# Chapter 1
# Inorganic Nanomaterials for Enhanced Therapeutic Safety


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## Contents



**Abstract Background/issues:** Nanomaterials have been effectively and widely utilized in a variety of scientific disciplines to enhance biomedical applications. These nanomaterials are often organic or inorganic and often comprised of polymers or metal derivatives. The therapeutic safety of these often-toxic materials, however, is of paramount importance to ensure therapeutic safety. The safety of nanomaterials is therefore a widely undertaken research discipline evaluated both in vitro and in vivo. **Major advances:** This review provides for the currently undertaken research for the determination of therapeutic safety in inorganic nanomaterials. The importance of therapeutic safety, toxicity, and regulation of nanomaterials has been provided prior to the review of the respective nanomaterials. Specific focus has been given to metal-derived nanomaterials including gold, silver, silica, copper, iron, zinc, and titanium nanomaterials. Toxicology profiling and cytotoxicity studies of these nanomaterials have also been provided in addition to the in vivo studies that

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have been undertaken and the potential for alternative nanomaterial safety assessments.

**Keywords** Multifunctional nanomaterials · Toxicity · Physicochemical properties · Gold · Silver · Silica · Copper · Iron · Zinc · Titanium dioxide

## 1.1 Introduction

Exploited mainly for drug delivery applications, advances in the field of nanomaterials have been exponential. Offering the advantages of enhanced and efficacious delivery, nanomaterials improve the in vivo stability and solubility of active pharmaceutical agents (APIs) (Moghimi et al. 2001; Jia et al. 2013). Their nanosize correlates with many biological structures and organelles, making the nanomaterials appropriate for interactions at the submicron scale, thereby assisting in improving intracellular delivery, circulation, biodistribution, and the crossing of biological membranes (Singh and Lillard Jr 2009; Hassan et al. 2017).

Beyond the conventional approach of using nanomaterials as delivery vehicles, nanomaterials have also been designed and developed to confer individual functionality in the fields of energy generation, devices, therapeutics, biomedical applications, and chemical assays including medical imaging and antimicrobial coatings. The advent of nanomaterials has also led to the development of functionalized nanotechnology-based drug delivery systems that are able to diagnose, image, sense, and deliver therapeutics via conjugating moieties, such as aptamers, small molecules, and peptides (Lee et al. 2012).

Gold nanoparticles, silver nanoparticles, copper nanoparticles, magnetic nanoparticles, and mesoporous silica are some examples of inorganic nanocarriers which are amenable to functionalization and in addition can provide tracking capabilities (Subbiah et al. 2010). Although nanomaterials offer attractive advantages, physicochemical properties such as shape, size, surface charge, structure, composition, functionalization, and dissolution could significantly affect their cytotoxicity and therapeutic safety (Sharifi et al. 2012; Nel et al. 2013). Organic-based nanomaterials such as polymeric micelles, nanoparticles, and liposomes, primarily consisting of biocompatible amphiphilic copolymers, are well-known for their therapeutic safety (Bi et al. 2008; Oh et al. 2008). Contrasting studies have been conducted in the past where some authors have demonstrated that inorganic nanoparticles are in fact suitable for in vivo applications, whereas others have proved otherwise. Undoubtedly, the ability of nanomaterials to impart both action and interference at the cellular level renders many toxicity implications for such materials. This chapter will therefore detail the use, safety, and toxicity of inorganic nanomaterials with emphasis provided on the toxicology profiling and cytotoxicity studies of these nanomaterials in addition to the results of the in vivo studies that have been undertaken. Also provided for is the effectiveness of current safety profiling in addition to the potential for alternative nanomaterial safety assessments.

## 1.2 Mechanism of Toxicity

Nanoparticle toxicity is dependent on dose, route of administration, size, shape, lipophilicity, and exposure time and may interrupt the chemical and biological processes at various parts of the human anatomy such as at the molecular, cellular, and tissue levels (Johnston et al. 2010; Schrand et al. 2010; Wolfram et al. 2015). Interactions between these nanoparticles and body's biomolecules instantaneously occur upon delivery of the nanoparticles. This is due to the nanoparticles' high surface free energy which results in the biomolecules coating the nanoparticles, forming the protein corona (Monopoli et al. 2012; Wolfram et al. 2014, 2015). Consisting of both a hard and soft layer, the protein corona can drastically influence the nanoparticle size, shape, and charge, ultimately changing the amount of protein interactions (Fig. 1.1; Wolfram et al. 2014). Additionally, the endogenous biomolecules may also undergo structural and functional alterations as a result.

As mentioned, nanomaterial size, composition, and surface chemistry of nanomaterials are key determinants in their interactions with biological systems and their subsequent toxicity (Mirshafiee et al. 2017). These physicochemical properties may result in random membrane insertion, thereby leading to a cascade of signaling transductions that result in cytokine production and proinflammatory responses or eventual cell death. At the cellular level, peroxidative product accumulation, in vitro apoptosis, and cell antioxidant depletion can occur as a result of overproduction of reactive oxygen species (ROS) (Shang et al. 2014; Wang et al. 2016). Consequently, the redox state of the cell becomes imbalanced resulting in oxidative stress which has detrimental effects on the cells through protein, lipid, and DNA damage, resulting in cellular apoptosis and mutagenesis (Khanna et al. 2015). In order to ensure in vivo safety, well-defined methods to characterize and evaluate nanomaterials are required. Cellular homeostasis can be affected by inorganic nanomaterials, thus

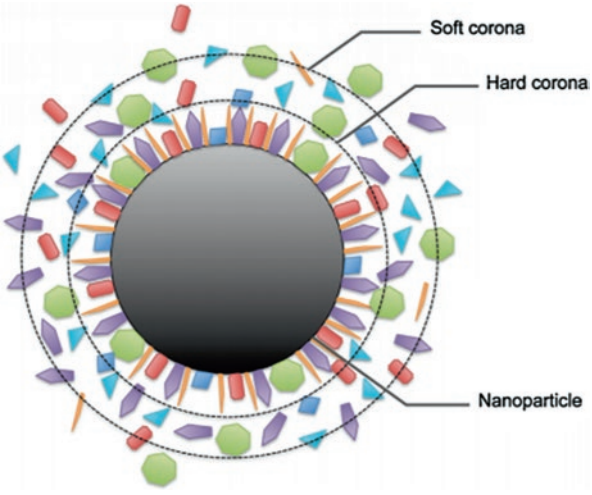


**Fig. 1.1** Schematic representation of the current protein corona hypothesis. A hard and soft layer of proteins covers the surface of the nanoparticle. The proteins in the hard corona are more tightly associated with the particle surface, making them less dynamic than the proteins in the soft corona. (Reproduced from Wolfram et al. 2014, © 2014 Elsevier B.V)

allowing for a cascade of possible effects. Several mechanisms may result in such effects which are detailed in Fig. 1.2.

