Assessing Nanoparticle Toxicity

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Abstract

Nanoparticle toxicology, an emergent field, works toward establishing the hazard of nanoparticles, and therefore their potential risk, in light of the increased use and likelihood of exposure. Analytical chemists can provide an essential tool kit for the advancement of this field by exploiting expertise in sample complexity and preparation as well as method and technology development. Herein, we discuss experimental considerations for performing in vitro nanoparticle toxicity studies, with a focus on nanoparticle characterization, relevant model cell systems, and toxicity assay choices. Additionally, we present three case studies (of silver, titanium dioxide, and carbon nanotube toxicity) to highlight the important toxicological considerations of these commonly used nanoparticles.

1. INTRODUCTION

Nanotoxicity: refers to the study of the potential toxic impacts of nanoparticles on biological and ecological systems

In vitro: describes experiments done in cells isolated from a living organism

In vivo: describes experiments done in a whole, living organism

Nanotoxicity, a term coined in 2004, refers to the study of the potential toxic impacts of nanoparticles on biological and ecological systems. Early nanotoxicity studies arose from aerosol studies examining size-dependent particle effects; the field continues to draw from that heritage as well as from diverse fields such as molecular toxicology, material science, molecular biology, analytical chemistry, and engineering. According to the National Nanotechnology Initiative, nanotechnology is "the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications" (1). Although this size definition is no longer explicitly followed in the categorization of nanomaterials, these unique properties make nanoparticles the subject of intense study and commercial/industrial interest. In fact, as of March 2011, nanoparticles were found in 1,317 commercially available products (2) and, as of September 2011, were responsible for almost 278,000 SciFinder scholar hits (search term: nanoparticle).

During an average day, people may be exposed to commercially available nanoparticles in many settings, including silver (Ag) nanoparticles in sheets and clothing, titanium dioxide (TiO_2) nanoparticles in cosmetics and sunscreens, carbon nanoparticles in bikes, and even clay nanoparticles in beer bottles (2). Over the past eight years, the field of nanotoxicity has grown significantly in response to and in hopes of addressing concerns (both public and regulatory) regarding the boom in nanoparticle technology and the subsequently increased possibility of exposure through consumer and medical applications (3).

A general goal of the nanotoxicity field is to build design rules for the synthesis of safe nanoparticles; therefore, systematic studies are essential and should be based on well-characterized physicochemical nanoparticle properties and their effects on cellular viability and function in relevant model systems. Risk assessment strives to determine risk on the basis of the possibility of exposure and the hazard of the potential toxic substance—in this case, nanoparticles—to make regulatory decisions. This review showcases some of the key roles analytical chemists can play in the field of nanotoxicity; we focus on recent work that considers the toxicity of engineered nanoparticles by use of in vitro mammalian models. We discuss several critical considerations, specifically the importance of nanoparticle physicochemical characterization along with cell model and toxicity assay selection, as they provide the foundation for systematic nanotoxicity studies. These areas are ripe for exploration by analytical chemists because, as with molecular toxicology, there is a need to increase the correlation between in vivo and in vitro studies. Additionally, analytical chemists provide a necessary tool set (e.g., sample preparation/characterization and method/technology development) to perform detailed analysis of complex systems that often present challenges in the assessment of nanotoxicity. Using nanoparticle-characterization methods, model cells, and toxicity assessments, we present three case studies for representative nanoparticle classes to examine the present body of literature; the goal is to highlight specific experimental considerations while drawing some general conclusions about current knowledge in the field of nanotoxicity.

2. NANOPARTICLE CHARACTERIZATION

Nanoparticles' physicochemical properties must be examined in detail to create nanoparticle design rules and to begin interpreting any results due to nanoparticle-induced toxicity. Because the field of nanotoxicity is relatively new and the specific nanoparticle properties that influence cellular toxicity are still not fully understood, a thorough characterization of the nanoparticle is essential. There is some agreement about the basic nanoparticle properties that should, at a minimum, be characterized (summarized in **Table 1**) to ensure thorough toxicity studies that produce sound conclusions (4–6).

Table 1 Important nanoparticle properties and common methods for characterization

Physiochemical properties	Common characterization methods[a,b]
Size (distribution)	TEM, AFM, DLS, NTA
Shape	TEM, AFM, UV-vis (for plasmonic nanoparticles)
Agglomeration or aggregation state	DLS, UV-vis (for plasmonic nanoparticles)
Crystal structure	XRD, ED
Surface chemistry/charge/area	AES, EELS, XPS, solid-state NMR, ζ-potential, BET
Stability over time/dissolution	DLS, UV-vis, ICP-AES, ICP-MS, colorimetric assays
Dosing metric	Variable
Uptake	ICP-AES, ICP-MS, TEM, fluorescence, flow cytometry, NAA

[a]Abbreviations: TEM, transmission electron microscopy; AFM, atomic force microscopy; DLS, dynamic light scattering; NTA, nanoparticle-tracking analysis; UV-vis, UV-visible spectroscopy; XRD, X-ray diffraction; ED, electron diffraction; AES, Auger electron spectroscopy; EELS, electron energy loss spectroscopy; XPS, X-ray photoelectron spectroscopy; NMR, nuclear magnetic resonance; BET, nitrogen adsorption/desorption isotherm; ICP-AES, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; NAA, neutron activation analysis.
[b]Not an exhaustive list of characterization approaches.

Various approaches are commonly used to characterize these properties. Size (distribution) determinations are typically assessed with one or more of the following: transmission electron microscopy (TEM) (7–9), dynamic light scattering (10, 11), and/or nanoparticle-tracking analysis (12). Shape determinations are generally established while size is investigated with TEM or atomic force microscopy. Determination of aggregation and/or agglomeration state is important and can be difficult (5). Some studies have approached this determination by perturbing the nanoparticle environment (e.g., pH and temperature) and examining changes in size distribution with dynamic light scattering (13), similar to stability studies in which changes are monitored over time (14). Crystal structure is generally studied by X-ray diffraction (9, 14–16), and surface area is determined with nitrogen adsorption/desorption isotherms (9, 15, 16). Surface chemical composition can be examined through various techniques and has recently been reviewed elsewhere (17). Although not a physical characteristic of the nanoparticle, characterization of the dose of nanoparticles is also critical for the interpretation of results. The determination of a dosing metric is inherently complicated because little is known about appropriate doses and how aggregation or stability influences effective dosing. Accordingly, there is little consistency in dosing metrics among studies; these metrics range from molar nanoparticle concentration to nanoparticle surface area.

Once initially characterized, nanoparticles are exposed to biological media where proteins are adsorbed to their surface, thereby altering the nanoparticles' original properties (12, 16). Ideally, nanoparticle characterization would take place before, after, and throughout the in vitro exposure to provide dynamic insight into any changes the nanoparticles undergo during exposure (e.g., altered aggregation state, adsorbed proteins). The most desired, and unavailable, characterization steps are for in situ measurements during exposure. Recently, nanoparticles have been characterized within various biological media at physiological temperature, which provides characterization data for nanoparticles in a more realistic state with the use of existing methods (12, 14, 16, 18). Analytical chemists have the potential to make significant contributions in nanoparticle characterization by developing methods to perform the desired in situ measurements, to improve upon current methods, or to help introduce more complex characterization methods to better model realistic in situ measurements. These characterization challenges are ripe for study and application of the collective expertise of analytical chemists.

Aggregation: an irreversible grouping of nanoparticles

Agglomeration: a reversible grouping of nanoparticles

3. IN VITRO MODEL SYSTEMS

Immortal: describes cells that proliferate indefinitely

Primary: describes cells collected directly from a live organism

There are four common routes through which a person can be exposed to nanoscale materials: ingestion, injection, transdermal delivery, and inhalation. Although various in vitro models are used in molecular toxicology (19, 20) to model critical portions of each of these four pathways, in vitro nanotoxicity studies often employ much simpler model systems. The vast majority of in vitro nanotoxicity assays examine nanoparticle influence on a single, homogeneous, immortal cell type. The cell types chosen often reflect a critical component of the exposure route and physiology of interest.

3.1. Ingestion

Many groups have used the undifferentiated human colon adenocarcinoma cell line known as Caco-2 to model the uptake and/or viability of cells following the ingestion of various engineered nanoscale materials, including metal oxide–, polymeric-, and carbon-based nanoparticles (21–23). RKO immortal colon cancer cells have also been employed; in one example study, cell viability was assessed following zinc oxide (ZnO) nanoparticle exposure (24). In an unusual case, primary-culture murine intestinal dendritic cells were employed to assess the toxicity of silicon dioxide (SiO_2) and TiO_2 nanoparticles; the investigators examined the secretion of inflammatory mediators from these cells (25).

3.2. Injection

Many nanoparticles for which injection toxicity is studied are intended for use as drug delivery or imaging agents. A small set of nanotoxicity studies have examined the direct effect of nanoparticles on primary-culture blood cells, including measures of stem cell viability, hemolysis, platelet activation, platelet aggregation, and coagulation time with, for example, hydroxyapatite-based contrast agents (26) or atomically thin graphene oxide (27). In some cases, nanoparticles yield false positives that can be avoided with careful controls (28). Other primary-culture cells employed in the study of injection nanotoxicity include (*a*) primary human umbilical vein epithelial cells following exposure to quantum dots (QDs) (29) and (*b*) rat skeletal myoblasts and bone marrow–derived mesenchymal stem cells following exposure to lanthanide-doped SiO_2 nanoparticles (30). Immortal cancer cell lines are also commonly employed because many nanoparticles are intended to target cancerous cells; the most commonly used cell lines are HeLa (31), MCF-7 (32), HCT-116 (33), BEAS-2B (34), and 3T3 fibroblasts (35). Although these lines are sometimes chosen on the basis of their organ of origin (and the eventual cancer target), few papers follow up with in vivo toxicity studies using the same cell type. One exception is a study performed to assess the in vitro cytotoxicity of polymeric nanoparticles in cultured MCF-7 and C26 cell lines, which was followed by an in vivo experiment with C26 solid tumors (36).

3.3. Transdermal Delivery

Although several ex vivo nanoparticle toxicity studies have been performed in which portions of skin are isolated intact, in vitro studies have focused mainly on keratinocytes or dermal fibroblast cells. Neither cell type is present in the outermost layer of skin, so any toxic effects are probably relevant only if the in vivo epidermal layer is damaged. For example, the human-derived keratinocyte HaCaT cell line has been used to assess (*a*) the role of TiO_2 and UV-irradiated TiO_2 in causing reactive oxygen species (ROS)-induced damage (37) and (*b*) whether TiO_2 and ZnO nanoparticles induce changes in the intracellular formation of radicals, cell morphology, mitochondrial activity,

or cell-cycle distribution (38). Sharma et al. (39) used primary human epidermal keratinocytes (HEKs) to examine whether ZnO nanoparticles compromise viability or induce DNA damage. A mouse keratinocyte cell line (HEL-30) was used to explore the role of both the size and the crystallinity of TiO_2 nanoparticles in cytotoxicity (40). Clearly, the most common nanoparticle types considered in transdermal exposure studies are metal oxide nanoparticles because of their common use in sunscreen and cosmetics; however, there are a few exceptions. For example, the influence of Ag nanoparticles on HaCaT cell viability and growth has been monitored (41), and HEKs have been used to study cell viability and the release of inflammatory mediators upon exposure to high–aspect ratio QDs (42).

