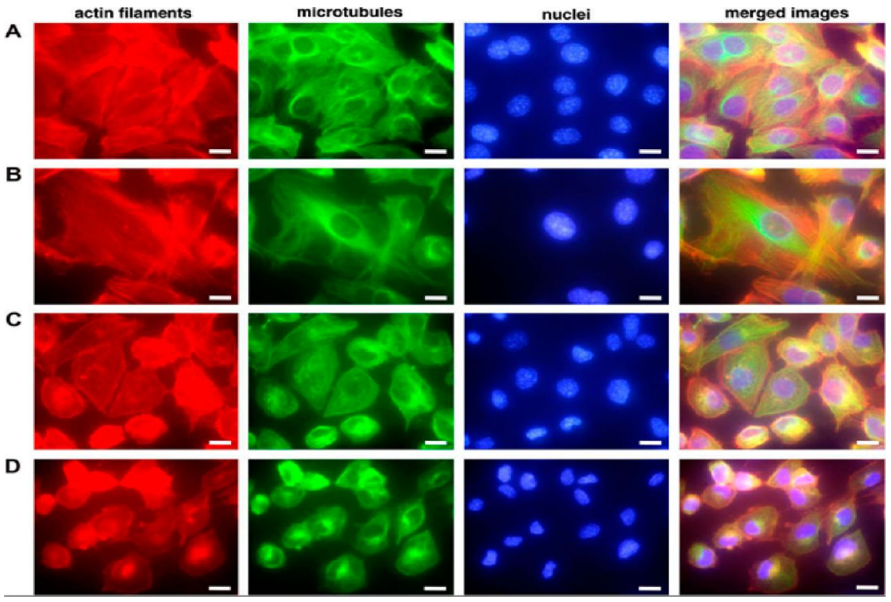

FIGURE 6. Representative fluorescence images showing structural changes in cytoskeleton and nuclei of 3T3 fibroblasts of (A) control cells; and cells treated with (B) 1 nM mercaptopropionic acid (MPA)-coated QDs, (C) 0.746 nM gum arabic/tri-*n*-octyl phosphine oxide (GA/TOPO)-QDs, and (D) 50 μM $CdCl_2$ for 6 h. Scale bars: 10 μm. Reprinted with permission from ref 42. Copyright 2010 Elsevier.

concentration from 0.15 to 15 mM, the cells appeared to possess little to no extended actin microfilaments within the soma, which made the cells spheroidal. It also caused the cells to exhibit minimal axonal/microtubule sprouting and failure to form mature neurites under the stimulation of nerve growth factor, indicating impaired cellular function due to cytoskeletal damage. Fe_2O_3 nanomaterials greatly disrupted actin fibers and tubulin network of human umbilical vein endothelial cells (HUVECs) and also impeded the maturation of focal adhesion complexes, which linked the cytoskeletal network to the extracellular matrix.[40] These cytoskeletal deformations also decreased the capacity of HUVECs to form vascular networks. A variety of Fe_2O_3 nanomaterials, including lipid-, dextran- and citrate-coated nanomaterials, induced actin and tubulin network deformations when high intracellular levels were reached in neural progenitor cells and primary human blood outgrowth endothelial cells.[41] It was hypothesized that the mere physical presence of high amounts of Fe_2O_3 nanomaterials enclosed in large and bulky lysosomal structures typically located in the perinuclear region, sterically hindered the cytoskeletal network and hereby induced the remodeling of the actin network. Moreover, with the destruction of the cytoskeletal network, the affected cells showed decreased expression of cell adhesion proteins as well as lower adhesion area, as the cytoskeleton is a key component for cell adhesion and migration. Furthermore, the cytoskeleton could be deformed after the uptake of QDs. There were significant structural changes in actin and tubulin networks of 3T3 fibroblasts after incubation with CdSe/ZnSe QDs (Figure 6).[42] It is also noted that the different surface modifications of QDs would lead to various degrees of cellular effects.

In short, the cytoskeleton plays an important role in the nanomaterial uptake process as well as cytotoxicity research. The disruption of cytoskeleton as well as its associated cytotoxicity is proportional to the properties of the nanomaterials, whose key parameters are exposure concentration, time, chemical composition, physical geometry, and surface modification, as mentioned in the discussion above. Better understanding of this nanomaterial–cytoskeleton interaction could shed light on pathologies that result from perturbation in cytoskeletal architecture due to nanomaterials.