At the cellular level, nanoparticles may disrupt membrane integrity resulting in cellular leakage and disruption or destruction of cellular function. Lysosomal membrane dysfunction has been reported to be caused by polycation particles (Molinaro et al. 2013), zinc oxide (Cho et al. 2011), and titanium dioxide (Hamilton et al. 2009), resulting in endoplasmic reticulum stress, mitochondrial dysfunction, oxidative stress, and protein aggregation (Stern et al. 2012). Organ-related nanoparticle toxicity also occurs as a result of nanoparticle accumulation due to toxicity occurring at the molecular and cellular levels as well as through immunological responses.

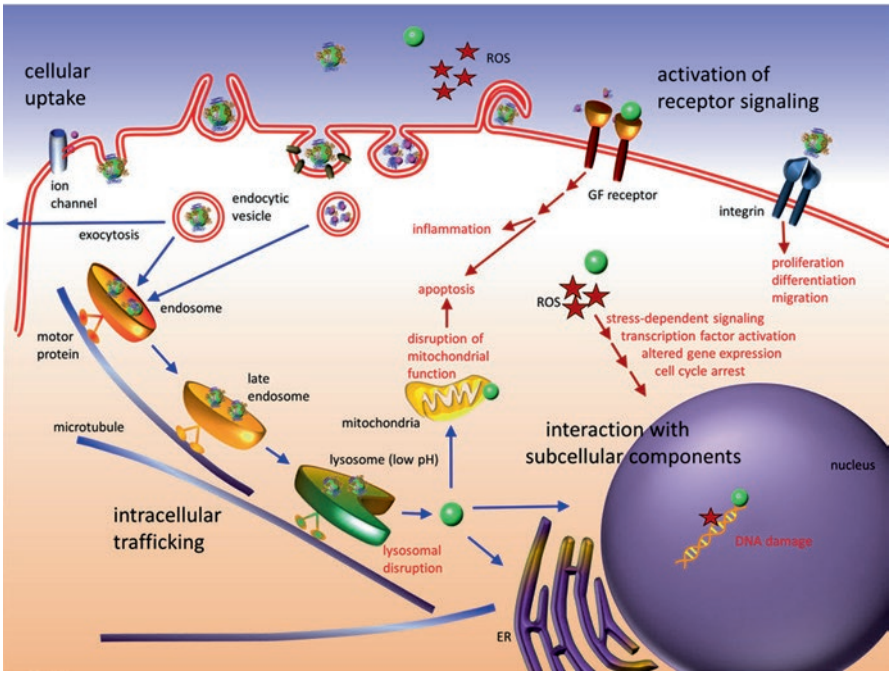


**Fig. 1.2** Cytotoxic effects of nanoparticles. In the biological environment, nanoparticles may trigger the production of reactive oxygen species (ROS). Elevated ROS levels may lead to (i) activation of cellular stress-dependent signaling pathways, (ii) direct damage of subcellular organelles such as mitochondria, and (iii) DNA fragmentation in the nucleus, resulting in cell cycle arrest, apoptosis, and inflammatory response. Nanoparticles may interact with membrane-bound cellular receptors, e.g., growth factor (GF) receptors and integrins, inducing cellular phenotypes such as proliferation, apoptosis, differentiation, and migration. After internalization via endocytic pathways, nanoparticles are trafficked along the endolysosomal network within vesicles with the help of motor proteins and cytoskeletal structures. To access cytoplasmic or nuclear targets, nanoparticles must escape from the endolysosomal network and traverse through the crowded cytoplasm. (Reproduced from Shang et al. 2014 © Shang et al.; licensee BioMed Central Ltd. 2014, distributed under a CC-BY 2.0)

## 1.3 Inorganic Nanomaterials Used in Drug Delivery

### *1.3.1 Metallic Nanomaterials*

#### Gold Nanoparticles

Possessing appreciable properties such as unique optical and electric properties and ease of functionalization with targeting moieties, drugs, and polymers using gold-thiol bonds, gold (Au) nanoparticles have been widely studied and employed in various applications such as drug delivery, photothermal delivery, and cellular and diagnostic imaging (Dreaden et al. 2012; Jia et al. 2013; Cheng et al. 2017). A study undertaken by Huo (2010) employed Au nanoparticle-based protein complex aggregation biomarker assays for the detection and diagnosis of cancer. Since these Au nanoparticles may be easily functionalized through simple bioconjugation techniques in a range of shapes and sizes, their cytotoxicity may thus be easily affected.

Below sizes of 4–5 nm in diameter, Au nanoparticles are catalytically active and may induce cytotoxicity (Falagan-Lotsch et al. 2016). Additionally, many toxicity studies have found Au nanoparticles with sizes greater than 4–5 nm in diameter to be nontoxic after acute exposure (Alkilany and Murphy 2010; Khlebtsov and Dykman 2011; Soenen et al. 2011). Particles of this size are considered mostly nontoxic to the mitochondrial cells; however, oxidative stress and mitochondrial damage can be incurred in cultured cells due to their high surface reactivity (Dreaden et al. 2012).

Au nanoparticles of 5 nm are generally used in nanomedicine and are presumed safe in drug delivery applications and photothermal therapy, with particles larger than 5 nm having the potential to result in cellular toxicity (Alkilany and Murphy 2010). Different cell type sensitivities and the use of high concentrations of Au nanoparticles may also attribute to other cases of acute toxicity (Patra et al. 2007; Mironava et al. 2010; Khlebtsov and Dykman 2011). After in vivo administration, Au nanoparticles have not been fully investigated for their long-term toxicological effects. Au nanoparticles have also been known to display an accumulation of degradation products as well as a reduced clearance of several months, possibly resulting in chronic toxicity (Khlebtsov and Dykman 2011; Kolosnjaj-Tabi et al. 2015). In addition, nephrotoxicity and erythrocytic cellular death have also been shown in vivo (Sereemaspun et al. 2008; Sopjani et al. 2008).

Studies have also shown that Au nanoparticle surface charge influences cellular uptake properties. The negatively charged cell surface residues have a higher affinity for cationic gold nanoparticles as compared to their anionic counterparts. Nevertheless, studies have demonstrated that the cationic nanoparticle surface charge can also result in an increased cytotoxicity in airway cells BEC and ASM and the ovarian cancer cells CP70, A2780 (Arvizo et al. 2010), and HeLa (Hauck et al. 2008) due to their altered surface properties associated with reduced particle size.