The toxicity profile of core-shell semiconductor QDs has also been explored through measurements of cell density, viability, and morphology in both HEKs and human dermal fibroblasts (HDFs) (43). HDFs have also been employed to explore the uptake mechanisms, localization, and toxicity of SiO_2 nanoparticles (44) as well as functionalized multiwalled carbon nanotubes (MWCNTs), with discouraging results (45). Another group examined MWCNT toxicity in various skin cells, including SZ95 sebocytes and immortal human keratinocytes; the authors found that appropriate dispersion of the MWCNTs minimized nanotoxicity (46).

3.4. Inhalation

Inhalation models are the most commonly employed of the four possible modes of nanoparticle uptake; cellular models are focused largely on readily available immortal lung cell lines. Of the various nanomaterials that have been studied in this context, TiO_2 is the most common. For example, Degussa AEROXIDE® P25 nanoparticles were added to two different immortal lung cell lines (A549 and H1,299) for correlated studies of uptake and viability (47). Another group (48) compared TiO_2 toxicity in normal human bronchial epithelial cells with that in two different epithelial cell lines (A549 and BEAS-2B), measuring both ROS and inflammatory mediator response in all three cultures. The differences in the results of this study highlight the importance of working with primary cells. Primary-culture, human nasal mucosa cells were exposed to TiO_2 for examination of cell uptake, viability, and genotoxicity (49).

From the inhalation perspective, carbon-based nanomaterials are also of general interest. One group studied the effect of carbon black nanoparticles on both a macrophage cell line (RAW 264.7) and primary human alveolar macrophages, examining caspase-1 activity and cell death (50). Another group compared in vitro and in vivo exposure of the lung to dispersed single-walled carbon nanotubes (SWCNTs) by using immortal lung fibroblasts (CRL1,490) for the in vitro work; the results show consistency between the in vitro and in vivo experiments (51).

Various other nanomaterials have been considered in inhalation-relevant monocultures, especially those using A549 and BEAS-2B cells. Nanotoxicity studies on A549 viability have focused on both TiO_2 and carbon nanotubes (CNTs) (52), Ag nanoparticles and Ag^+ (53), $NiFe_2O_4$ nanoparticles (54), and MWCNTs (55). BEAS-2B cells have been employed in studies on the uptake and/or toxicity of SWCNTs (56), hydroxyapatite (57), TiO_2 (58), SiO_2 (59), and graphite fibers (60).

3.5. Coculture Models

Although the vast majority of in vitro nanotoxicity work uses monocultures of cells, a small but growing segment of the literature is making use of coculture models. The natural cellular and chemical complexity of cocultures makes them more realistic models of mammalian physiology, and the results of coculture nanotoxicity experiments often differ from the results of monoculture

studies. For example, one group used a coculture of immortal Caco-2 and epithelial M cells to model the human intestinal epithelium and assess the toxicity of ingested Ag nanoparticles of various sizes (61). Another group cocultured immortal adipocytes and macrophages to simulate relevant cells in inflammatory response, then showed the antioxidant properties of introduced fullerenes (62). Also relevant to inflammation is a primary-culture, murine peritoneal mast cell (MPMC)–3T3 fibroblast coculture that has been systematically used to study mast cell degranulation following coculture exposure to gold (Au), Ag, SiO_2, and TiO_2 nanoparticles (7–9, 11). Another group (63) modeled inhalation toxicity by using the cocultured epithelial cell line H441 and the endothelial cell line ISO-HAS-1 to mimic the alveolar-capillary barrier; exposure of these cells to amorphous SiO_2 nanoparticles led to DNA damage and endoplasmic reticulum stress. These authors compared these results directly to those in each monoculture and found significant differences in toxicity markers (63). In another inhalation model, a triple-cell coculture model (composed of A549 cells, human blood monocyte–derived macrophages, and dendritic cells) simulating the alveolar lung epithelium was exposed to aerosolized Au nanoparticles; all the cells took up the nanoparticles without showing any cytotoxic effects (64). Going forward, analytical chemists will offer sample platforms, particularly through the development of microfluidic devices, to better replicate in vivo conditions in an in vitro environment.

4. BIOLOGICAL NANOTOXICITY ASSESSMENT

Common in vitro methods used to assess nanoparticle toxicity fall into two general categories: functional assays and viability assays. Herein, we classify functional assays as those that seek to assess the effects of nanoparticles on various cellular processes, whereas viability assays are concerned solely with whether a given nanoparticle causes death in a cell or a system of cells. A thorough review of both in vivo and in vitro assays can be found in Reference 65.

4.1. Uptake

Although uptake analysis does not specifically assess toxicity in a traditional sense, it is inherently linked to any assessment of toxicity. Furthermore, it measures a nanoparticle's ability to bypass cellular membranes, thereby influencing the nanoparticle's capacity to induce toxic effects. Common methods for assessing uptake include TEM, inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and fluorescence imaging. TEM, which has the added benefit of indicating the exact localization of particles within a cell (although it is generally not used to assess uptake quantitatively), has been used, for instance, to examine uptake of Au, SiO_2, and TiO_2 nanoparticles in MPMCs (7, 9). ICP-AES and ICP-MS, however, allow quantitative measurement of nanoparticle uptake and have been used to compare uptake differences arising from (*a*) variations in the size and shape of Au nanoparticles (66) and (*b*) the surface chemistry of Au (11, 67), Ag (11), and cobalt (Co) nanoparticles (68). Finally, fluorescence imaging is becoming an increasingly important method for assessing uptake and localization of nanoparticles as it can be used to quantify both uptake and localization of nanoparticles within a cell (when the nanoparticles under consideration are fluorescent). Using confocal microscopy, Ruan et al. (69) followed TAT peptide–conjugated QDs in HeLa cells over 24 h and assessed the effects of temperature on uptake.

4.2. Functional Assays

In this section, we describe commonly used functional assays, grouped by the (similar) cellular processes studied.

4.2.1. DNA synthesis and damage. DNA synthesis assays give critical information about the proliferative state and general health of dividing cells. Such assays are commonly used to assess cell proliferation or to quantify the number of cells in each stage of the cell cycle (which can subsequently reveal cell-cycle arrest at a given point). The incorporation of BrdU (5-bromo-2′-deoxyuridine) into newly synthesized DNA has been frequently employed to quantify DNA synthesis in nanotoxicity assays. This technique has been utilized to assess the genotoxicity of Ag nanoparticles and polyethylene glycol (PEG)-coated cadmium selenide/zinc sulfide (CdSe/ZnS) QDs on A549 cells and skin epithelial cells (HSF-42), respectively (70, 71). Although less common, a similar method of measuring thymidine incorporation has also been used to assess DNA synthesis of macrophages following Au nanoparticle exposure (72).

Damage to DNA is a fundamental example of cellular toxicity, and it is critical to assess such damage for any nanoparticle that is likely to come in contact with humans, given that damage to DNA is highly correlated with an increased risk of cancer. By far the most common method to assess DNA damage is the comet assay (single-cell gel electrophoresis assay), which is utilized to measure the number of single-strand breaks in DNA. This assay has been used to assess DNA damage in cells exposed to cerium oxide (CeO_2) (73), Co (74), Ag (75), and SiO_2 nanoparticles (76). Other methods to assess DNA damage include checking for the presence of micronuclei or other chromosomal aberrations and measuring the expression of proteins implicated in DNA repair (**Figure 1*a***). Oostingh and colleagues (70) used the cytokinesis-blocked micronucleus assay to show the influence of cobalt oxide, Au, Fe_3O_4, or CeO_2 on the amount of micronuclei in peripheral blood mononuclear cells. Others have monitored chromosomal aberrations that arose after exposure to Co/Cr alloy particles by visual inspection after fluorescence in situ hybridization in human fibroblasts (77). Finally, an increase in expression and activation of the DNA repair–related proteins was observed upon cellular exposure to MWCNTs (78).

4.2.2. Altered gene expression. Understanding the effect of nanoparticles on the cellular genome is a critical step toward achieving a real understanding of any nanoparticle's toxicity profile. The activity of functional genes implicated in various cellular processes can be quantitatively assessed through the use of techniques such as DNA microarray (general) or polymerase chain reaction (PCR; specific) analysis. DNA microarray analysis has been used to assess changes in gene expression upon exposure to Au nanorods (67), SWCNTs (79), and SiO_2-coated CdSe/ZnS QDs (71). Bregoli et al. (80) analyzed gene expression with PCR to study the effects of antimony trioxide (Sb_2O_3) nanoparticles in erythroblasts, whereas Park et al. (34) studied CeO_2 nanoparticle impact on the expression of genes related to oxidative stress and cell structure.

4.2.3. Immunogenicity. The ability of a given nanoparticle to evoke an immune response is a critical indicator of its toxicity to physiological systems, one that is not necessarily being explored by standard cellular toxicity studies. Cytokine levels can be accurately detected at minute volumes (picograms per milliliter) by an enzyme-linked immunosorbent assay. Using this technique, investigators have studied proinflammatory cytokines [e.g., interleukin (IL)-6 and IL-8] in various cell types following exposure to metal oxide nanoparticles (81, 82). A different approach used real-time PCR to quantify the degree of messenger RNA expression of IL-18 and its receptor, IL-18Rα, upon exposure to AgO nanoparticles (70).

4.2.4. Oxidative stress. An increase in the presence of ROS in the cellular environment has the potential to damage or disrupt a host of key cellular processes. This increase in ROS may result either from an innate immune response to a nanoparticle or from the ability of a specific nanoparticle (e.g., a fullerene or a metal oxide) to autocatalyze ROS formation (83, 84).

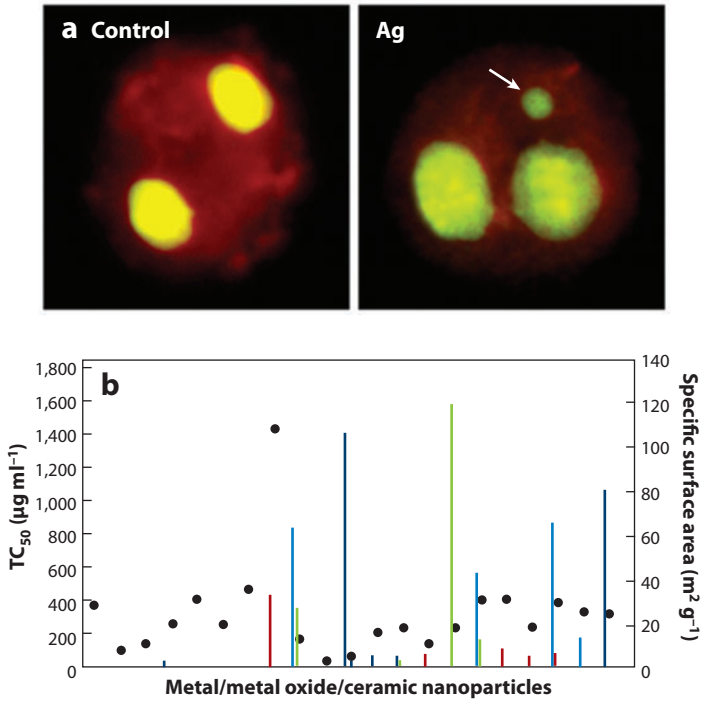


Figure 1

Examples of functional and viability assays used to assess nanoparticle toxicity. (*a*) Functional assay. Micronucleus analysis of human lung fibroblasts after exposure to control and 100 μg ml^{-1} Ag nanoparticles for 48 h. The arrow in the nanoparticle-exposed cell (*right*) highlights the micronucleus, which indicates nanoparticle-induced chromosomal breakage. Panel *a* adapted and reprinted with permission from Reference 75. (*b*) Viability assay. The toxic concentration necessary to cause mortality of 50% of the examined population (TC_{50}) values for 24 commercially available nanoparticles in A549 and THP-1 cells as determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and neutral red assays. Panel *b* adapted and reprinted from Reference 92. These assay results, and others like them, illustrate the complexity of drawing nanoparticle-specific conclusions about toxicity among various studies.

