

The Potential Toxicity of Nanomaterials—The Role of Surfaces

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Nanotechnology has attracted considerable attention in the scientific community ever since its emergence as a powerful basic and applied science tool. While beneficial aspects of nanomaterials are well visioned, several reports have suggested the negative impact of nanomaterials on living cells. The diverse array of surface properties achieved due to reduction in particle size that catalyzes the surface chemistry of nanoparticles is responsible for their toxic potential. Physical parameters such as surface area, particle size, surface charge, and zeta potential are very important for providing mechanistic details in the uptake, persistence, and biological toxicity of nanoparticles inside living cells. This short review provides insights into the physical, chemical, and interfacial parameters on the toxic potential of nanomaterials. While nanotechnology has promised invaluable progress in science and technology, the onus rests on the scientific community to predict the unknown outcome on the biological system for its safe proliferation.

INTRODUCTION

As evident from recent findings, nanotechnology can dramatically change the properties and applications of industrial and research materials. The selectivity and reactivity achieved due to very small size have produced a wide variety of applications of nanomaterials. Such extraordinary physicochemical properties bring along a concern about the adverse effects of nanostructures on biological systems. Research in the field of biomaterials and biotechnology along with other sources of human interaction with nanomaterials has thus attracted considerable attention. For example, carbon nanotubes (CNTs) have been

shown to be selectively separated by DNA and to further the potential for designing a biosensor.¹ However, CNTs have the potential to interact with the DNA, which can lead to undesirable results when present inside a living human body.

Materials surface chemistry is vital in biological interaction. Parameters such as zeta potential, dielectric constant, and

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type of surface charge could be useful in predicting the sustainability/mobility and reaction of materials inside the human body in terms of toxic evaluation. Scientists want to avoid the repetition of the experience with asbestos, which was once considered a miracle fiber and is now a major pollutant.² The proliferation of nanotechnology at an alarming rate could present new challenges to the environment.

The results on the unknowns of nanotechnology have been mixed and outcry of banning nanotechnology has so far been a concern, but not a threat. The discussion in this article is directed toward providing an insight into the biological toxicity of nanomaterials. The role of surface and interfaces in the toxic

potential of nanomaterials is presented.

CLASSIFICATION OF NANOMATERIALS

Human beings are exposed to airborne nanosized particles every day. Nanosized particles are variably called ultrafine particles by toxicologists at the U.S. Environmental Protection Agency, Aitken mode and nucleation mode particles by atmospheric scientists³ at the National Research Council in 1983, and engineered nano-structures by material scientists (National Nanotechnology Initiative 2004).⁴ Nanoparticles can originate from several sources classified as intentional, resulting from nanotechnology-based synthesis, and unintentional, originating from such sources as engine exhaust, volcanoes, and forest fires.⁴ These particles can be classified into three categories: nanosized particles (NSP) <100 nm, engineered nanoparticles of spherical shapes, and ultra-fine particles that include ambient and laboratory-generated NSPs that are not produced in a controlled, engineered way.⁴ In addition to these spherical nanoparticles are various shapes and morphologies such as rods, tubes, fibers, rings, and wires, which can be classified separately.

See the sidebar for details on how nanoparticles enter humans.

MECHANISMS BEHIND TOXICITY OF NANOMATERIALS

Nanosized particles can penetrate the human body via various routes and could persist in the system because of the incapability of the macrophages to phagocytose them. Whether these persisting nanomaterials react with the body, stay inert, or interact with the system