Additionally, due to their high biocompatibility and bioinert character, Au nanoparticles have also been extensively applied in diagnostic applications and gene delivery. However, for endothelial and epithelial applications, the presence of stabilizers such as sodium citrate residues on the Au nanoparticles was revealed to affect the proliferation and induce cytotoxicity in human alveolar type II (AT II)-like cells, human cerebral microvascular endothelial cells (hcMEC/D3), and human dermal microvascular endothelial cells (HDMEC). The study was carried out to determine if these effects were related to the varying degree of internalization of the Au nanoparticles, to surface sodium citrate on the Au nanoparticles, or to nanoparticle size. Differing uptake behaviors for citrate-stabilized Au nanoparticles were observed for the epithelial and endothelial cells. Concentration-dependent cytotoxicity was also observed after exposure to the Au nanoparticles (Fig. 1.3). It was demonstrated that the lower the degree of purification, the less cell viability and proliferation occurred concluding that the safety of Au nanoparticles may be enhanced with the abridged addition of sodium citrate (Freese et al. 2012).

Falagan-Lotsch and coworkers (2016) investigated the long-term in vitro effect on human dermal fibroblasts of two shapes of Au nanoparticles. The study was conducted on both nanorods and nanospheres under both chronic and nonchronic conditions. It was determined that the oxidative stress and inflammation gene expression could be modified with a subcytotoxic dose of Au nanoparticles, with the effect lasting over 20 weeks. The results indicated that the cell stress response is not reversible over time upon removal of the nanoparticles after acute exposure and that the cells can adaptively respond to chronic, low-level nanoparticle insults (Falagan-Lotsch et al. 2016). Interestingly, in the study undertaken by Falagan-Lotsch et al. (2016), the surface chemistry of polyethylene glycol was found to be not as benign as is generally assumed.

These studies investigating the toxicological effects of Au nanoparticles therefore suggest that long-term studies are warranted, rather than their acute counterparts, to elucidate better, safety profiles of gold nanomaterials.

### Silver Nanoparticles

Used in various antimicrobial applications, silver (Ag) nanoparticles have been implicated in major health concerns due to their toxicological impacts on various organs (Mirshafiee et al. 2017). A notable effect of chronic silver exposure is argyria in humans (Wijnhoven et al. 2009). Ag nanoparticles release toxic silver ions following particle dissolution resulting in significant cytotoxicity via ROS generation (Wang et al. 2014; Zhornik et al. 2014; Osborne et al. 2015; Zhang et al. 2015).

Furthermore, these Ag nanoparticles may migrate to the brain, lungs, kidneys, liver, and spleen following detachment from colloidal silver wound dressings (Ahamed et al. 2010). Peripheral multiorgan inflammation caused by Ag nanoparticles was demonstrated by Guo and coworkers (2016). Focusing on interendothelial junctions, the mechanisms of action of Ag nanoparticles and silver nitrate ($AgNO_3$) were compared employing primary human umbilical vein endothelial

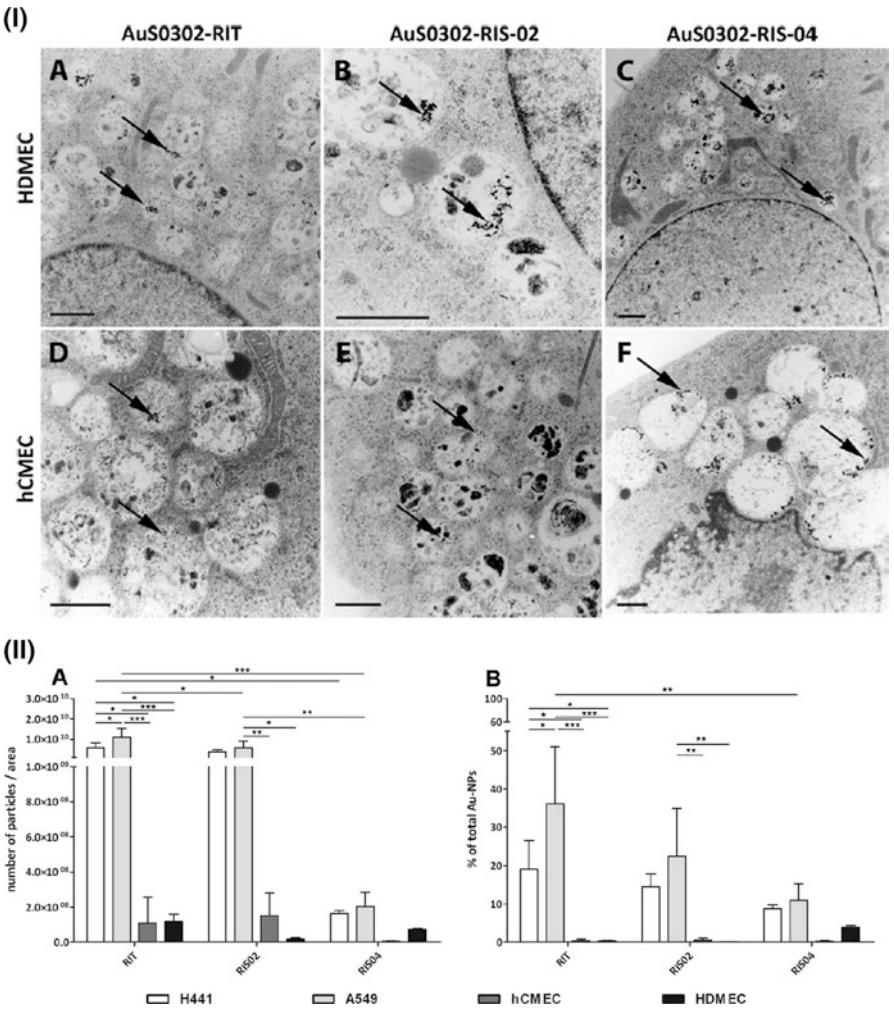


**Fig. 1.3** **(I)** Internalization of gold nanoparticles in HDMEC and hCMEC/D3 analyzed by transmission electron microscopy. HDMEC (**a**–**c**) and hCMEC/D3 (**d**–**f**) were incubated with 300 μM gold nanoparticles for 24 h. After exposure, cells were extensively washed, fixed with paraformaldehyde, and examined by transmission electron microscopy (TEM). AuS0302-RIT, AuS0302-RIS02, and AuS0302-RIS04 were found in intracellular vesicles which were mostly located in the perinuclear region. The arrow heads indicate the gold nanoparticles within the vesicles. Scale bar: 1 μm. **(II)** Quantification of internalized gold nanoparticles in endothelial and epithelial cells by ICP-AES. Both epithelial cells (H441 and A549) and endothelial cells (HDMEC and hCMEC/D3) were incubated with 50 μM gold nanoparticles at 37 °C for 24 h. Cells were extensively washed, lysed by aqua regia (3:1 hydrochloric acid/nitric acid), and analyzed for gold concentration by ICP-AES. In (**a**) the total number of particles per area was calculated, while in (**b**) the percentage uptake of particles into cells, as a function of the total amount applied, was determined. (Reproduced with permission from Freese et al. 2012 © Freese et al.; licensee BioMed Central Ltd. 2012, distributed under a CC-BY 2.0)