Generally, the presence of ROS is assessed either directly (by quantifying the amount of ROS present in a given cell population) or indirectly (by monitoring the secondary effects of prolonged oxidative stress). Direct measurements usually employ a spectrofluorimetry/flow cytometry– or spectrophotometry-based system to monitor the ROS-induced formation of the fluorescent product fluorescein from 2′,7′-dihydrodichlorofluorescein diacetate (DCFDA), the superoxide-induced conversion of dihydroethidium (DHE) from the blue fluorescent form to the red fluorescent form, or the superoxide-induced conversion of nitroblue tetrazolium (NBT) to blue formazan. Recent examples have employed the DCFDA and DHE assays to show changes in ROS levels in MPMCs (11) or human fibroblasts (75) exposed to Au or Ag nanoparticles with different surface functionalities. The NBT assay has been employed to study the effects of ultrasmall superparamagnetic iron oxide nanoparticles (85) and cationic lipid–coated Fe_3O_4 nanoparticles (86) in human monocyte macrophages and 3T3 cells, respectively. Measurement of the secondary effects of increased cellular ROS has been performed predominantly by assaying for lipid peroxidation or antioxidant depletion, which allowed for the detection of 8-hydroxy deoxyguanosine (8-OHdG) and superoxide dismutase (SOD) activity. To assess lipid peroxidation in the presence of Cd/Te QDs, Choi et al. (87) used a green fluorescent dye that inserts into the cell membrane and turns red in the

presence of oxidized lipids. Other groups have detected 8-OHdG by either high-performance liquid chromatography or immunochemical methods to assess oxidative stress caused by Au (88) and Co/Cr alloy nanoparticles (89).

4.2.5. Cell proliferation. The rate of cell growth is an important indicator of overall cell health and of the potential for nanoparticles to interfere with proliferative processes. Two quantitative assays have emerged as the standard for assessing cell proliferation: (*a*) cell counting by flow cytometry or high-content image analyzers and (*b*) the colony-forming efficiency (CFE) assay. Flow cytometry has been utilized to determine the effect of SWCNTs (79) and PEG-silane-modified CdSe/ZnS QDs (71) on the proliferation of HEK293 and human lung and skin epithelial cells, respectively. The CFE assay has been used to assess the effects of polymeric entrapped thiol–coated Au nanorods (90) and of Au, TiO_2, Fe_2O_3, Fe_3O_4, Ag, Co, and Sb_2O_3 nanoparticles (80) on murine fibroblasts and human hematopoietic progenitor cells.

4.2.6. Exocytosis. The effect of nanomaterials on vital cellular processes, such as exocytosis, should be intimately understood before any nanomaterial is deemed safe. Recently, a novel method, carbon-fiber microelectrode amperometry, has been employed to study the effect of various nanoparticles on the secretion of electroactive small molecules (e.g., serotonin, epinephrine). Use of this single-cell measurement method allows one to quantify the number of chemical messenger molecules released per vesicle, the specific release kinetics, and the frequency of vesicle fusion with high sensitivity and time resolution. Studies in MPMCs and adrenal chromaffin cells have utilized this method to reveal detailed changes in quantal content and frequency of vesicle fusion in response to SiO_2 (9), TiO_2 (9), Ag, and Au (8), as well as functionalized (with either positive or negative side chains) Au and Ag nanoparticle exposure (11).

4.3. Viability Assays

This section describes the assays commonly used to assess cell viability following nanoparticle exposure.

4.3.1. Metabolic activity. By a considerable margin, assays of metabolic activity are the most common methods used to determine cell viability following nanoparticle exposure. Of these assays, the most popular is the MTT assay—in live cells, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is reduced to purple formazan, which can be detected spectrophotometrically. However, due to the possibility that various nanomaterials may interfere with this assay, differing water solubility, and investigator preference, several similar assays (e.g., MST, MTS, XTT, WST-1) have also been employed (91). Lanone et al. (92) utilized the MTT assay to make comparisons between the toxicities of 24 manufactured nanoparticles (**Figure 1*b***). Another dye, alamar blue (resazurin), which is reduced by living cells to the fluorescent product resorufin, has also been extensively utilized to measure cell viability, for example, following exposure to SiO_2-coated CdSe QDs (93) and amino acid–functionalized Au (94). Finally, AshaRani et al. (75) assessed total ATP content to determine the toxicity of starch-capped Ag nanoparticles.

4.3.2. Hemolysis. The lysis of erythrocytes in response to nanoparticles can be a measure of both membrane disruption and extreme cellular toxicity (i.e., necrosis) and is especially important for nanoparticles that are intended to be directly introduced into the bloodstream. The spectrophotometric detection of hemoglobin is an extremely sensitive technique and has been exploited in a study of Stöber and mesoporous SiO_2 (95). Goodman et al. (96) have also used this approach

to determine the median lethal dose values for functionalized Au nanoparticles. Recent studies in our lab have focused on the hemolytic potential of functionalized Au nanoparticles while assessing their effects on ROS production in neutrophils and thrombotic capabilities (S.A. Love, J.W. Thompson & C.L. Haynes, manuscript submitted).

4.3.3. Apoptosis and necrosis. Measurements of the indicators of programmed cell death (i.e., apoptosis) and/or necrosis directly reveal nanoparticles' ability to induce intracellular suicide mechanisms or destroy cells. Such assays focus largely on measuring membrane integrity, but some also attempt to measure apoptotic protein levels/activation and DNA fragmentation. Five main techniques are used to determine membrane integrity: phosphatidylserine (which migrates to the extracellular surface of apoptotic cells) labeling with annexin V (75), propidium iodide exclusion by intact membranes (75), Trypan blue exclusion by intact membranes (67, 96), neutral red staining (which undergoes a color change due to protonation in intact lysosomes) (85, 92), and determination of total lactate dehydrogenase (LDH) content in the extracellular medium (89, 97). Another common assay looks for the exclusion of red fluorescent ethidium homodimer 1 from live cells while measuring uptake of calcein-AM (which fluoresces green after modification by intracellular esterases). Kirchner et al. (98) and Chang et al. (99) employed the last method to visualize dose-dependent cell death in response to CdSe, CdSe/ZnS, or Au nanoparticles and CdSe/CdS QDs, respectively. Attempts to quantify apoptotic proteins (or their activation) have been confined mostly to measurement of caspase-3 and caspase-9. For example, Park et al. (34) detected cytosolic capase-3 following exposure to CeO_2 nanoparticles, and Jiang et al. (100) investigated changes in caspase-3 and caspase-9 levels following exposure to Au nanoparticles. Finally, assessment of the level of DNA fragmentation with TUNEL (terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling) can be used to identify apoptosis, as demonstrated by studies of SWCNTs (79) and $Eu(OH)_3$ nanoparticles (101).

When choosing one of these assays, or others not reviewed above, it is important to consider the challenges that have emerged in the presence of nanoparticles. That is, several assays produce erroneous results when used with nanoparticles. In the MTT assay, CNTs can cause the solubility of the formazan to be modified through adsorption of the reduced crystals, thereby falsely lowering viability results (102). Failure of the MTT assay can also occur during assessment of mesoporous silicon nanoparticles due to the spontaneous reduction of MTT by the silicon surface, which causes artificially high viability results and potentially masks nanoparticle effects (103). Such spontaneous reduction may also occur in graphene particles (104). The LDH assay has also failed for some nanoparticles, including Cu (LDH was inactivated) and TiO_2 (LDH was adsorbed) (105). These examples of nanoparticle assay interference suggest that careful controls, a specialty of the analytical chemist, are needed to ensure accurate results and that multiple assessments of viability are necessary (91, 106). In addition to developing techniques in which nanoparticles do not interfere, as in the aforementioned viability assays, the expertise of analytical chemists in performing sensitive measurements to determine toxicological markers that may be unique to nanotoxicity will be required to advance the field.

5. CASE STUDIES

In this section, we present three case studies of nanoparticles that are commonly used and studied via various methods to showcase the types of work done with in vitro models and to attempt to draw conclusions from recent toxicity assessments. These case studies focus on one representative of each of the three most common and widely studied nanoparticle classes: metal-, metal oxide–, and carbon-based nanoparticles. For each nanoparticle class, numerous studies have examined toxicity

within both in vivo and in vitro systems via various approaches by use of the above-described model systems for mammalian and other systems. Note that generalized conclusions for mammalian in vitro studies are drawn in each of the case studies discussed below, despite the wide variety of nanoparticles, assays, and model systems used.

5.1. Metal: Silver

According to the Project on Emerging Nanotechnologies, Ag nanoparticles are the single most commonly used nanoparticle in consumer applications (2); therefore, we use Ag to represent the metal nanoparticle class. Ag nanoparticles are often employed for their desirable optical properties, which arise from the surface plasmon (a collective surface electron oscillation supported by the nanoparticle) that gives them their characteristic intense color and strong Rayleigh scattering. Because one can readily modulate the surface plasmon by tailoring nanoparticle properties (i.e., size, shape, aggregation or agglomeration state, etc.), Ag nanoparticles are being studied for a wide variety of applications (2). Ag in many forms has been used as an effective antimicrobial agent, and as such, Ag nanoparticles are being studied for use in the same and extended applications. Accordingly, one of the most investigated properties of Ag nanoparticles is the possible release of Ag^+, a species thought to contribute to Ag's antimicrobial activity (107–109). In this capacity, Ag nanoparticles can be found in diverse commercial products, including socks, pants, sheets, and washing machines, which allows for either unintentional or intentional exposure to Ag nanoparticles (2). In 2007, Benn & Westerhoff (110) found that commercially available socks release both colloidal and ionic Ag upon normal washing conditions. For this reason, a significant number of Ag-related toxicity studies focus on the antibacterial efficacy and ecological impacts of Ag nanoparticles. Because a complete discussion of the current state of bacterial work is beyond the scope of this review, we direct interested readers to two review articles (107, 109). Another study has examined the impact of Ag nanoparticles in both bacterial and eukaryotic cells; the authors found that Ag nanoparticles embedded in a chitosan polymer are not toxic to eukaryotic cells but are to microbes (108).