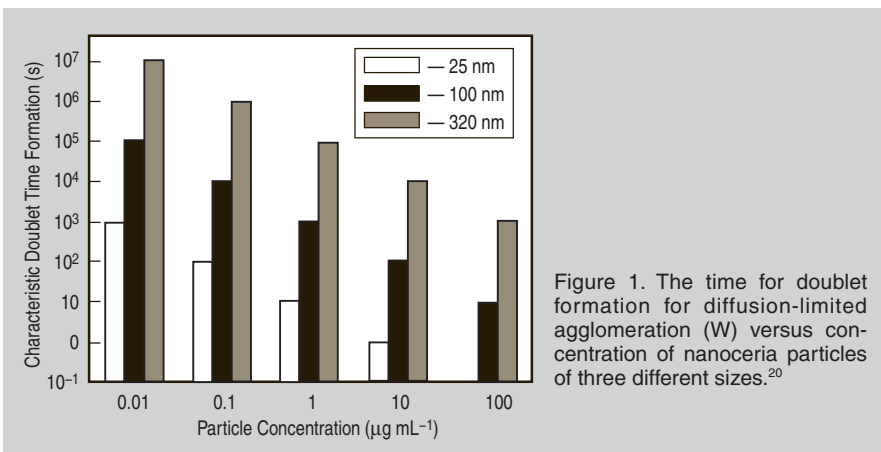


Figure 1. The time for doublet formation for diffusion-limited agglomeration (W) versus concentration of nanoceria particles of three different sizes.²⁰

will govern their toxic properties and is primarily dependent on their surface properties.

Surface Chemistry

It seems that the role of surface chemistry had been underemphasized in the present research of nanotoxicity. Whether the particle remains suspended as an individual particle or as an aggregate depends upon its surface chemistry. A small aggregate or single particle is presumed to be more toxic than an aggregate of NSPs as the relative surface area could change, determining whether the material has a good wetting characteristic, or has a surface characteristic that catalyzes specific chemical reactions or remains passive and allows fibrous tissue to grow on its surface. It was shown¹⁸ that the rats, when treated to polymeric vapors of polytetrafluoroethylene (PTFE) having a diameter of 18 nm, suffered severe lung injury with high mortality rate of within 4 h after a 15 min. inhalation exposure to 50 µg/m³. The aging¹⁹ of PTFE fume particles for 3 min. increased their size to >100 nm due to agglomeration, which resulted in loss of toxicity.

Particle Size

Nanoparticles may be surrounded by water molecules and may or may not get agglomerated when present in fluid medium, which, in turn, will govern the diffusion of species. The diffusion coefficient of the particles can be derived using the well-known Stokes Einstein equation (Equation 1), where R is the hydrodynamic radius, T is the temperature, η is the viscosity, and k_b is the Boltzmann constant (all equations are given in the Equations table on page 80).

The aggregation of the particles can also be quantified using a simple formulation.²⁰ If n_i is the monodisperse particle population and β is the aggregation rate constant, then Equations 2 and 3 can be used to derive the characteristic time of a doublet formation τ from initial particle concentration n_0 (see Equation

2),²⁰ where W is the stability ratio relating the steric and electronic hindrance toward the aggregation and gives the ratio of the aggregation constant of diffusion limited cluster aggregation and the observed aggregation constant. The time for doublet formation which gives a measure of the agglomeration is given in Equation 3.

In a study of the ceria nanoparticles, particle concentration and particle size have been found to significantly influence the stability ratio and hence the doublet formation time. The doublet formation time of large particles was reported to be a few orders of magnitude higher than the nanoparticles even at a very low concentration, as shown in Figure 1.²⁰

Surface Charge

It can be noted that the particle size has an effect on the stability constant and this can be altered by small changes

NANOPARTICLE ENTRY ROUTES INTO HUMANS

Airborne inhalation of nanosized particles (NSPs) and entry through the respiratory tract is the most likely route of exposure to nanoparticles. It has been shown⁵ in a model workplace that a very low concentration of <50 µg/m³ can enter the human body upon significant exposure to airborne singlet-engineered carbon nanotubes or C₆₀ fullerenes. Exposure via other routes has not been studied in detail and is less plausible unless it is by direct ingestion through food or drug delivery, dermal contact through application of oils and skin creams, or as a contaminant in water through a nanoparticle-treated membrane system.