cells (HUVEC). It was shown that endothelial endocytosis was primarily due to Ag nanoparticles as opposed to $AgNO_3$. This study determined increased intracellular ROS and VE-cadherin downregulation resulting in the disruption of the integrity of the endothelial layer between the endothelial cells caused by Ag nanoparticles which interestingly could be remedied by N-acetylcysteine (Fig. 1.4). However, $AgNO_3$ (>20 μg/mL) resulted in direct cell death without ROS induction at lower concentrations. Notably, peripheral inflammation was induced in the liver, lungs, and kidneys from Ag nanoparticle release with the severity increasing in relation to the diameter of the Ag nanoparticles used.

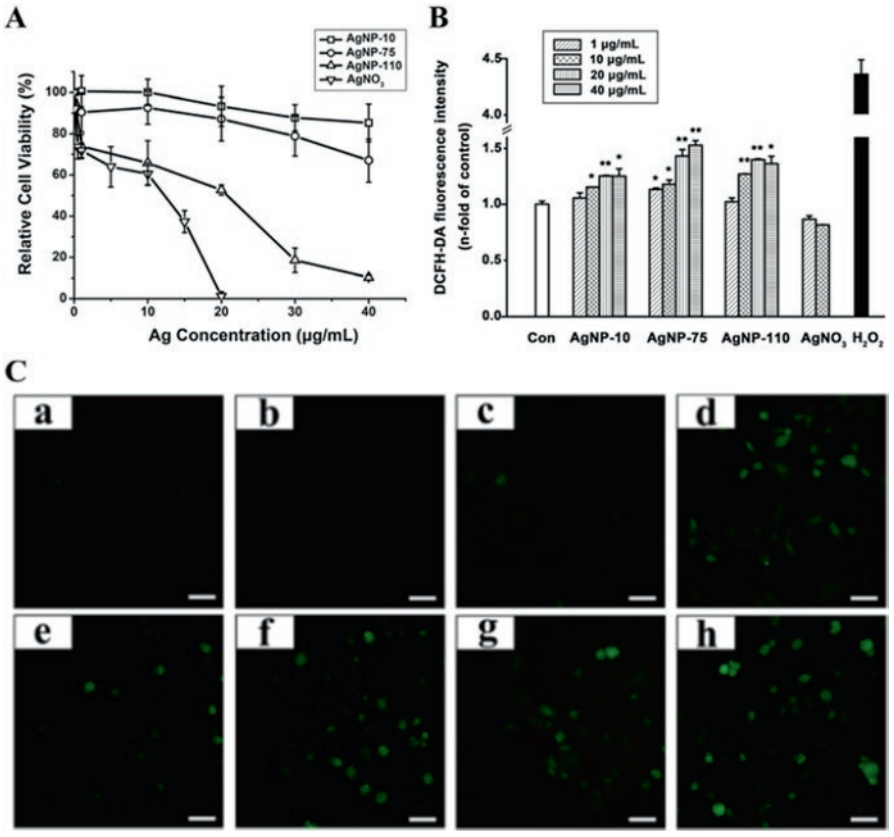


**Fig. 1.4** The viability and intracellular ROS of cells exposed with Ag nanoparticles or $AgNO_3$: (A) Cell viability from CCK-8 assay. (B) The intracellular ROS level caused by Ag nanoparticle or $AgNO_3$ exposure for 1 h. The $H_2O_2$ group was set as the positive control. The * represents significant difference between control group and Ag nanoparticle-75-treated group (*: $p < 0.05$, **: $p < 0.01$). (C) Representative fluorescence images of cells stained by DCFH-DA, in which (a) control group, (b and c) cells incubated with $AgNO_3$ at 1 μg/mL and 10 μg/mL of Ag, (d) cells exposed to 7.5 mg/mL $H_2O_2$, (e–h) cells treated with 1, 10, 20, and 40 μg/mL Ag nanoparticle-75. The scale bar represents 50 μm. Ag nanoparticle toxicity depends on surface chemistry and particle size. (Reproduced from Guo et al. 2016 © Guo et al. 2016, distributed under a CC-BY 4.0 license)

Studies conducted by Wang and coworkers (2014) determined that polyvinylpyrrolidone- and citrate-coated Ag nanoparticles (20 nm) cause more oxidative stress and cellular toxicity than larger particles (110 nm) due to their bioavailability and higher rate of dissolution. The pulmonary impact was assessed in vivo, where the large Ag nanoparticles were shown to cause more significant subchronic lung injury at 21 days due to a slower dissolution rate, whereas the smaller silver particles (20 nm) induced higher acute lung inflammation. This study has demonstrated the size and dissolution effects on biopersistence and lung inflammation.

### Copper Nanoparticles

A vital micronutrient in all tissue, copper (Cu), is mandatory for various cellular functions: cellular pigment formation, neurotransmitter biosynthesis, connective tissue strength, respiration, and peptide amidation (Araya et al. 2003; Desai and Kaler 2008; Ude et al. 2017). The preservation in Cu homeostasis is essential in preventing possible neurological diseases such as Huntington's and Alzheimer's disease (Kaler 1998; Gaggelli et al. 2006). Used in an array of products such as cosmetics, textiles, inks, antimicrobials, and food contact materials, it is pertinent that copper-containing nanomaterials be evaluated for possible toxicity.