Investigators have characterized Ag nanoparticle uptake by mammalian cells; nanoparticles are internalized, localizing within various cellular compartments. Arora et al. (111) used TEM to show that Ag nanoparticles localize to the mitochondria in primary murine fibroblasts and to mitochondria and endosomes in liver cells. Also, AshaRani et al. (112) found that in immortalized human glioblastoma cells (U251), starch-coated nanoparticles are distributed throughout the cytoplasm and are found in the nucleus and the mitochondria. Several other studies, including those by Foldbjerg et al. (113) and Hsin et al. (114), used atomic absorption spectroscopy and flow cytometry to demonstrate that nanoparticles are taken up by various cell types in a time-dependent manner. Additional studies have found concentration-dependent uptake (8) and differential uptake for varied surface charges (11). Overall, although Ag nanoparticles are internalized, differences in nanoparticle and model cell choice can lead to differential uptake and localization within the cells, which suggests that there are complex uptake mechanisms worthy of further systematic study.

Because Ag^+ is a known toxicant, investigations must consider the possibility of Ag nanoparticle dissolution during exposure. Additionally, because nanoparticles apparently make their way into cells, ending up in various cellular compartments with a range of pHs, the possibility of nanoparticle dissolution and Ag^+ release seems likely. Recent work has begun to examine the dissolution rate of several types of Ag nanoparticles, focusing on solution parameters (e.g., pH, dissolved O_2) (115) and varied surface coatings (116). Chen and coworkers (18) have found that when environmental factors (e.g., pH, dissolved O_2) are kept constant, Ag^+ release rates depend on primary nanoparticle concentration and size (**Figure 2*a***). As the concentration of O_2 and protons

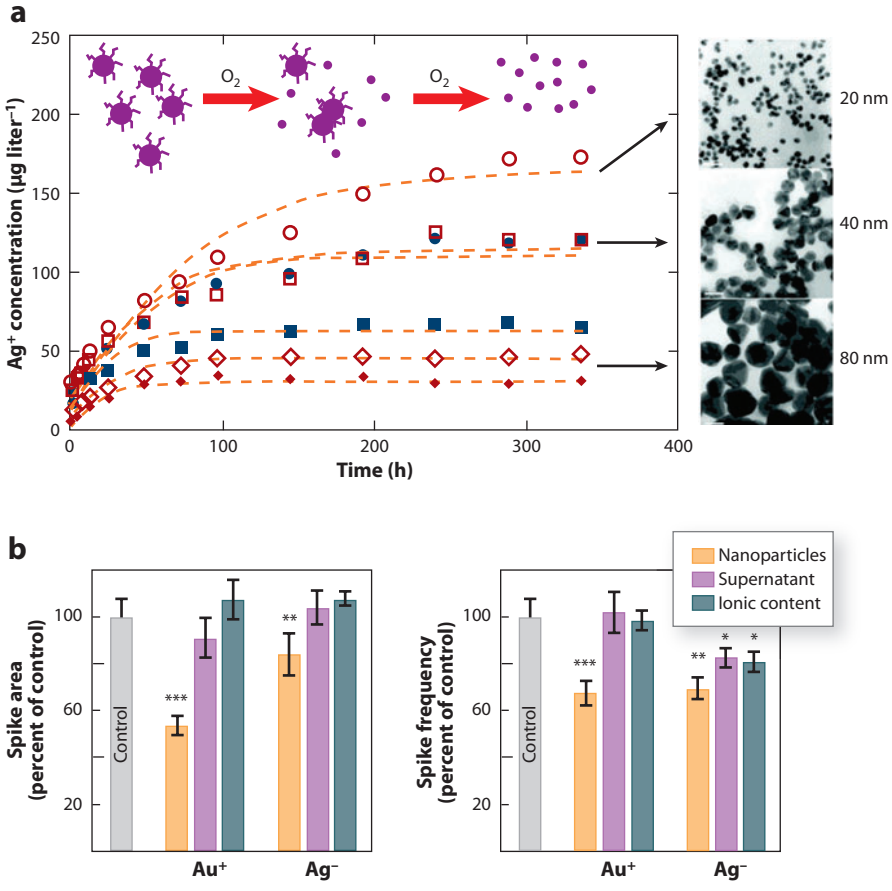


Figure 2

Ag^+ release from nanoparticles is one of the greatest causes of Ag nanoparticle toxicity. (*a*) Dissolution of Ag^+ from Ag nanoparticles of varied size, over time, and in different O_2 environments (*large red arrows*), as measured with inductively coupled plasma mass spectrometry. Panel *a* reproduced with permission from Reference 18. (*b*) Results demonstrating the effect of Ag^+ on the exocytotic cell function of murine peritoneal mast cells following exposure to Ag nanoparticles with positively or negatively charged surface molecules. The results indicate a significant decrease in the number of chemical messenger molecules (serotonin) secreted by the cells (as denoted by the spike area) and the frequency of exocytotic events (denoted by the spike frequency) following exposure to either Ag nanoparticle; the ionic content caused a significant decrease in frequency for Ag^-. Single asterisk, $p < 0.05$; double asterisk, $p < 0.01$; triple asterisk, $p < 0.005$. Panel *b* reproduced with permission from Reference 11. These two examples of Ag^+ ion release experiments highlight the need to utilize careful controls to interpret nanoparticle toxicity results, an essential consideration already commonly utilized by analytical chemists.

affects the rate of Ag nanoparticle dissolution (18, 115), surface modifications help control the dissolution process (117). In the case of MUA (11-mercaptoundecanoic acid) modification, Hurt and coworkers (117) found that Ag dissolution was completely abrogated. However, Marquis et al. (11) found that an ion control (**Figure 2*b***) did not completely account for the changes in exocytosis observed when the cells were exposed to the nanoparticles; this finding implicates a nanoparticle-specific mechanism of toxicity. All of these studies suggest that Ag nanoparticles probably release Ag^+ during exposure—which highlights the need for careful controls to compare

ion effects with nanoparticle effects—but that the Ag nanoparticles themselves are important to measured cytotoxicity.

Numerous studies have examined Ag nanoparticle toxicity in vitro; the majority focused on nanoparticle size–variation effects. Studies have examined individual cell and monolayer morphology and found that cellular morphology appears to change upon nanoparticle exposure, leaving cells shriveled or deformed (111, 112, 118, 119). Many studies conducted with MTT, LDH, and apoptosis/necrosis assays have found that Ag nanoparticles lead to dose- and size-dependent decreases in viability (111, 112, 118, 119). Zanette et al. (41) found that cell proliferation is also altered upon exposure and does not return to normal even after a week of recovery.

To further assess the possible mechanism of these changes in cell structure and viability, studies have examined cytokine release (118), membrane permeability, and oxidative stress (119), finding changes that indicate increased stress (i.e., increased proinflammatory mediator release, altered membrane potentials, and increased ROS and oxidative stress markers) in a time-, size-, and dose-dependent manner. In a systematic study, Park et al. (120) examined poly(*N*-vinyl-2-pyrrolidone)-stabilized Ag nanoparticles measuring 4, 20, and 70 nm in diameter in immortalized U937 (human monocyte) cells. These authors monitored exposure effects, with cells incubated in 1 to 50 $\mu g\ ml^{-1}$ nanoparticles for up to 24 h, on viability (cell counting, propidium iodide/annexin V), oxidative stress (DCFDA), and cytokine release (e.g., IL-8, tumor necrosis factor α) (120). Park et al. found decreased cell viability for the smallest nanoparticles; IL-8 and ROS were increased but could be abolished either with *N*-acetylcysteine (antioxidant) pretreatment or by blocking nanoparticle uptake (120).

Some general trends emerge from these recent studies of Ag nanoparticles using in vitro approaches. Generally, cells exposed to Ag nanoparticles show increased indicators of cellular stress and functional changes that do not necessarily lead to cell death. Careful consideration of Ag^+ dissolution and its physicochemical properties is crucial to inform rules for safe nanoparticle design.

5.2. Metal Oxide: Titanium Dioxide

TiO_2 nanoparticles are some of the most abundantly produced nanomaterials and are found in diverse everyday and nanotechnology-enabled products and applications (2). Their wide use in applications ranging from cosmetics and sunscreens (121) to heterogeneous catalysts (122) results in an increased likelihood of either intentional or unintentional exposure (see Reference 123 for a review of in vitro toxicity and Reference 124 for a review of aquatic in vivo toxicity). As a semiconductor nanoparticle, TiO_2 is known for its considerably large bandgap of 3.2 eV, which gives rise to the nanoparticle's photocatalytic activity. Under UV illumination, the nanoparticle becomes a strong oxidizing agent. An important characteristic of TiO_2 nanoparticles that distinguishes them from many other commonly used materials is that they are crystalline. The three crystal forms of TiO_2 are rutile, anatase, and brookite; whereas bulk TiO_2 is thermodynamically most stable in the rutile form, nanoparticles tend to have significant anatase character. Both the crystallinity and the oxidizing potential of TiO_2 nanoparticles are important considerations in the assessment of their cytotoxicity.

Characterization of TiO_2 nanoparticle uptake is critical to understanding this material's toxicity modes. TiO_2 nanoparticles tend to aggregate in solution without surface modification (9, 16, 47), which influences their effective size and may affect their induced cytotoxicity. Such aggregation or agglomeration is readily observed in uptake and localization studies. That is, in many studies utilizing TEM, TiO_2 nanoparticles are taken up into the cells and tend to localize within the lysosomes or vesicles of the cells as aggregates (9, 40, 125–127). Hackenberg et al. (49)