Respiratory Tract

Inhalation of airborne nanoparticles through the respiratory tract is a common means of entry in humans. Degradation of engineered nanomaterials that are available commercially can be one such route. It has been shown that the inhaled nanoparticles are efficiently deposited by diffusional mechanisms in all regions of the lungs. Hoet et al.⁶ summarized that most nanosized spherical solid materials easily enter the lungs. In a preliminary study by two independent groups^{7,8} the pulmonary effects of single-walled carbon nanotubes (SWCNTs) were demonstrated. It was concluded that CNTs can reach the lungs and can be even more dangerous than carbon black or quartz. Recently, Zhang et al.⁹ compared the deposition of nano- and micro-sized particles in a human upper airway system. Deposition of nanoparticles occurs at greater concentrations around the carinal ridges when compared to the straight segments in the bronchial airways; however, deposition distributions are much more uniform along the airway branches. The deposition enhancement factors vary with bifurcation, particle size, and inhalation flow rate. The relatively larger uniform distribution of nanoparticles can lead to greater toxicity as it presents a greater area to react with the cell membranes and absorb and transport toxic substances.

It has also been demonstrated that NSPs are not always cleared effectively by alveolar macrophages. Figure A displays the results of several studies of exposure of rats to different-sized particles of polystyrene beads.⁴ It is observed that as opposed to 80% retention of the micrometer-sized particle in the macrophages, only 20% of NSPs were retrieved by the macrophages, resulting in greater toxicity.

It has been reported that long fibers are not effectively cleared from the respiratory tract, thereby increasing their bio-persistence.⁶ According to Stanton et al.,^{10,11} for mesothelioma induction in rats, the peak activity of glass fibers was greatest for fibers longer than 8 µm and less than 1.5 µm in diameter. The hypothesis commonly known as the Stanton hypothesis, valid for micrometer-size particles, can be very much applicable to the aspect ratio variation in nanofibers. Various studies showed that CNTs can cause toxicity in cellular systems,^{5,7,8,12-14} where the aspect ratio of CNT plays an important role in biointeraction. For example, SWCNTs induce more stress than multi-walled CNTs.¹²

on the particle surface, such as charge distribution. The surface potential (ΔV) of a monolayer spread at the air/water interface can be interpreted using the Helmholtz Equation 4, where μ_n is the effective molecular dipole moment at the interface, ϵ is the vacuum dielectric permittivity, and A is the surface area per molecule.²¹ When a particle is present inside a human body, the most common surfaces it comes across are the phospholipid membranes. Protein adsorption onto alumina NSPs caused a shift in the iso-electric point after a monolayer of protein was adsorbed onto the surface.²¹ Nanosized particles are found to overcome the blood-brain barrier (BBB) and the surface charge influences the integrity of the BBB and permeability of the NSPs in the system.²² Whether NSPs can cross this phospholipid membrane barrier and/or interact with it will decide the level of toxicity. For

example, a particle having a high affinity for phosphates may or may not react with the phospholipid membranes. Such being the case, monolayers of particles spread at the phospholipid membrane can be treated as a Vogel Mobius two-capacitor model; an effective molecular dipole moment is given by Equation 5, where μ_w represents the contribution of the tail group and μ_a represents the contribution from the head group of a phospholipid.²² These contributions can be directly influenced by the surface charge present on the molecule and the complex forming ability of the particle with the phospholipid. Zeta potential and ultraviolet-visible-spectroscopy measurements may thus provide an estimate of adsorption of proteins, etc. on NSPs.²³ Zeta potential (ζ) of the particles having mobility v_E in body fluid media thus becomes an important parameter and is given by the Smoluchowski Equa-

tion 6, where ϵ_r and ϵ_0 are the relative dielectric constant and the electrical permittivity of a vacuum respectively, η is the solution viscosity, r is the particle radius, and $\kappa = (2n_0z^2e^2/\epsilon_r\epsilon_0k_B T)^{1/2}$ is the Debye-Hückel parameter, n_0 is the bulk ionic concentration, z is the valence of the ion, e is the charge of an electron, k_B is the Boltzmann constant, and T is the absolute temperature.

It can be noted that in all the equations mentioned for relating the stability, zeta potential, or agglomeration, particle radius is directly or inversely related and hence an important parameter in bio/material interaction. Thus, from a material scientist's point of view, these parameters can be evaluated and compared to derive a hypothetical uptake of NSPs and their agglomeration behavior inside the body.