Of the metallic nanomaterials, copper oxide nanomaterials (42 nm) were deemed to be the most cytotoxic in comparison to iron complexes ($CuZnFe_2O_4$, $Fe_3O_4$, and $Fe_2O_3$), titanium oxide ($TiO_2$), and zinc oxide (ZnO). They were seen to exhibit the most DNA damage to the A459 human lung epithelial cell line (Karlsson et al. 2008). However, studies on the toxicity of ingested copper oxide (CuO) nanomaterials are few. A recent study by Ude and coworkers (2017) has exploited the cytotoxic impact of CuO nanomaterials on intestinal epithelial cells (Fig. 1.5). Employing undifferentiated Caco-2 intestinal cells, CuO nanomaterials, and $CuSO_4$, the study evaluated the toxicity comparability of both CuO nanomaterials and $CuSO_4$ in vitro, suggesting particle- and ion-mediated mechanism effects due to the less soluble CuO nanomaterial. The CuO nanomaterials displayed concentration-dependent decreases in undifferentiated cell viability, yet no discernable difference was seen between the cytotoxicity of CuO nanomaterials and $CuSO_4$. Additionally, important for risk assessment, CuO nanomaterials were proven to be no more potent than the $CuSO_4$.

An interesting study by Murugan and coworkers (2017) investigated the function of geometrical structure of copper nanoparticles (Fig. 1.6). Nanoparticles were synthesized with a dual functionality comprising the ability to induce cytotoxicity on proliferating cells as well as geometric attributes for enhanced cellular uptake. Extensive cellular internalization studies were conducted using HeLa and NHEK cell models. The primary toxicity factor was attributed to the effect of the nanogeometry of the copper nanoparticles. Cell viability was also observed to be dose dependent. Interestingly, results displayed a significant difference in toxicity between the two cell lines and the geometrical nanoparticles. On the NHEK cell line, cell viability was observed to be 33.33% at the highest Cu nanoparticle

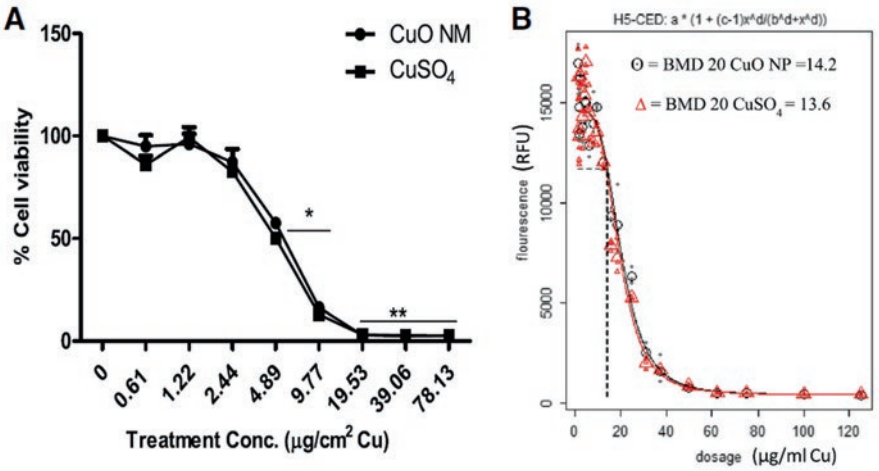


**Fig. 1.5** Cytotoxicity of CuO nanomaterials and $CuSO_4$ to undifferentiated Caco-2 cells. Viability of undifferentiated Caco-2 cells was assessed using the Alamar Blue assay following exposure of cells to cell culture medium (control), CuO nanomaterials, or $CuSO_4$ at concentrations ranging from 0.61 to 78.13 µg/cm$^2$ Cu for 24 hours. (**a**) Viability of Caco-2 cells following CuO nanomaterial or $CuSO_4$ exposure expressed as a % of the control. (**b**) Determination of 20% benchmark dose (BMD 20) in µg/ml following exposure of undifferentiated Caco-2 cells to CuO nanomaterials or $CuSO_4$ exposure. Data was analyzed using Proast 38.9 software to obtain the BMD 20. Data are expressed in mean ± SEM (n = 3), and * represents significance compared to control at $P < 0.05$. (Reproduced from Ude et al. 2017 © Ude et al. 2017, distributed under a CC-BY 4.0 license)

concentration with the median lethal concentration ($LC_{50}$) values occurring at approximately 12.5 µg/ml and 25 µg/ml, respectively, for the NHEK and HeLa cell lines.

## Iron Nanoparticles

There are currently several iron nanoparticles approved by the FDA for therapeutics and imaging purposes (Hassan et al. 2017). Iron nanoparticles may be functionalized for different therapies but have primarily been used for targeted drug delivery, protein separation, magnetic hyperthermia, and MRI (Mahmoudi and Shokrgozar 2012; Schladt et al. 2012). Superparamagnetic iron oxide nanoparticles (SPIONs) display lower toxicity in comparison to other contrast agents (Mirshafiee et al. 2017). AAs an example, in hepatic imaging, following administration, the SPION particles are expected to be phagocytosed by the hepatic Kupffer cells (Wang 2011). Since lower uptake is expected in the diseased hepatic region, a concentrated signal will be generated by the SPIONs to more aptly identify lesions. Following intracellular uptake, SPIONs dissolve into a nonsuperparamagnetic form of iron ions which is further hepatically metabolized and subsequently excreted via kidneys or utilized in red blood cell formation (Weissleder et al. 1989).