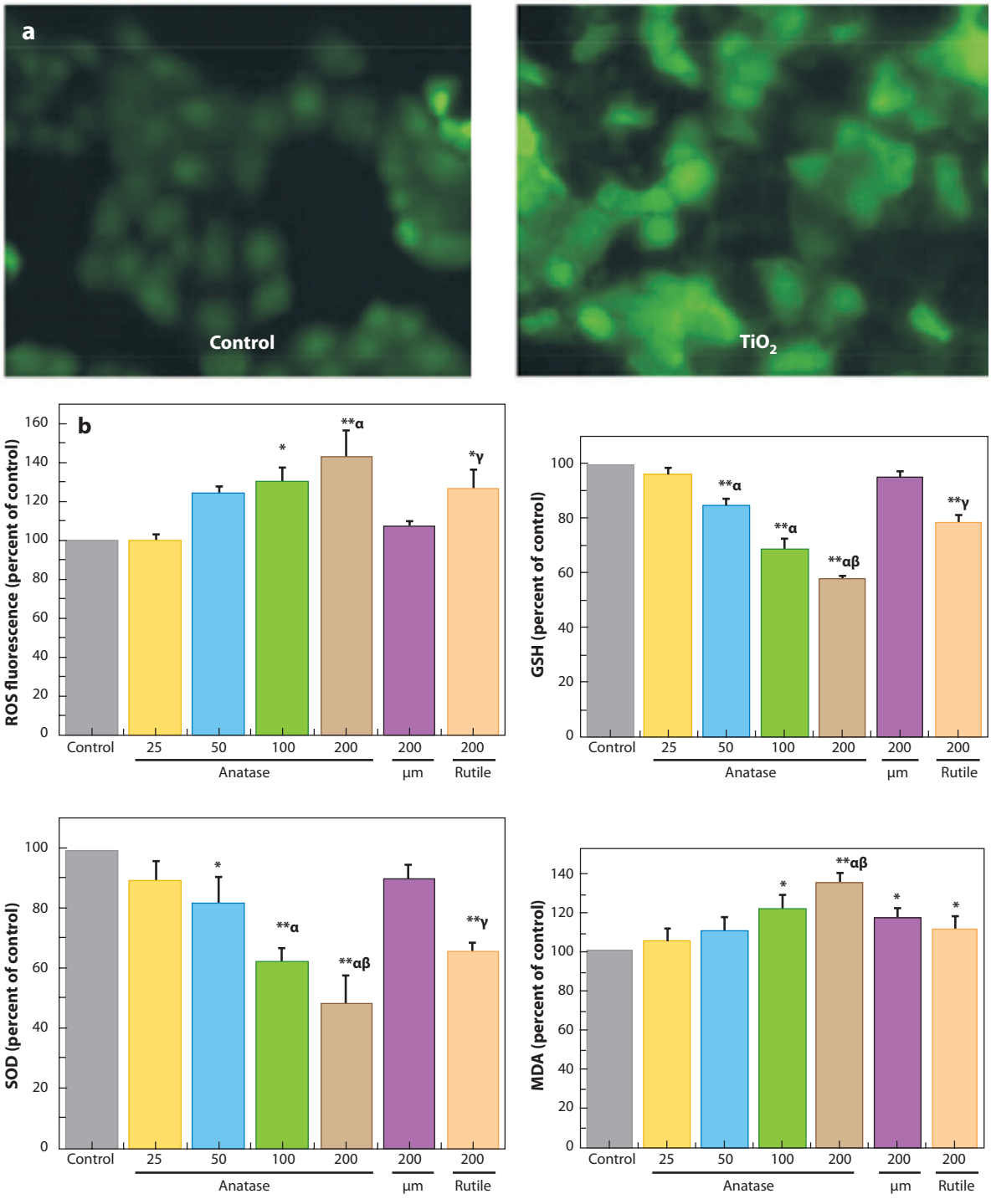
and Simon-Deckers et al. (128) observed nanoparticle uptake; however, nanoparticles remained localized in the cytoplasm. Electron microscopy [both TEM and scanning electron microscopy (SEM)] can also be used to observe morphological changes in the cell upon exposure. Using SEM imaging, Pan et al. (127) showed that TiO_2 nanoparticles are aggregated and cause morphological changes in human dermal fibroblasts. Although TiO_2 nanoparticles aggregate and are localized in the cell as aggregates, to our knowledge no systematic studies of the impact of TiO_2 nanoparticle aggregates on toxic responses have been done.

In vitro studies performed to better understand the effects of TiO_2 crystallinity on cells have revealed that the anatase crystal form causes a greater toxic response than the rutile form does (40, 127, 129). Elucidation of the toxic response has involved the MTS (129), MTT (82, 126, 128, 130), cell-staining (130), and cell-proliferation (40) assays, which revealed that TiO_2 nanoparticles cause a dose- and time-dependent decrease in cell viability; the anatase form induces the greatest decrease in viable cells. In addition to decreased cellular viability, TiO_2 nanoparticles, particularly the anatase form, cause increased levels of inflammatory indicators such as LDH (82, 126, 129, 130) and IL-8 (130); again, anatase nanoparticles cause a greater inflammatory response than do rutile nanoparticles. Schanen et al. (82) utilized a novel simulated immune system coupled with a multiplex cytokine array to measure proinflammatory mediators secreted from the system; they determined that all TiO_2 nanoparticles initiate an inflammatory response and that, again, anatase nanoparticles cause greater proinflammatory cytokine secretion than do other TiO_2 nanoparticle forms.

The anatase crystal form may be more toxic because of its greater oxidizing potential, which would generate a greater amount of reactive species (100). The effect of (any crystalline form of) TiO_2 nanoparticles' oxidizing potential is commonly assessed as cellular oxidative stress through direct detection of ROS (**Figure 3*a***) (125, 129, 131) and/or the indirect measurement of oxidative stress indicators such as glutathione (129) and SOD (**Figure 3*b***) (129, 132). A dose- and time-dependent increase in oxidative stress has been observed for TiO_2 nanoparticles; the anatase form generates the greatest amount (**Figure 3*b***). A hypothesized product of TiO_2 nanoparticle–induced oxidative stress is DNA damage, as measured with the comet assay; however, minimal genotoxicity has been observed upon exposure to TiO_2 nanoparticles but even then only at high doses (49, 126). Wu et al. (129) have investigated the oxidative stress effects induced by TiO_2 nanoparticles on the mitochondrial membrane potential of PC12 cells; they identified a dose-dependent decrease in membrane potential following exposure to anatase nanoparticles. However, Hussain et al. (131) found no decrease in mitochondrial membrane potential in bronchial epithelial cells following nanoparticle exposure. In addition to membrane potential reduction, increased levels of signaling molecules such as caspase-3 have been observed, which indicates that TiO_2 may trigger apoptosis (131, 132); other work suggests that anatase nanoparticles also cause necrosis (40, 129).

Figure 3

Oxidative stress induced by TiO_2 nanoparticles. (*a*) Bronchial epithelial cells exposed to nanoparticles demonstrate nanoparticle-induced reactive oxygen species (ROS) after 4 h exposure and imaging with the fluorescent ROS probe 2′,7′-dihydrodichlorofluorescein diacetate (DCFDA). Higher-intensity fluorescence indicates greater amounts of ROS. Panel *a* modified with permission from Reference 131. (*b*) TiO_2-induced oxidative stress in PC12 cells, as revealed by various assays. Results indicate that anatase TiO_2 nanoparticles cause a dose-dependent increase in ROS (as measured with DCFDA), a decrease in the antioxidant glutathione (GSH), decreased superoxide dismutase (SOD) activity, and an increase in the oxidative stress marker malonaldehyde (MDA). Additionally, these assays reveal that anatase and nanosized particles cause greater oxidative stress than do rutile or micrometer-sized nanoparticles, respectively. These two examples of TiO_2-induced ROS generation illustrate a mode of toxicity commonly thought to be critical in nanotoxicity. Panel *b* modified with permission from Reference 129.



Important observations about the cytotoxicity of TiO_2 nanoparticles have emerged, although challenges to drawing generalized conclusions persist. To this end, some investigators have attempted multilab studies (92), and many TiO_2 toxicity studies utilize commercially available Degussa AEROXIDE P25 nanoparticles that are approximately 30 nm in diameter and ~80% rutile and ~20% anatase (133). However, these nanoparticles do not represent an accurate model based on particles currently utilized in commercial products (134). In general, nanoparticles' characteristics of crystallinity, oxidizing potential, and aggregation are probably key contributors to the observed in vitro cellular toxicity for TiO_2 nanoparticles and require continued careful work.

5.3. Carbon: Carbon Nanotubes

Since CNTs were discovered in by Iijima (136) in 1991, they have been studied extensively because of interest in their extremely high strength-to-weight and aspect ratios, high surface area, tensile strength, thermal stability, and conductivity (135). CNTs are allotropes of carbon composed of either a single graphite sheet (termed SWCNTs) or multilayered graphene sheets (termed MWCNTs). To date, CNTs are some of the most abundantly produced engineered nanomaterials for industrial use. CNTs have been used as carriers for drug and gene delivery and as scaffolds for tissue engineering (137, 138). The global market for CNTs was recently predicted to amount to approximately $1 billion by 2014 (139), which indicates that a huge number of CNT-related products are likely to appear in the future. A complete review of all the CNT toxicity studies is beyond the scope of this article, but comprehensive reviews of in vitro and in vivo CNTs toxicity can be found in two recently published papers (140, 141). Due to different synthesis and dispersion methods as well as nanoparticle diversity in the CNT class, residual metal catalysts, surface chemistry, aggregation state, and structural differences must be considered in CNT toxicity studies.

Cellular uptake of CNTs is important in interpreting the cytotoxicity of CNTs; uptake of CNTs can be monitored with optical microscopy, TEM, or fluorescence microscopy. The cellular uptake and morphological changes of individual CNT or CNT agglomerates in various cells have been confirmed with optical microscopy and TEM (55, 142, 143). The morphological changes (144, 145), vacuole formation in cells (143, 146), and loss of membrane integrity (147) following cells' exposure to CNTs have also been observed with optical microscopy and TEM. TEM images of cellular ultrastructure, which reveal the presence of cytoplasmic protrusions and CNT-containing phagolysosomes, further demonstrate that CNTs increase the phagocytic activity of macrophages (145). Using fluorescence microscopy, investigators have also observed fragmented nuclei and balloon-like nuclear morphology (**Figure 4**) in CNT-exposed cells, which indicates the presence of apoptotic and necrotic cells, respectively (142, 147).

Here, we discuss several major factors that affect the cytotoxicity results of CNTs, using representative examples. Pulskamp et al. (148) showed that commercially available CNTs do not cause acute toxicity (WST-1 and PI staining) but that they do induce ROS generation in human A549 lung cells and rat macrophages. However, the authors attributed the increased ROS levels to the metal residuals in the CNTs. To exclude the impurity effect, Tsukahara & Haniu (142) used highly purified MWCNTs to demonstrate that, even with no significant intracellular ROS generation, the cellular uptake of MWCNT still increases cell death (alamar blue assay), increases membrane damage (LDH assay), and induces the release of cytokines (e.g., tumor necrosis factor α, IL-12, IL-10, IL-6, IL-1β, and IL-8) in BEAS-2B cells. These authors also speculated that CNT exposure induces BEAS-2B cell necrosis (142).

To study the influence of surface chemistry and particulate state of CNTs in the cytotoxic response, Bottini et al. (149) compared the cytotoxicity of pristine versus oxidized MWCNTs and

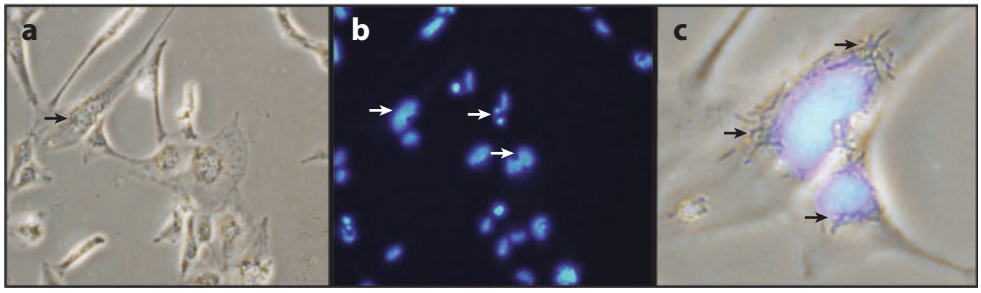


Figure 4

Morphological analysis of BEAS-2B cells following 24 h exposure to multiwalled carbon nanotubes (MWCNTs). (*a*) Bright-field image of exposed cells. (*b*) Cells stained with Hoechst 33,342. (*c*) Merged image of panels *a* and *b*. The arrows indicate nuclei with ballooned morphology, which is associated with necrotic cell death. Additionally, these images reveal that MWCNTs are internalized in the cytoplasm of the cell near the nucleus and show the importance of examining the uptake of nanoparticles, which can be a key contributor, although it is not directly a measure of toxicity. Modified with permission from Reference 142.

found that oxidized MWCNTs induced greater human T cell apoptosis, as shown by Trypan blue and annexin V assays. Wang et al. (150) screened the cytotoxicity of nine different SWCNTs from various vendors. They found that only two SWCNTs are highly toxic to normal rat kidney cells, and both are carboxylic acid functionalized. They also demonstrated that additional purification can remove the cytotoxic species (small oxidized carbon fragments) from carboxylate SWCNTs (150). In addition, Wick et al. (144) investigated the effect of CNT agglomeration in mesothelial cells and found that well-dispersed SWCNTs are less toxic than agglomerated SWCNTs.