Surface Area

Surface area also plays a major role in the interaction of the materials with biocells. One must appreciate that nanomaterials have a high surface-area-to-volume ratio and thus are more reactive than their coarse counterparts. One such example was presented²⁴ in a preliminary study where pulmonary inflammatory response to anatase and rutile TiO_2 was characterized. When the dose-to-neutrophil response was expressed as a function of mass, the two forms of titania showed entirely different responses while when expressed as a function of surface area the response was similar for both the particles. It can thus be assumed safely that surface area for particles of different sizes but the same chemistry is a better dosimetric than particle mass or number.

In aerosols, the degree to which engineered NSPs aggregate in ambient aerosol and subsequently aggregate or remain suspended following inhalation and deposition in lungs can influence the particle deposition rates and interaction behavior with lungs.²⁵ If the particles do not aggregate they may induce enhanced inflammatory reaction, directly influencing the entire area being in contact with the cells. The surface area concentration "S" of an aerosol represents an attractive method of estimating the aerosol surface area from combined number and mass concentration measurements and is given by $S = N\pi d_s^2$,²⁶ where d_s is the average

Intestinal Media

Nanosized particles cleared from the respiratory tract can enter the gastrointestinal (GI) tract. They can also be ingested directly if present in food or water or through drug delivery. There is considerable literature on the intestinal uptake of materials but the cellular uptake of nanomaterials is less studied and most of them have shown that NSPs pass through the GI tract and are eliminated rapidly.

Dermal Layer

Skin is a barrier structured in three layers: epidermis, dermis, and the subcutaneous layer. Dermal exposure is another important uptake source for NSPs especially because of the increased interest in the use of TiO_2 , ZnO , and other nanoparticles for protection against ultraviolet rays in various dermal creams, lotions, and cosmetics. Nanosized particles can enter through the unbroken skin during the flexing of the wrist.¹⁵ The flexing of the skin can lead to uptake of micrometer-long fluorescent beads. TiO_2 particles (5–20 nm) have been argued to penetrate into the skin cells and interfere with the immune system. Anatase TiO_2 nanoparticles (10 nm and 20 nm) induced oxidative DNA damage, lipid peroxidation, and micronuclei formation. They also increased hydrogen peroxide and nitric oxide production in BEAS-2B cells, a human bronchial epithelial cell line, in the absence of photo activation.¹⁶ However, the treatment with anatase (≥ 200 nm) particles did not induce oxidative stress in the absence of light irradiation. Thus, it is easy to hypothesize in the present case that the shorter the size of the nanoparticles the easier it

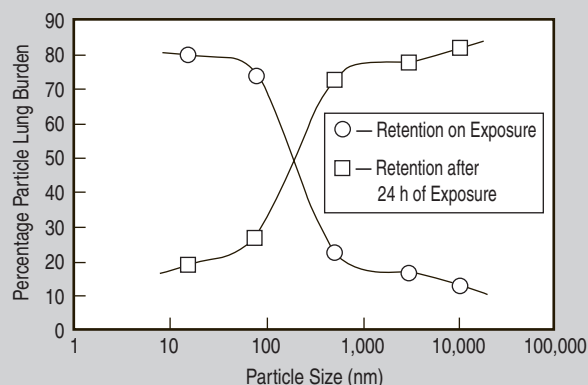


Figure A. The in-vivo retention of inhaled nanosized and larger particles in alveolar macrophages and in exhaustively lavaged lungs (epithelial and interstitial retention) upon exposure and 24 h after exposure.⁴

becomes for the NSPs to permeate through skin and induce damage. It was reported that contrary to popular belief, the photocatalytic activity of the anatase is higher than that of the rutile. In fact, 200 nm rutile titania can induce H_2O_2 and oxidative DNA damage in the absence of light while the anatase titania remains inert. The uptake of materials via skin is a rather complex process and there are both exogenous and endogenous factors involved.¹⁷

diameter is given by Equation 7 and the count mean diameter (CMD) and N are defined from the unimodal longnormal particle size distribution function F as $F(x_1, x_2, \dots, x_m, d) = N\Phi(\text{CMD}, s_g, d)$ where $x_1 = n$, $x_2 = \text{CMD}$, $x_3 = \sigma_g$ (geometric standard deviation), and d is the diameter of the particle (the x_1, x_2, x_3 , etc. represent the size distribution), L is the layer thickness, Φ is the normalized longnormal function, and n is the number of independent measurements made.