4. Disruption of Nucleus

Lastly, due to the minute size, charge, and high specific surface area of nanomaterials, coupled with other physicochemical features such as metal contaminants, these nanomaterials may be able to enter cell nuclei and induce unpredictable genotoxicity. For instance, gold nanomaterials with smaller sizes (about 1.4 nm) with the ability to penetrate the nuclear compartment could potentially bind to negatively charged DNA due to gold's electronegativity.[43]

The interaction of nanomaterials with nuclei may go through similar substitution and removal processes like other well studied synthetic DNA binding drugs such as Cisplatin. Platinum in Cisplatin binds specifically to DNA and stalls the DNA replication complex during S-phase of

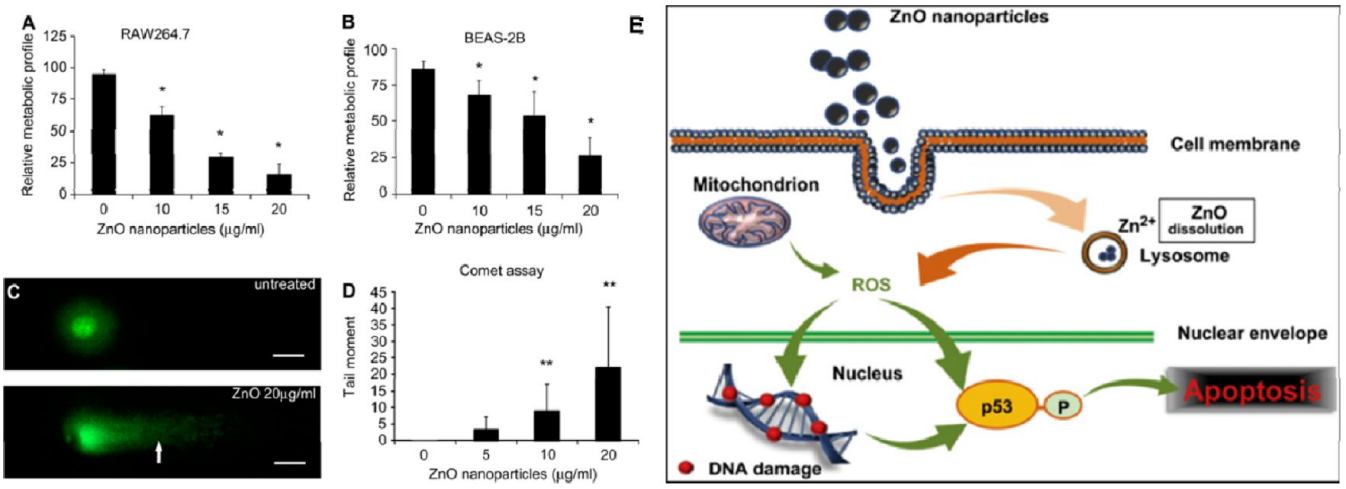

FIGURE 7. Cytotoxic and genotoxic effects of ZnO nanoparticles. WST-8 assay performed on (A) RAW264.7 and (B) BEAS-2B cells showed cytotoxic effects of ZnO nanoparticles in a dose dependent manner after 24 h exposure. (C) DNA damage in BEAS-2B cells was observed using the comet assay. Scale bars: 30 μm. (D) Plot of tail moments indicated that there was increasing DNA damage in BEAS-2B cells with increasing ZnO nanoparticle concentration. (E) Proposed cellular response mechanism involving p53 pathway. Reprinted with permission from ref 45. Copyright 2011 Elsevier.