Cytotoxicity comparisons between SWCNTs and MWCNTs have been performed in several studies; in general, both material classes produce time- and concentration-dependent changes in cellular morphology, viability, and oxidative stress (143, 147), although there are some notable differences. Jia et al. (146) found that purified SWCNTs are more toxic than MWCNTs, quartz, and C_{60} in primary guinea pig alveolar macrophages. SWCNTs also significantly impaired the phagocytotic activity of alveolar macrophage at a very low dosage (0.38 $\mu g\ cm^{-2}$). Di Giorgio et al. (145) showed that SWCNTs and MWCNTs are cytotoxic (Trypan blue exclusion assay) and genotoxic (cytokinesis-block micronucleus and comet assays) to mouse macrophages (RAW 264.7). These authors also found that CNTs (*a*) cause necrosis in RAW 264.7 cells, (*b*) are more genotoxic than carbon black, and (*c*) also cause chromosomal breakage and changes in chromosome number.

On the basis of our present knowledge of CNT cytotoxicity, it is difficult to make a generalized conclusion because of conflicting results. To date, investigators generally agree that CNTs are toxic and adversely affect a variety of cells. Factors such as metal impurities, particulate state, structural differences, and the surface properties of CNTs greatly influence their apparent cytotoxicity. To advance the field, both thorough material characterization of CNTs prior to toxicity studies and standardized and reliable methods to assess the cytotoxicity of CNTs are needed.

6. CONCLUSIONS

A fundamental concept in toxicity assessment is that risk is a combination of both exposure and hazard. With the increased use of nanoparticles in commercial products, there has been a significant increase in the possibility of nanoparticle exposure to both the public and industrial workers. As discussed above, scientists in a broad range of disciplines have been working to establish the

hazards of nanoparticles by using a myriad of viability and functional assays, although few have been from the analytical chemistry community. However, the current state of in vitro toxicity studies and of the nanotoxicity field at large has limitations, particularly in technology for the characterization of nanoparticles throughout exposure and in assays that are better at predicting the in vivo toxic response; therefore, this area is ripe for development. In light of these challenges, this field could greatly benefit from the skills of analytical chemists, as highlighted herein, to develop better methods, technologies, and models.

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LITERATURE CITED

1. US Natl. Nanotechnology Initiat. 2011. *Nanotechnology 101*. Washington, DC: US Natl. Nanotechnol. Initiat. **http://www.nano.gov/nanotech-101/nanotechnology-facts**
2. Proj. Emerg. Nanotechnol. 2011. *Project on Emerging Nanotechnologies Consumer Product Inventory*. Washington, DC: Woodrow Wilson Int. Cent. Sch. **http://www.nanotechproject.org/inventories/**
3. Maynard AD, Warheit DB, Philbert MA. 2011. The new toxicology of sophisticated materials: nanotoxicology and beyond. *Toxicol. Sci.* 120(Suppl. 1):109–29
4. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, et al. 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol.* 2:8
5. Powers KW, Brown SC, Krishna VB. 2006. Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. *Toxicol. Sci.* 90:296–303
6. Grassian VH, Hamers RJ. 2011. Nanomaterials and the environment: the chemistry and materials perspective. *Natl. Sci. Found. Workshop Rep.* **http://nsfenv-nano.chem.wisc.edu/sites/nsfenv-nano.chem.wisc.edu/files/report/workshop_report_FINAL_august_26_2011.pdf**
7. Marquis BJ, Maurer-Jones MA, Braun KL, Haynes CL. 2009. Amperometric assessment of functional changes in nanoparticle-exposed immune cells: varying Au nanoparticle exposure time and concentration. *Analyst* 134:2293–300
8. Love SA, Haynes CL. 2010. Assessment of functional changes in nanoparticle-exposed neuroendocrine cells with amperometry: exploring the generalizability of nanoparticle-vesicle matrix interactions. *Anal. Bioanal. Chem.* 398:677–88
9. Maurer-Jones MA, Lin Y-S, Haynes CL. 2010. Functional assessment of metal oxide nanoparticle toxicity in immune cells. *Am. Chem. Soc. Nano* 4:3363–73
10. Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM. 2008. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol. Sci.* 101:239–53

11. Marquis BJ, Liu Z, Braun KL, Haynes CL. 2011. Investigation of noble metal nanoparticle ζ-potential effects on single-cell exocytosis function in vitro with carbon-fiber microelectrode amperometry. *Analyst* 136:3478–86
12. Montes-Burgos I, Walczyk D, Hole P, Smith J, Lynch I, Dawson K. 2009. Characterisation of nanoparticle size and state prior to nanotoxicological studies. *J. Nano Res.* 12:47–53
13. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V. 2010. Time evolution of the nanoparticle protein corona. *Am. Chem. Soc. Nano* 4:3623–32
14. Lin YS, Abadeer N, Haynes CL. 2011. Stability of small mesoporous silica nanoparticles in biological media. *Chem. Commun.* 47:532–34
15. Lin Y-S, Haynes CL. 2010. Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *J. Am. Chem. Soc.* 132:4834–42
16. Ji Z, Jin X, George S, Xia T, Meng H, et al. 2010. Dispersion and stability optimization of TiO_2 nanoparticles in cell culture media. *Environ. Sci. Technol.* 44:7309–14
17. Sapsford KE, Tyner KM, Dair BJ, Deschamps JR, Medintz IL. 2011. Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques. *Anal. Chem.* 83:4453–88
18. Zhang W, Yao Y, Sullivan N, Chen Y. 2011. Modeling the primary size effects of citrate-coated silver nanoparticles on their ion release kinetics. *Environ. Sci. Technol.* 45:4422–28
19. Davila JC, Rodrigues RJ, Melchert RB, Acosta D. 1998. Predictive value of in vitro model systems in toxicology. *Annu. Rev. Pharmacol. Toxicol.* 38:63–96
20. Zucco F, De Angelis I, Testai E, Stammati A. 2004. Toxicology investigations with cell culture systems: 20 years after. *Toxicol. In Vitro* 18:153–63
21. Gerloff K, Albrecht C, Boots AW, Förster I, Schins RPF. 2009. Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. *Nanotoxicology* 3:355–64
22. Jos A, Pichardo S, Puerto M, Sánchez E, Grilo A, Cameán AM. 2009. Cytotoxicity of carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicol. In Vitro* 23:1491–96
23. Thubagere A, Reinhard BM. 2010. Nanoparticle-induced apoptosis propagates through hydrogen-peroxide-mediated bystander killing: insights from a human intestinal epithelium in vitro model. *Am. Chem. Soc. Nano* 4:3611–22
24. Moos PJ, Chung K, Woessner D, Honeggar M, Cutler NS, Veranth JM. 2010. ZnO particulate matter requires cell contact for toxicity in human colon cancer cells. *Chem. Res. Toxicol.* 23:733–39
25. Winter M, Beer H-D, Hornung V, Krämer U, Schins RPF, Förster I. 2010. Activation of the inflammasome by amorphous silica and TiO_2 nanoparticles in murine dendritic cells. *Nanotoxicology* 5:326–340
26. Ashokan A, Chandran P, Sadanandan AR, Koduri CK, Retnakumari AP, et al. 2011. Development and haematotoxicological evaluation of doped hydroxyapatite based multimodal nanocontrast agent for near-infrared, magnetic resonance and X-ray contrast imaging. *Nanotoxicology.* In press. doi: 10.3109/17435390.2011.600839
27. Singh SK, Singh MK, Nayak MK, Kumari S, Shrivastava S, et al. 2011. Thrombus inducing property of atomically thin graphene oxide sheets. *Am. Chem. Soc. Nano* 5:4987–96
28. Dobrovolskaia MA, Clogston JD, Neun BW, Hall JB, Patri AK, McNeil SE. 2008. Method for analysis of nanoparticle hemolytic properties in vitro. *Nano Lett.* 8:2180–87
29. Yan M, Zhang Y, Xu K, Fu T, Qin H, Zheng X. 2011. An in vitro study of vascular endothelial toxicity of CdTe quantum dots. *Toxicology* 282:94–103
30. Abdul Jalil R, Zhang Y. 2008. Biocompatibility of silica coated $NaYF_4$ upconversion fluorescent nanocrystals. *Biomaterials* 29:4122–28
31. Lu B, Xu X-D, Zhang X-Z, Cheng S-X, Zhuo R-X. 2008. Low molecular weight polyethylenimine grafted *N*-maleated chitosan for gene delivery: properties and in vitro transfection studies. *Biomacromolecules* 9:2594–600
32. Kong T, Zeng J, Wang X, Yang X, Yang J, et al. 2008. Enhancement of radiation cytotoxicity in breast-cancer cells by localized attachment of gold nanoparticles. *Small* 4:1537–43
33. He X, Ma J, Mercado A, Xu W, Jabbari E. 2008. Cytotoxicity of paclitaxel in biodegradable self-assembled core-shell poly(lactide-co-glycolide ethylene oxide fumarate) nanoparticles. *Pharmacol. Res.* 25:1552–62