A bird's-eye view of the relative adsorption and agglomeration of the particles inside the human body, whether inhaled or injected in the body, can be obtained following the various physical parameters described. As surface area and the ultrafine size of nanoparticles are recognized for their superior properties, the same can be attributed to their ill effects. However, it must be pointed out that there are mixed reports of the toxicity being dependent upon the particle size and the surface area. Though most of the research has pointed out that the NSPs are more toxic than their coarse counterparts, there are contradicting reports available. Recently it has been shown that the toxicity in the case of pulmonary instillation studies using titania nanorods and nanoparticles is not dependant upon the particle size or the surface area.²⁵ Table I lists various parameters for different nanomaterials

and their relative biological effects in the light of toxicity.

Oxidative Stress and Reactive Oxygen Species

It is established that there is a direct correlation between the ROS generating capability, the surface area of NSPs, and the inflammatory response in the lung.^{4,30-33} Nel et al.³³ summarized from various resources^{4,30-34} that ROS generation is the best-developed paradigm for the explanation of toxic effects of inhaled nanoparticles. As opposed to normal coupling conditions in mitochondrion (low ROS generation), under excess ROS generation due to nanoparticle exposure, the natural antioxidant defense mechanisms may be inundated.³⁵ The role of the surface groups is paramount to the behavior of nanomaterials in vivo. Surface groups, as pointed out earlier, can make the material hydrophilic, hydrophobic, lipophilic, lipophobic, or catalytically active or passive. An illustration of the various possibilities of reaction of surface groups with biological tissue is presented in Figure 2.³³ The figure emphasizes the importance of various factors contributing to the interaction with biological system. Oxidative stress due to exposure to NSPs of TiO₂, quartz, carbon black, etc. results in airway inflammation and interstitial fibrosis.^{4,30-33,36} Oxidative stress is considered to be a potential

EQUATIONS	
$D = \frac{k_B T}{6\pi\eta R}$	(1)
$\frac{dn_t}{dt} = -\frac{1}{2} \frac{\beta}{W} n_t^2$	(2)
$\tau = \frac{2W}{\beta} = \frac{3\eta W}{4k_B T n_0}$	(3)
$\Delta V = \frac{\mu_n}{\epsilon_0 A}$	(4)
$\mu_n = \mu_a + \mu_w$	(5)
$v_E = 4\pi\epsilon_0\epsilon_r \frac{\zeta}{6\pi\eta} (1 + \kappa r)$	(6)
$d_s = \text{CMD}e^{L n \sigma}$	(7)

element for screening the toxicity of NSPs. Responses at each level of oxidative stress such as large antioxidant enzyme expression or cytokines in the lungs of animals have been incorporated as screening assays in-vivo.³³

Nanoceria in Biological Cells

Though the previous discussion raises concerns about the ROS-generating capabilities of nanomaterials, the authors' research group is working on ceria nanoparticles/rods which have shown exceptional radical scavenging properties and promote cell longevity owing to their powerful antioxidant properties.³⁷ In one such study, specially fabricated surface-modified nanoceria particles were compared with a control sample of surfactant (in toluene) in two different cell lines: A549 (human lung carcinoma cell line) and SH-SY5Y (human neuroblastoma cell line). Neutral red (NR) assay was carried out 24 h after the treatment of these cells with ceria nanoparticles.

A cytotoxic material is suspected to interfere with the normal and continuous multiplication and proliferation of healthy cells. Preliminary studies have indicated that the viability of neurons treated with 3-5 μm spherical ceria is higher than the surfactant (control sample) at concentrations below 10 nM, indicating a positive effect of nanoceria particles compared to surfactant alone and surfactant-coated nanoceria. The