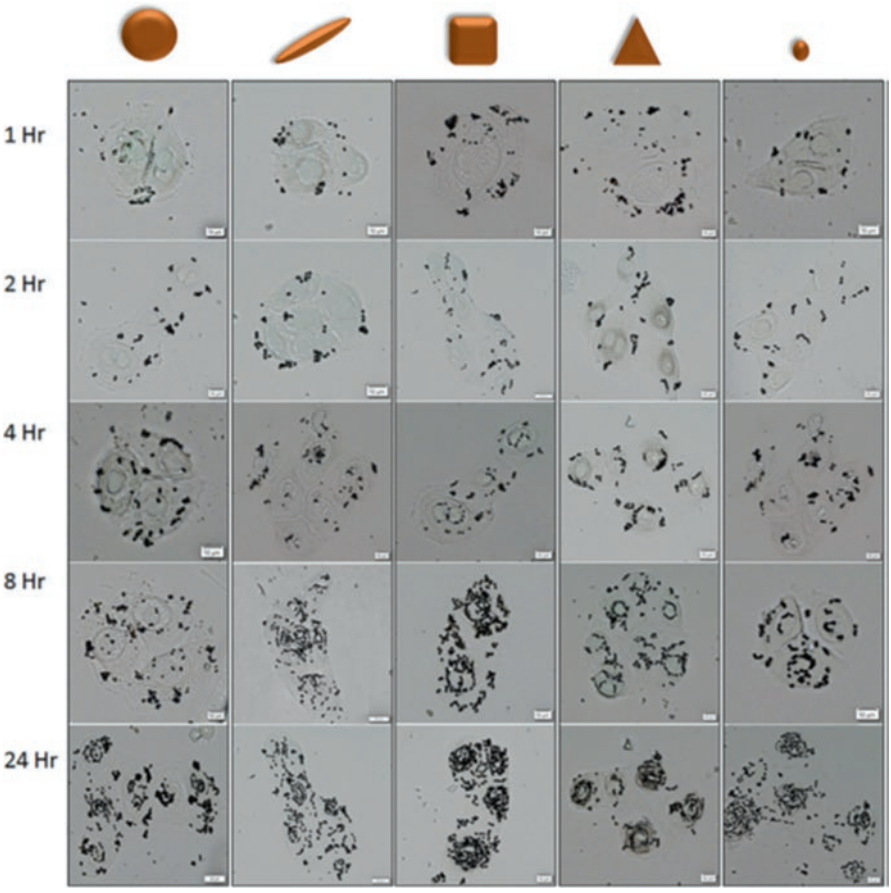


**Fig. 1.6** Phase contrast images of geometric Cu nanoparticle internalization over a 24 hour incubation period. (Reproduced with permission from Murugan et al. 2017, © 2017 Elsevier B.V)

Lunov and coworkers (2010) purport increased ROS production by SPIONs that eventually lead to Kupffer cell apoptosis via the ferrous ions ($Fe^{2+}$) released via the Fenton reaction. This, in turn, reacts with mitochondrial hydrogen peroxide and oxygen ultimately inducing oxidative stress. Moreover, with frequent administration or prolonged treatment, SPION accumulation can result in elevated lipid metabolism, disruption of iron homeostasis, as well as liver dysfunction. Thus, to combat or possibly reduce such adversities associated with SPION use, surface coatings (e.g., dextran and silicon) have been applied to improve biocompatibility but do not address iron accumulation issues in the body (Mirshafiee et al. 2017).

Interestingly, DeLoid and coworkers (2017) reported on the evaluation of nanoparticle biokinetics and toxicity using an iron oxide ($Fe_2O_3$) and corn oil in phosphate buffer emulsion. This nanoenabled food was passed through a GIT simulator. The study determined the influence of food and GIT components on

nanoparticle biokinetics, transport, and toxicological profile. $Fe_2O_3$ was found to be nontoxic with the $Fe_2O_3$ translocation after 4 h being <1% and ~2% for digesta with and without serum, respectively. Results from this study suggest the alteration of nanomaterial biokinetics by serum proteins, raising concerns about the neglect of such food–GIT interactions.

Producing a multifunctional nanomaterial, Zhang and coworkers (2012) exploited the use of $SiO_2$-coated magnetic $Fe_3O_4$ nanoparticles based on the premise of avoiding iron leaching in acidic biological environments. Addressing chemotherapeutic applications, these nanorattles were produced through an ion exchange process and consisted of hydrophilic, rare-earth-doped $NaYF_4$ shells. Displaying appreciable drug-loading capacity and excellent water dispersibility, this system allowed for both upconversion magnetic and luminescent properties and was found to shrink tumors in vivo by simultaneously delivering doxorubicin (DOX) and enhancing tumor targeting (Fig. 1.7).

## Zinc Nanoparticles

Zinc oxide (ZnO) nanoparticles have been utilized in cosmetics as well as in sunscreen lotions due to their UV-blocking ability. These nanoparticles have been purported to induce cytotoxicity through ROS generation, affecting endothelial cell function and causing possible damage to intracellular organelles (Abukabda et al. 2016).

Kura and coworkers (2015) evaluated for acute oral toxicity in Sprague Dawley rats using a zinc–aluminum–LDH–levodopa nanocomposite (ZAL) and zinc–aluminum nanocomposite (ZA). Employing a layered double hydroxide (LDH) nanocarrier system, the results suggested that acute toxicity in the rats was not induced by ZAL and ZA at 2000 mg/kg body weight, suggesting safe, acute, oral administration of zinc–aluminum.

In another study by Kolesnikova and coworkers (2011), nanocomposite microcapsules with zinc oxide nanoparticles in their shells were fabricated using layer-by-layer assembly. Constituent components of the microcapsule shell included both poly(allylamine hydrochloride) solution (PAH) and poly(sodium styrene sulfonate) solution (PSS). Results indicated that the acute toxicity effect in comparison with the constituent components was significantly decreased for the suspension of the microcapsules.

**Fig. 1.7** (continued) H22 xenograft tumor were injected with DOX-loaded MUC-F-NR (1 mg/kg) and subjected (+MF) or not subjected (−MF) to the magnetic field for 1 h. At 24 h postinjection, mice were imaged in vivo. (**c**) The luminescence signal was measured from the whole tumor in vivo and ex vivo. (**d**) Tumor volume changes of saline-treated mice compared to mice treated with MUC-F-NR, DOX, and DOX-loaded MUC-F-NR over 21 days in the absence and presence of magnetic field. Data show mean ± SD (n = 5, *p e 0.05). (Adapted with permission from Zhang et al. 2012 ©, 2011 American Chemical Society)