the cell cycle. Similarly, intranuclear localized modified nanomaterials can bind to DNA in a way that either results in mutations or totally stalls replication of chromosomal DNA. This issue is of prime importance in normal replicative cells like epidermal stem cells in the gut and skin, stem cells in the gametes, developing embryo and fetus, and abnormally replicative cancer cells. Besides directly binding to DNA, nanomaterials may cause DNA damage indirectly, by promoting oxidative stress and inflammatory responses.[44] Whichever the origin of DNA damage, in the short term, nanomaterial induced DNA damage converges to the activation of p53.[45] p53 then activates either antioxidative stress genes in the case of reactive oxygen species (ROS) mediated DNA damage or apoptosis when the DNA damage exceeds reparable limits.[46] ZnO nanomaterials with the ability to release transition metal ions could induce the formation of ROS inside the cell by using intracellular oxygen (Figure 7).[45] In fact, the toxicity effect of ZnO is accentuated by ROS.[47] The generation of ROS was found to be closely associated with the oxidation of DNA and proteins inside the nucleus, as well as DNA breakage or transverse coupling. This hypothesis was also strongly supported by the findings that the distribution of TiO_2 nanoparticles, with the ability to induce DNA damage and genetic instability in mice,[48] was consistent with the site of ROS production in the cell.[49] During this process, the accumulation of oxidative stress induced by TiO_2 nanoparticles activated cell inflammatory response, which caused increased DNA damage, chromosome breaks, and point mutations; as well as inhibition of DNA repair, induced by abnormal methylation of bases, causing abnormal gene expression. In the long term, as the nucleus produces all the necessary proteins to maintain the physical properties of cell, any damage in the nucleus by nanomaterials could indirectly induce the biophysical response of cells.

Due to the ability of semiconductor nanomaterials to penetrate nuclear membranes as a result of their small size, they showed direct damage to chromosomes or nucleoproteins.[50] On the other hand, nanomaterials could also cause nuclear damage though indirect effects. The oxidative stress or inflammation induced by nanomaterial uptake by cells serves to cause nuclear damage. For instance, quantum dots were able to enter the nucleus through the nuclear pore, inducing nuclear protein aggregation, as well as the inhibition of gene transcription and cell proliferation.[51] In addition to the ability to cause oxidative damage to cells, nanomaterials were experimentally proven to also affect genes associated with genomic stability and DNA repair.

In short, small nanomaterials or those with the ability to release transition metal ions are capable of penetrating nuclear membrane and producing damage to genomic component inside nucleus. Such damage to nuclear components has a direct link with nanotoxicity, which reveals the importance of studying the integrity of cell nucleus at the nano–bio interface.

5. Future Prospects

The study of interactions between nanomaterials and biological soft interfaces allows researchers to understand the underlying mechanisms of nanotoxicity, thus aiding in

designing safer nanomaterials. The above-mentioned nanotoxicity at nano–bio interfaces relies heavily on the physical property changes in cellular structures as well as cell mechanics, and is thus a hotspot of research within this field of nanotoxicology. The success in using biophysical properties as a model to distinguish the cell state opens up a new possibility to exploit them for nanotoxicity research.

While the effects of nanomaterials on cellular biophysical responses have only recently garnered attention, the underlying mechanisms and subsequent consequences have not yet been investigated in-depth. This Account highlights the susceptibility of cellular physical structural and functional changes due to specific nanomaterial exposure and directs the limelight on the causality of biophysical changes in nanotoxicity. These responses indicate that the cell's physical behavior can change during the binding, uptake or intracellular accumulation of nanomaterials, which are closely related to toxicity. Hence, the biophysical responses of cells at the nano–bio interface could present a new angle to study nanotoxicity. It also sheds light on the ways to utilize biophysical properties as markers to quantify the effect of chemical stimulus (nanomaterials) on cells. For example, the nanomaterial–cytoskeleton interaction mentioned above could lead to a change in cellular stiffness and contractility of the cell.[52] Measuring these properties can therefore serve as a sensitive tool for probing the organization of the cytoskeleton,[17,53,54] as well as the signaling pathways that control the cytoskeleton. However, biophysical responses have not been extensively researched, which provide a novel method to study nanotoxicity. We envision that increasing collaboration between practitioners in the fields of biology, medicine, physics, and chemistry will yield new fundamental insights into nanotoxicity research. Future explorations of mechanobiology at the nano–bio interface will help to identify pathogenic nanomaterial species and contribute to a better understanding of the effects of nanomaterial exposure to human health. New developments in nanotechnology tools and analytical methods,[55,56] such as plasmonic spectroscopy, microscopes with superhigh spatial and temporal resolution, along with simulation and modeling, will promote and ignite the exploration of health and safety considerations for nanotechnology.

BIOGRAPHICAL INFORMATION

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FOOTNOTES

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The authors declare no competing financial interest.

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