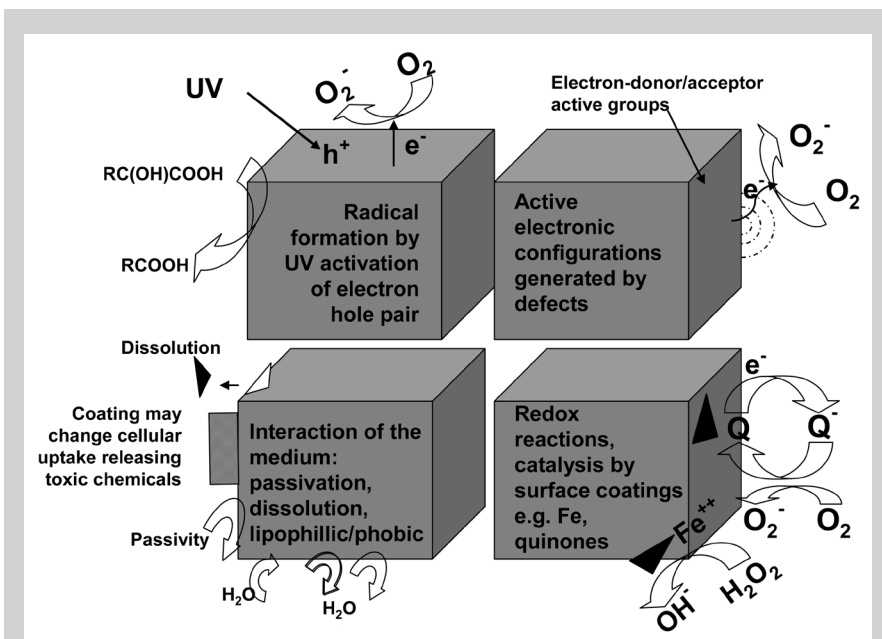


Figure 2. The mechanisms of interaction of nanomaterials with biological tissues, illustrating the importance of material chemistry, electronic structure, bonding, active or passive surface coatings, solubility, and interactions with other environmental factors.³³

cells treated with 5 mM commercially available nanoceria (~10–15 nm, cubical shape) in water seem to have the lowest cell viability overall compared to the viability of cells treated with all the other samples (Figure 3). This shows the same pattern as seen on A549 cells (not shown). The radical scavenging properties of ceria are currently being explored for developing a radical sensor. Thus, a careful consideration of all the properties and parameters must be made before a statement is passed for the possible behavior of nanomaterials in biosystems. In the authors' belief, all the NSPs currently used should be categorized into groups based on their reactivity and properties and a model should be developed for studying the toxicity.

FUTURE RESEARCH AND TRENDS

No clinically relevant toxicity has yet been reported for NSPs but there is a growing concern among researchers worldwide about the possible outcomes of the ill effects (if any). Most of the data have reported the toxicity in terms of the blockage of airways leading to asphyxiation from unrealistically high dosages of materials. It has been suggested that the granulomatous inflammation observed during the bio-persistence of ODNs particles could have a similar affect as that of asbestos fibers and the metal impurities present could account for the

toxicity.^{8,33} There are issues that need to be addressed as to whether there could be substantial uptake of nanomaterials from the workplace, both on the laboratory and industrial scales.

Research efforts are also being made toward screening the toxicity of materials.³⁸ The National Nanotechnology Initiative (NNI), a U.S. federal research and development program for nanotechnology, has a section devoted to identifying the potential exposure, possible toxicity, and need for personal protective equipment when working with nanoscale materials. Several other U.S. agencies (eg., the National Science Foundation Nanoscale Science and Engineering

technology, the NNI Nanotechnology Environment and Health Implications working group, the Environmental Protection Agency, and the National Institute for Occupational Safety and Health) are working to identify support and monitor the impact of nanotechnology on environment and health. The National Institutes of Health–National Toxicology Program funds research on the toxicity of nanomaterials. A recent report on the environmental risks of nanotechnology discussed the funding and the focus of the NNI on the direct and indirect impacts of the nanotechnology on environment.³⁹ Not only does this encompass the full life-cycle analysis approach for identify-