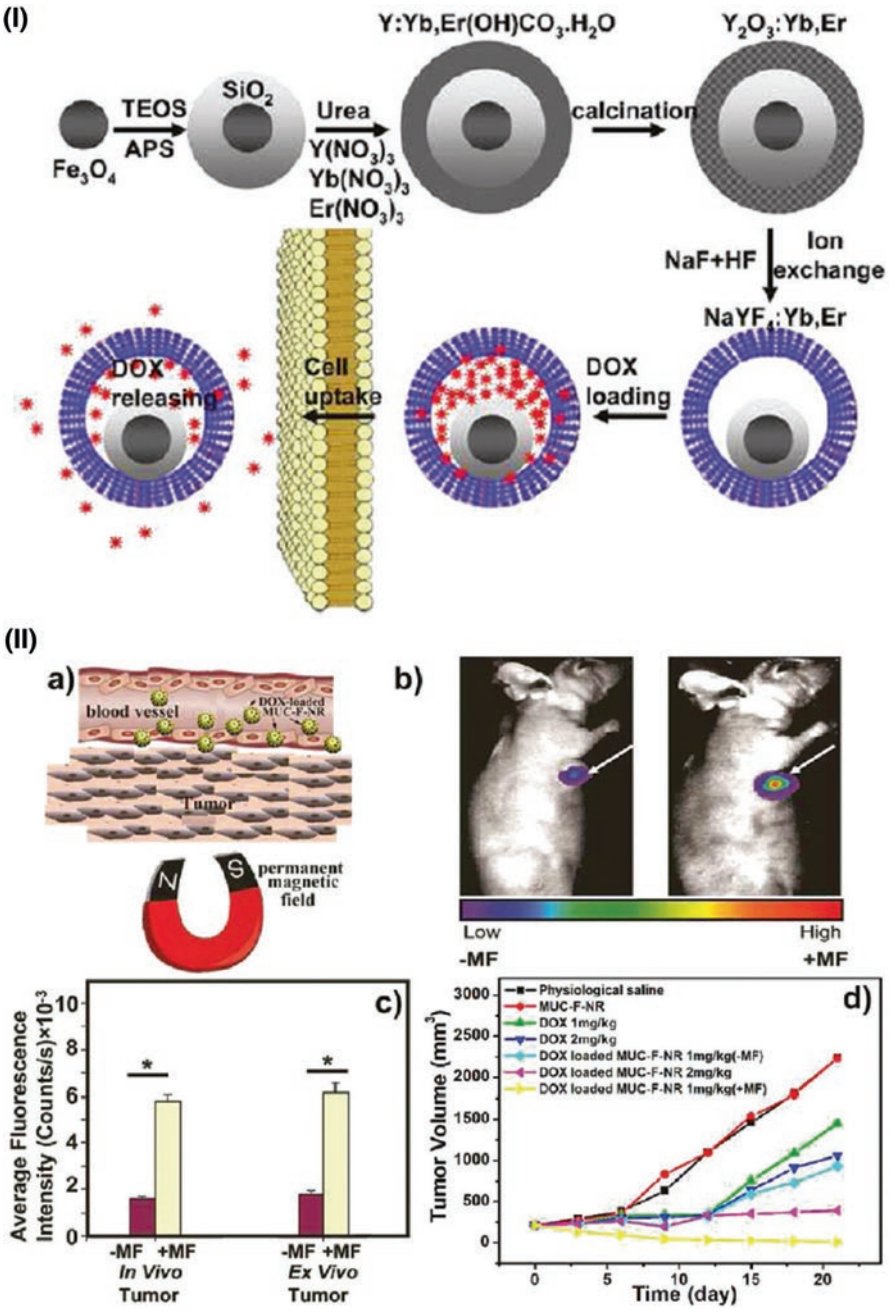


**Fig. 1.7** **(I)** Synthetic procedure for the drug-loaded $Fe_3O_4$@$SiO_2$@α-NaYF/Yb, Er nanorattles (DOX-MUC-F-NR). **(II)** (**a**) Schematic illustration of targeting of DOX-loaded multifunctional drug carrier to tumor cells assisted by an externally applied magnetic field (MF). (**b**) Tumor location as defined by MUC-F-NR intensity increases with 1 h magnetic field treatment. Mice bearing-

(continued)

**Titanium Dioxide**

The literature available on titanium dioxide $TiO_2$ is vast as this metal oxide nanoparticle is widely exploited. $TiO_2$ is a white pigment with a very high refractive index and is thus commonly included in inks, paints, papers, pharmaceuticals, medicines, food products, and toothpaste and along with zinc oxides in sunscreens and cosmetics (Shi et al. 2013). Several investigations have focused on the dermal penetration and toxicity of $TiO_2$. In a study by Schulz and coworkers (2002), particle size, coating, and shape were evaluated for its effect on $TiO_2$ skin penetration (4 mg/cm$^2$). Several $TiO_2$-coated sunscreens including aluminum oxide ($Al_2O_3$), silica ($SiO_2$) (10–15 nm), and trimethyloctylsilane (20 nm) were exposed topically to human skin for 6 h. Results indicated that $TiO_2$ did not penetrate the skin. Similarly, a study conducted by Mavon and coworkers (2007) determined $TiO_2$ (20 nm) distribution both in vitro and in vivo. Five hours post direct topical application (2 mg/cm$^2$), tape stripping was used to determine the dermal penetration of $TiO_2$. It was found that there was minimal $TiO_2$ distribution within the epidermis.

Concern over the potential cytotoxicity of $TiO_2$ nanoparticles stems mainly from that of the pulmonary adverse effects of $TiO_2$ with many dermal $TiO_2$ distribution studies concluding that $TiO_2$ nanoparticles are not systemically available to a significant extent after dermal exposure. Originally emanating from studies by Ferin and coworkers (1990, 1992) and Oberdorster and coworkers (1990), ultrafine $TiO_2$ was demonstrated to enhance pulmonary inflammation and particle retention and translocation. These studies have led to the reassessment of $TiO_2$ as a negative control in pulmonary toxicology studies when assessing the toxicity of pathogenic particulates such as alpha-quartz (Johnston et al. 2009). The limit for fine particles in the air is 50 μg/m$^3$ for an average human of 70 kg (Simko and Mattsson 2010). Acute toxicity information for $TiO_2$ nanoparticles in humans, however, is currently lacking (Shi et al. 2013).

### *1.3.2 Nonmetallic Nanomaterials*

**Silica-Derived Nanoparticles**

Silica (Si) nanoparticles offer exemplary characteristics such as rapid in vivo degradation, chemical conjugation-mediated camouflage (Parodi et al. 2013), metal incorporation for theragnostic applications (Lee et al. 2011), and regulation of pore sizes (2–10 nm) for drug encapsulation (Gao et al. 2011). Cutaneous absorption of this metalloid nanomaterial through the skin often simultaneously occurs with exposure to other environmental allergens as well as other chemical compounds, yet these potential associated hazards have not been thoroughly investigated (Li et al. 2008).

A study conducted by Hirai and coworkers (2015) investigated the concurrent topical application of amorphous silica nanoparticles and mite extract on human

atopic dermatitis and allergic sensitization in NC/Nga mice. Low-level production of allergen-specific IgGs was observed after concurrent cutaneous exposure of the nanoparticles and allergens. Additionally, following exposure to the allergen–silica nanoparticle agglomerates, low-level IgG production was induced in the mice, but this was not observed when exposed to well-dispersed nanoparticles or nanoparticles applied separately from the allergen. This research conducted suggests that the allergen-specific immune response is not directly affected by silica nanoparticles. However, it should be noted that the Si nanoparticles led to a key risk factor of atopic allergies in humans as well as a low IgG/IgE ratio, when present in allergen-adsorbed agglomerates.