34. Park E-J, Choi J, Park Y-K, Park K. 2008. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. *Toxicology* 245:90–100
35. Shi X, Sitharaman B, Pham QP, Spicer PP, Hudson JL, et al. 2008. In vitro cytotoxicity of single-walled carbon nanotube/biodegradable polymer nanocomposites. *J. Biomed. Mater. Res. A* 86:813–23
36. Senthilkumar M, Mishra P, Jain NK. 2008. Long circulating PEGylated poly(D, L-lactide-co-glycolide) nanoparticulate delivery of docetaxel to solid tumors. *J. Drug Target.* 16:424–35
37. Xue C, Wu J, Lan F, Liu W, Yang X, et al. 2010. Nano titanium dioxide induces the generation of ROS and potential damage in HaCaT cells under UVA irradiation. *J. Nanosci. Nanotechnol.* 10:8500–7
38. Kocbek P, Teskač K, Kreft ME, Kristl J. 2010. Toxicological aspects of long-term treatment of keratinocytes with ZnO and TiO_2 nanoparticles. *Small* 6:1908–17
39. Sharma V, Singh SK, Anderson D, Tobin DJ, Dhawan A. 2011. Zinc oxide nanoparticle induced genotoxicity in primary human epidermal keratinocytes. *J. Nanosci. Nanotechnol.* 11:3782–88
40. Braydich-Stolle LK, Schaeublin NM, Murdock RC, Jiang J, Biswas P, et al. 2009. Crystal structure mediates mode of cell death in TiO_2 nanotoxicity. *J. Nanopart. Res.* 11:1361–74
41. Zanette C, Pelin M, Crosera M, Adami G, Bovenzi M, et al. 2011. Silver nanoparticles exert a long-lasting antiproliferative effect on human keratinocyte HaCaT cell line. *Toxicol. In Vitro* 25:1053–60
42. Zhang LW, Yu WW, Colvin VL, Monteiro-Riviere NA. 2008. Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicol. Appl. Pharmacol.* 228:200–11
43. Romoser AA, Chen PL, Berg JM, Seabury C, Ivanov I, et al. 2011. Quantum dots trigger immunomodulation of the NFκB pathway in human skin cells. *Mol. Immunol.* 48:1349–59
44. Zhang Y, Hu L, Yu D, Gao C. 2010. Influence of silica particle internalization on adhesion and migration of human dermal fibroblasts. *Biomaterials* 31:8465–74
45. Patlolla A, Patlolla B, Tchounwou P. 2010. Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube. *Mol. Cell. Biochem.* 338:225–32
46. Vankoningsloo S, Piret J-P, Saout C, Noel F, Mejia J, et al. 2009. Cytotoxicity of multi-walled carbon nanotubes in three skin cellular models: effects of sonication, dispersive agents and corneous layer of reconstructed epidermis. *Nanotoxicology* 4:84–97
47. Tedja R, Marquis C, Lim M, Amal R. 2011. Biological impacts of TiO_2 on human lung cell lines A549 and H1299: particle size distribution effects. *J. Nano Res.* 13:3801–13
48. Ekstrand-Hammarström B, Akfur CM, Andersson PO, Lejon C, Österlund L, Bucht A. 2011. Human primary bronchial epithelial cells respond differently to titanium dioxide nanoparticles than the lung epithelial cell lines A549 and BEAS-2B. *Nanotoxicology*. In press. doi:10.3109/17435390.2011.598245)
49. Hackenberg S, Friehs G, Froelich K, Ginzkey C, Koehler C, et al. 2010. Intracellular distribution, geno- and cytotoxic effects of nanosized titanium dioxide particles in the anatase crystal phase on human nasal mucosa cells. *Toxicol. Lett.* 195:9–14
50. Reisetter AC, Stebounova LV, Baltrusaitis J, Powers L, Gupta A, et al. 2011. Induction of inflammasome-dependent pyroptosis by carbon black nanoparticles. *J. Biol. Chem.* 286:21844–52
51. Wang L, Mercer RR, Rojanasakul Y, Qiu A, Lu Y, et al. 2010. Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts. *J. Toxicol. Environ. Health A* 73:410–22
52. Wadhwa S, Rea C, O'Hare P, Mathur A, Roy SS, et al. 2011. Comparative in vitro cytotoxicity study of carbon nanotubes and titania nanostructures on human lung epithelial cells. *J. Hazard. Mater.* 191:56–61
53. Foldbjerg R, Dang D, Autrup H. 2011. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. *Arch. Toxicol.* 85:743–50
54. Ahamed M, Akhtar MJ, Siddiqui MA, Ahmad J, Musarrat J, et al. 2011. Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* 283:101–8
55. Thurnherr T, Brandenberger C, Fischer K, Diener L, Manser P, et al. 2011. A comparison of acute and long-term effects of industrial multiwalled carbon nanotubes on human lung and immune cells in vitro. *Toxicol. Lett.* 200:176–86
56. Pacurari M, Schwegler-Berry D, Friend S, Leonard SS, Mercer RR, et al. 2011. Raw single-walled carbon nanotube–induced cytotoxic effects in human bronchial epithelial cells: comparison to asbestos. *Toxicol. Environ. Chem.* 93:1045–72

57. Fan Q, Wang YE, Zhao X, Loo JSC, Zuo YY. 2011. Adverse biophysical effects of hydroxyapatite nanoparticles on natural pulmonary surfactant. *Am. Chem. Soc. Nano* 5:6410–16
58. Gibbs-Flournoy E, Bromberg P, Hofer T, Samet J, Zucker R. 2011. Dark field–confocal microscopy detection of nanoscale particle internalization by human lung cells. *Part. Fibre Toxicol.* 8:2
59. Eom H-J, Choi J. 2009. Oxidative stress of silica nanoparticles in human bronchial epithelial cell, BEAS-2B. *Toxicol. In Vitro* 23:1326–32
60. Lindberg HK, Falck GCM, Suhonen S, Vippola M, Vanhala E, et al. 2009. Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. *Toxicol. Lett.* 186:166–73
61. Bouwmeester H, Poortman J, Peters RJ, Wijma E, Kramer E, et al. 2011. Characterization of translocation of silver nanoparticles and effects on whole-genome gene expression using an in vitro intestinal epithelium coculture model. *Am. Chem. Soc. Nano* 5:4091–103
62. Xiao L, Aoshima H, Saitoh Y, Miwa N. 2010. The effect of squalane-dissolved fullerene-C_{60} on adipogenesis-accompanied oxidative stress and macrophage activation in a preadipocyte-monocyte co-culture system. *Biomaterials* 31:5976–85
63. Kasper J, Hermanns M, Bantz C, Maskos M, Stauber R, et al. 2011. Inflammatory and cytotoxic responses of an alveolar-capillary coculture model to silica nanoparticles: comparison with conventional monocultures. *Part. Fibre Toxicol.* 8:6
64. Brandenberger C, Rothen-Rutishauser B, Mühlfeld C, Schmid O, Ferron GA, et al. 2010. Effects and uptake of gold nanoparticles deposited at the air-liquid interface of a human epithelial airway model. *Toxicol. Appl. Pharmacol.* 242:56–65
65. Marquis BJ, Love SA, Braun KL, Haynes CL. 2009. Analytical methods to assess nanoparticle toxicity. *Analyst* 134:425–39
66. Chithrani BD, Ghazani AA, Chan WC. 2006. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.* 6:662–68
67. Hauck TS, Ghazani AA, Chan WC. 2008. Assessing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells. *Small* 4:153–59
68. Patil S, Sandberg A, Heckert E, Self W, Seal S. 2007. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of ζ potential. *Biomaterials* 28:4600–7
69. Ruan G, Agrawal A, Marcus AI, Nie S. 2007. Imaging and tracking of tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. *J. Am. Chem. Soc.* 129:14759–66
70. Oostingh GJ, Casals E, Italiani P, Colognato R, Stritzinger R, et al. 2011. Problems and challenges in the development and validation of human cell–based assays to determine nanoparticle-induced immunomodulatory effects. *Part. Fibre Toxicol.* 8:8
71. Zhang TT, Stilwell JL, Gerion D, Ding LH, Elboudwarej O, et al. 2006. Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. *Nano Lett.* 6:800–8
72. Bastus NG, Sanchez-Tillo E, Pujals S, Farrera C, Lopez C, et al. 2009. Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response. *Am. Chem. Soc. Nano* 3:1335–44
73. Auffan M, Rose J, Orsiere T, De Meo M, Thill A, et al. 2009. CeO_2 nanoparticles induce DNA damage towards human dermal fibroblasts in vitro. *Nanotoxicology* 3:161–71
74. Ponti J, Sabbioni E, Munaro B, Broggi F, Marmorato P, et al. 2009. Genotoxicity and morphological transformation induced by cobalt nanoparticles and cobalt chloride: an in vitro study in Balb/3T3 mouse fibroblasts. *Mutagenesis* 24:439–45
75. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *Am. Chem. Soc. Nano* 3:279–90
76. Barnes CA, Elsaesser A, Arkusz J, Smok A, Palus J, et al. 2008. Reproducible Comet assay of amorphous silica nanoparticles detects no genotoxicity. *Nano Lett.* 8:3069–74
77. Bhabra G, Sood A, Fisher B, Cartwright L, Saunders M, et al. 2009. Nanoparticles can cause DNA damage across a cellular barrier. *Nat. Nanotechnol.* 4:876–83
78. Zhu L, Chang DW, Dai L, Hong Y. 2007. DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett.* 7:3592–97

79. Mu Q, Du G, Chen T, Zhang B, Yan B. 2009. Suppression of human bone morphogenetic protein signaling by carboxylated single-walled carbon nanotubes. *Am. Chem. Soc. Nano* 3:1139–44
80. Bregoli L, Chiarini F, Gambarelli A, Sighinolfi G, Gatti AM, et al. 2009. Toxicity of antimony trioxide nanoparticles on human hematopoietic progenitor cells and comparison to cell lines. *Toxicology* 262:121–29
81. Veranth JM, Kaser EG, Veranth MM, Koch M, Yost GS. 2007. Cytokine responses of human lung cells (BEAS-2B) treated with micron-sized and nanoparticles of metal oxides compared to soil dusts. *Part. Fibre Toxicol.* 4:2
82. Schanen BC, Karakoti AS, Seal S, Drake DR III, Warren WL, Self WT. 2009. Exposure to titanium dioxide nanomaterials provokes inflammation of an in vitro human immune construct. *Am. Chem. Soc. Nano* 3:2523–32
83. Babior BM. 1987. The respiratory burst oxidase. *Trends Biochem. Sci.* 12:241–43
84. Xia T, Kovochich M, Brant J, Hotze M, Sempf J, et al. 2006. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett.* 6:1794–807
85. Muller K, Skepper JN, Posfai M, Trivedi R, Howarth S, et al. 2007. Effect of ultrasmall superparamagnetic iron oxide nanoparticles (ferumoxtran-10) on human monocyte–macrophages in vitro. *Biomaterials* 28:1629–42
86. Soenen SJH, Brisson AR, De Cuyper M. 2009. Addressing the problem of cationic lipid–mediated toxicity: the magnetoliposome model. *Biomaterials* 30:3691–701
87. Choi AO, Cho SJ, Desbarats J, Lovric J, Maysinger D. 2007. Quantum dot–induced cell death involves Fas upregulation and lipid peroxidation in human neuroblastoma cells. *J. Nanobiotechnol.* 5:1
88. Li JJ, Zou L, Hartono D, Ong C-N, Bay B-H, Yung L-YL. 2008. Gold nanoparticles induce oxidative damage in lung fibroblasts in vitro. *Adv. Mater.* 20:138–142
89. Papageorgiou I, Brown C, Schins R, Singh S, Newson R, et al. 2007. The effect of nano- and micron-sized particles of cobalt-chromium alloy on human fibroblasts in vitro. *Biomaterials* 28:2946–58
90. Franchini MC, Ponti J, Lemor R, Fournelle M, Broggi F, Locatelli E. 2010. Polymeric entrapped thiol–coated gold nanorods: cytotoxicity and suitability as molecular optoacoustic contrast agent. *J. Mater. Chem.* 20:10908–14
91. Stone V, Johnston H, Schins RPF. 2009. Development of in vitro systems for nanotoxicology: methodological considerations. *Crit. Rev. Toxicol.* 39:613–26
92. Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, et al. 2009. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. *Part. Fibre Toxicol.* 6:14
93. Selvan ST, Tan TT, Ying JY. 2005. Robust, non-cytotoxic, silica-coated CdSe quantum dots with efficient photoluminescence. *Adv. Mater.* 17:1620–25
94. Ghosh PS, Kim C-K, Han G, Forbes NS, Rotello VM. 2008. Efficient gene delivery vectors by tuning the surface charge density of amino acid–functionalized gold nanoparticles. *Am. Chem. Soc. Nano* 2:2213–18
95. Lin Y-S, Haynes CL. 2009. Synthesis and characterization of biocompatible and size-tunable multifunctional porous silica nanoparticles. *Chem. Mater.* 21:3979–86
96. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. 2004. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjug. Chem.* 15:897–900
97. Tkachenko AG, Xie H, Liu YL, Coleman D, Ryan J, et al. 2004. Cellular trajectories of peptide-modified gold particle complexes: comparison of nuclear localization signals and peptide transduction domains. *Bioconjug. Chem.* 15:482–90
98. Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, et al. 2005. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Lett.* 5:331–38
99. Chang E, Thekkek N, Yu WW, Colvin VL, Drezek R. 2006. Evaluation of quantum dot cytotoxicity based on intracellular uptake. *Small* 2:1412–17
100. Jiang J, Oberdörster G, Elder A, Gelein R, Mercer P, Biswas P. 2008. Does nanoparticle activity depend upon size and crystal phase? *Nanotoxicology* 2:33–42