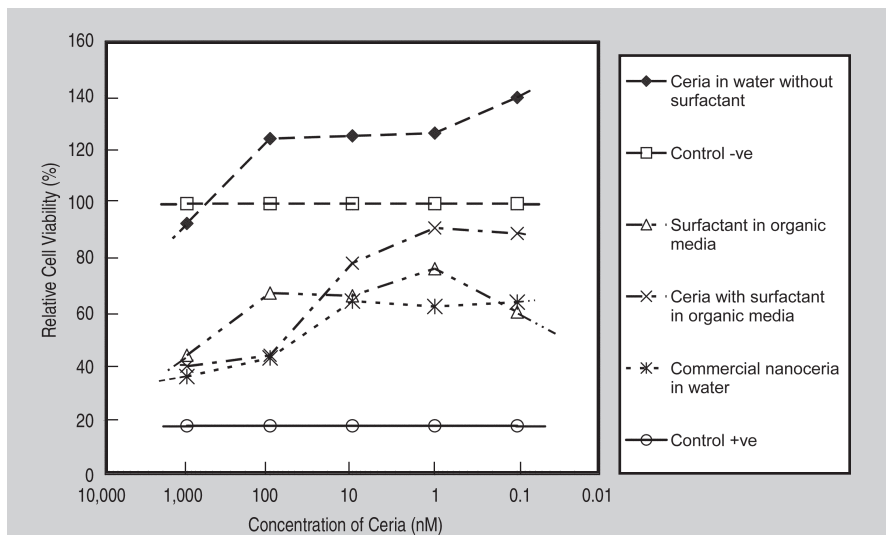


Figure 3. The relative neurons cell (SH-SY5Y) viability of different concentrations of ceria solution.

Table I. Toxic Evaluation of Nanostructures as a Function of Surface Properties*					
Type of Material	Particle Size	Surface Area	Charge/Zeta Potential	Biological Toxicity	Ref.
Alumina	116 nm	13.37 m ² /g	45-50 mV (pH 5.5–6.5)	Protein (BSA) adsorption with time IEP shifts with pH and surface area	23
PEG Quantum (Q) Dots	10 nm	—	—	Retention of Q dots in liver, spleen, and bone marrow	27
MWCNT	10–20 nm	40–300 m ² /g	—	Cytotoxicity: alveolar macrophages at high dose	14
SWCNT	1.4 nm	270 m ² /g	—	Cytotoxicity: alveolar macrophages at low dose; transient inflammatory and cell injury	14 8
Titania	300 nm (rutile)	6 m ² /g	—	Short-term reversible inflammation	25
	Rods (20–233 nm) (anatase rods)	26.5 m ² /g	—	Short-term reversible inflammation, minor adverse lung tissue reaction	25
	Spherical (5–6 nm) (anatase spherical powder)	169.4 m ² /g	—	Short-term reversible inflammation, minor adverse lung tissue reaction	25
Quartz	1.5 μm	4 m ² /g	—	High pulmonary toxicity	25
PTFE	20 nm	—	—	Cell death—15 min. exposure	24
	130 nm	—	—	No ill effects	
Emulsifying Wax	74.7 ± 53.4 nm (neutral)	—	-14.1 ± 2.1 mV	No BBB permeation ability in low conc.	22
	127.1 ± 70.6 nm (anionic)	—	-59 ± 2.9 mV	No BBB permeation ability in low conc.	22
	97.2 ± 68.9 nm (cataionic)	—	45.2 ± 3.5 mV	Toxic at brain microvasculature endothelium	22
Ceria	3–5 nm	—	—	Radio protection, nontoxic at low/medium dose	28
Yttria	50 nm	—	—	Neuroprotection against oxidative stress	29

*IEP—iso-electric point; SWCNT—single-wall carbon nanotubes; MWCNT—multi-wall carbon nanotubes; BBB—blood-brain barrier; PTFE—polytetrafluorethylene; PEG—polyethylene glycol

ing the risks of nanotechnology but also assesses the economic and environmental tradeoffs in the process design.

A very good example of a systematic approach toward identifying the possible effects of nanotoxicity is further reviewed.⁴⁰ A risk score was determined based on toxicity, water solubility, flammability, and emissions. A relative estimate of the adsorption and uptake of NSPs and their agglomeration behavior can be found out by calculating of parameters such as surface area, particle size, surface charge, zeta potential, and surface functional groups. The mechanism, being dependent on too many parameters, is hard to visualize and characterize at present. The aim of nanotechnology is to provide better solutions to humanity but not at the cost of human lives. Chances cannot be taken by refuting the preliminary findings as too diminutive to be a threat as human lives will be at the receiving end.

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