101. Patra CR, Bhattacharya R, Patra S, Vlahakis NE, Gabashvili A, et al. 2008. Pro-angiogenic properties of europium(III) hydroxide nanorods. *Adv. Mater.* 20:753–756
102. Worle-Knirsch JM, Pulskamp K, Krug HF. 2006. Oops, they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett.* 6:1261–68
103. Laaksonen T, Santos H, Vihola H, Salonen J, Riikonen J, et al. 2007. Failure of MTT as a toxicity testing agent for mesoporous silicon microparticles. *Chem. Res. Toxicol.* 20:1913–18
104. Liao KH, Lin YS, Macosko CW, Haynes CL. 2011. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *Am. Chem. Soc. Appl. Mater. Interfaces* 3:2607–15
105. Han X, Gelein R, Corson N, Wade-Mercer P, Jiang J, et al. 2011. Validation of an LDH assay for assessing nanoparticle toxicity. *Toxicology* 287:99–104
106. Monteiro-Riviere NA, Inman AO, Zhang LW. 2009. Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicol. Appl. Pharmacol.* 234:222–35
107. Samberg ME, Orndorff PE, Monteiro-Riviere NA. 2011. Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. *Nanotoxicology* 5:244–53
108. Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, et al. 2009. Non-cytotoxic silver nanoparticle–polysaccharide nanocomposites with antimicrobial activity. *Biomacromolecules* 10:1429–35
109. Marambio-Jones C, Hoek EMV. 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J. Nanopart. Res.* 12:1531–51
110. Benn TM, Westerhoff P. 2008. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* 42:4133–39
111. Arora S, Jain J, Rajwade JM, Paknikar KM. 2009. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. *Toxicol. Appl. Pharmacol.* 236:310–18
112. AshaRani PV, Hande MP, Valiyaveettil S. 2009. Anti-proliferative activity of silver nanoparticles. *BMC Cell Biol.* 10:65
113. Foldbjerg R, Dang DA, Autrup H. 2010. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. *Arch. Toxicol.* 85:743–50
114. Hsin Y-H, Chen C-F, Huang S, Shih T-S, Lai P-S, Chueh PJ. 2008. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicol. Lett.* 179:130–39
115. Liu J, Hurt RH. 2010. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ. Sci. Technol.* 44:2169–75
116. Zook JM, Long SE, Cleveland D, Geronimo CLA, MacCuspie RI. 2011. Measuring silver nanoparticle dissolution in complex biological and environmental matrices using UV-visible absorbance. *Anal. Bioanal. Chem.* 6:1993–2002
117. Liu JY, Sonshine DA, Shervani S, Hurt RH. 2010. Controlled release of biologically active silver from nanosilver surfaces. *Am. Chem. Soc. Nano* 4:6903–13
118. Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, et al. 2010. Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. *Toxicol. Sci.* 118:160–70
119. Carlson C, Hussain SM, Schrand AM, Braydich-Stolle LK, Hess KL, et al. 2008. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *J. Phys. Chem. B* 112:13608–19
120. Park J, Lim D-H, Lim H-J, Kwon T, Choi J-S, et al. 2011. Size dependent macrophage responses and toxicological effects of Ag nanoparticles. *Chem. Commun.* 47:4382–84
121. Nohynek GJ, Lademann J, Ribaud C, Roberts MS. 2007. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Crit. Rev. Toxicol.* 37:251–77
122. Pruden AL, Ollis DF. 1983. Photoassisted heterogeneous catalysis—the degradation of trichloroethylene in water. *J. Catal.* 82:404–17
123. Iavicoli I, Leso V, Fontana L, Bergamaschi A. 2011. Toxicological effects of titanium dioxide nanoparticles: a review of in vitro mammalian studies. *Eur. Rev. Med. Pharmacol. Sci.* 15:481–508
124. Menard A, Drobne D, Jemec A. 2011. Ecotoxicity of nanosized TiO_2. Review of in vivo data. *Environ. Pollut.* 159:677–84

125. Migdal C, Rahal R, Rubod A, Callejon S, Colomb E, et al. 2010. Internalisation of hybrid titanium dioxide/para-amino benzoic acid nanoparticles in human dendritic cells did not induce toxicity and changes in their functions. *Toxicol. Lett.* 199:34–42
126. Barillet S, Simon-Deckers A, Herlin-Boime N, Mayne-L'Hermite M, Reynaud C, et al. 2010. Toxicological consequences of TiO_2, SiC nanoparticles and multi-walled carbon nanotubes exposure in several mammalian cell types: an in vitro study. *J. Nanopart. Res.* 12:61–73
127. Pan Z, Lee W, Slutsky L, Clark RAF, Pernodet N, Rafailovich MH. 2009. Adverse effects of titanium dioxide nanoparticles on human dermal fibroblasts and how to protect cells. *Small* 5:511–20
128. Simon-Deckers A, Gouget B, Mayne-L'Hermite M, Herlin-Boime N, Reynaud C, Carriere M. 2008. In vitro investigation of oxide nanoparticle and carbon nanotube toxicity and intracellular accumulation in A549 human pneumocytes. *Toxicology* 253:137–46
129. Wu J, Sun J, Xue Y. 2010. Involvement of JNK and P53 activation in G_2/M cell cycle arrest and apoptosis induced by titanium dioxide nanoparticles in neuron cells. *Toxicol. Lett.* 199:269–76
130. Sayes CM, Wahi R, Kurian PA, Liu YP, West JL, et al. 2006. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. *Toxicol. Sci.* 92:174–85
131. Hussain S, Thomassen LCJ, Ferecatu I, Borot M-C, Andreau K, et al. 2010. Carbon black and titanium dioxide nanoparticles elicit distinct apoptotic pathways in bronchial epithelial cells. *Part. Fibre Toxicol.* 7:10
132. Ge Y, Bruno M, Wallace K, Winnik W, Prasad RY. 2011. Proteome profiling reveals potential toxicity and detoxification pathways following exposure of BEAS-2B cells to engineered nanoparticle titanium dioxide. *Proteomics* 11:2406–22
133. Rushton EK, Jiang J, Leonard SS, Eberly S, Castranova V, et al. 2010. Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics. *J. Toxicol. Environ. Health A* 73:445–61
134. Lewicka ZA, Benedetto AF, Benoit DN, Yu WW, Fortner JD, Colvin VL. 2011. The structure, composition, and dimensions of TiO_2 and ZnO nanomaterials in commercial sunscreens. *J. Nanopart. Res.* 13:3607–17
135. Schnorr JM, Swager TM. 2011. Emerging applications of carbon nanotubes. *Chem. Mater.* 23:646–57
136. Iijima S. 1991. Helical microtubules of graphitic carbon. *Nature* 354:56–58
137. Edwards SL, Werkmeister JA, Ramshaw JAM. 2009. Carbon nanotubes in scaffolds for tissue engineering. *Exp. Rev. Med. Dev.* 6:499–505
138. Beg S, Rizwan M, Sheikh AM, Hasnain MS, Anwer K, Kohli K. 2011. Advancement in carbon nanotubes: basics, biomedical applications and toxicity. *J. Pharm. Pharmacol.* 63:141–63
139. BCC Res. 2010. *Carbon Nanotubes: Technologies and Global Markets.* Wellesley, MA: BCC Res. **http://www.bccresearch.com/report/carbon-nanotubes-technology-nan024d.html**
140. Uo M, Akasaka T, Watari F, Sato Y, Tohji K. 2011. Toxicity evaluations of various carbon nanomaterials. *Dent. Mater. J.* 30:245–63
141. van der Zande M, Junker R, Walboomers XF, Jansen JA. 2011. Carbon nanotubes in animal models: a systematic review on toxic potential. *Tissue Eng. B* 17:57–69
142. Tsukahara T, Haniu H. 2011. Cellular cytotoxic response induced by highly purified multi-wall carbon nanotube in human lung cells. *Mol. Cell. Biochem.* 352:57–63
143. Guo YY, Zhang J, Zheng YF, Yang J, Zhu XQ. 2011. Cytotoxic and genotoxic effects of multi-wall carbon nanotubes on human umbilical vein endothelial cells in vitro. *Mutat. Res. Gene Toxicol. Environ. Mutagen.* 721:184–91
144. Wick P, Manser P, Limbach LK, Dettlaff-Weglikowska U, Krumeich F, et al. 2007. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol. Lett.* 168:121–31
145. Di Giorgio ML, Di Bucchianico S, Ragnelli AM, Aimola P, Santucci S, Poma A. 2011. Effects of single and multi walled carbon nanotubes on macrophages: cyto and genotoxicity and electron microscopy. *Mutat. Res. Gene Toxicol. Environ. Mutagen.* 722:20–31
146. Jia G, Wang HF, Yan L, Wang X, Pei RJ, et al. 2005. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. *Environ. Sci. Technol.* 39:1378–83

147. Wang JY, Sun PP, Bao YM, Liu JW, An LJ. 2011. Cytotoxicity of single-walled carbon nanotubes on PC12 cells. *Toxicol. In Vitro* 25:242–50
148. Pulskamp K, Diabate S, Krug HF. 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol. Lett.* 168:58–74
149. Bottini M, Bruckner S, Nika K, Bottini N, Bellucci S, et al. 2006. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicol. Lett.* 160:121–26
150. Wang RH, Mikoryak C, Li SY, Bushdiecker D, Musselman IH, et al. 2011. Cytotoxicity screening of single-walled carbon nanotubes: detection and removal of cytotoxic contaminants from carboxylated carbon nanotubes. *Mol. Pharmacol.* 8:1351–61

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