Mesoporous silica nanoparticles, the advancement of silica (Si) nanoparticles, have been utilized to overcome the issues associated with biocompatibility, degradability, and drug release rates related to metallic or other inorganic nanomaterials (Hassan et al. 2017). Mesoporous silica nanoparticles have since been functionalized to regulate biodistribution and reduce systemic toxicity. "Cloaking" of the mesoporous silica nanoparticles, i.e., coating with leucocyte membranes, has been found to reduce cytotoxicity while enhancing the delivery of doxorubicin in vivo (Parodi et al. 2013). Kim and coworkers (2016) investigated functionalized poly(ethylene glycol)-coated (PEGylated) near-infrared (NIR) fluorescent silica nanoparticles that were functionalized with melanoma-targeting peptides. This hybrid organosilica particle demonstrated the ability to induce cell death and ferroptosis as well as the inhibition of tumor growth and tumor regression with high-dose particle delivery.

### Nanoclay-Derived Delivery

The implementation of nanoclays in industrial and commercial commodities has increased exponentially over the years. In the pharmaceutical industry, nanoclay–polymer-based composites have allowed for improved mechanical strength and reinforcement properties. Due to their fine and nanoparticulate nature, nanoclays have been investigated for toxic effects on lung health (Wagner et al. 2017). Studies have shown that nanoclays, on a cellular level, display mitochondrial damage, ROS generation, and membrane and cellular damage effects (Wagner et al. 2017). Most clays have been deemed as non-toxic and have thus been extensivley studied for their biomedical applications such as drug delivery, preparation of scaffolds and tissue engineering. Studies by Wang and Tong (2008) and Michael and coworkers (2016) have investigated the effects of nanoclays, for bone cement applications. Results of both studies have determined increased bioactivity and mechanical properties. Yang and coworkers (2017) developed semi-IPN sericin/poly(NIPAm/LMSH) (HSP) nanocomposite hydrogels for wound healing applications. The nanocomposites were shown to result in almost complete recovery by day 13 of the study.

## 1.4 Safety of Nanotheragnostic Agents

Theragnostics synergistically employs the use of both diagnostics and therapeutics culminating into more safe and efficacious personalized disease management. Commonly used in ultrasound, single photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetic resonance imaging (MRI), theragnostic nanoparticles can be categorized into therapeutic payload, payload carrier, signal emitter, and targeting ligand (Fang and Zhang 2010). Theragnostic nanoparticles possess qualities that allow for sufficient, targeted drug delivery; specific, rapid, and selective targeting; reporting of biochemical and morphological disease characteristics; and rapid, efficient clearance without the formation of toxic by-products (Jokerst and Gambhir 2011; Chen et al. 2014). Though studies on theragnostic nanomaterial toxicity are limited, many studies have employed theragnostic approaches with functionalized engineered magnetic nanoparticles for MRI-guided therapeutic cell replacement and MRI-assisted diagnosis and surgeries (Shubayev et al. 2009).

Notably, the superior superparamagnetic theragnostic qualities of iron oxide engineered magnetic nanoparticles have been safely and effectively used in MRI with many dextran-coated nanoformulations being approved for clinical use as MRI contrast agents, i.e., ferumoxtran, ferucarbotran, and ferumoxides (Shubayev et al. 2009). Even though iron deposits have been associated with many neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis, Parkinson's disease, and Huntington's disease, iron oxide engineered magnetic nanoparticles were shown in studies to be the safest of the metal oxide nanoparticles only producing cytotoxic effect at 100 μg/ml or higher (Hussain et al. 2005; Jeng and Swanson 2006; Gojova et al. 2007). Dextran-coated magnetite nanoparticles were found to exert cytotoxic effects at 400 mg/kg in rats (Lacava et al. 1999; Lacava et al. 2004).

## 1.5 Nanomaterial Hazard Assessment

Relative to the toxicity potential of nanomaterials, risk assessments in this subject matter have been exponential (OECD 2005, 2007, 2010a, b, 2012a, b, c, 2014, 2015, 2016a, b, c, d, e, f). Depending on particular nanomaterial characteristics such as various biological indicators, structure activity, physicochemical, in vitro test results, or in vivo test results, predictive toxicological modeling is achieved (Schulte et al. 2018). In vivo exposure systems have been extensively utilized to address these concerns and have been instrumental in addressing the potential safety concerns regarding nanomaterials. Since the ethical support of the replacement of animals with more human-relevant alternatives, the principle of the 3Rs – Replacement, Reduction, and Refinement – has become an increasing mandate (Tornqvist et al. 2014; Drasler et al. 2017). A stepwise approach to categorize the need for validated in vivo studies based on positive effects of in vitro cell experiments involving

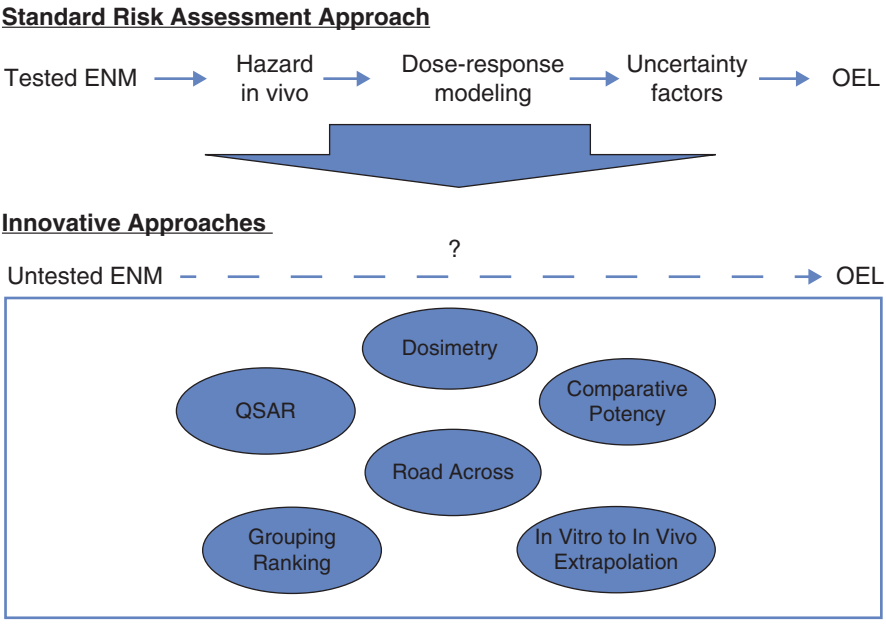


**Fig. 1.8** Frontier of risk assessment for developing occupational exposure limits for engineered nanomaterials. Abbreviations: *ENM* engineered nanomaterial, *OEL* occupational exposure limit, *QSAR* quantitative structure–activity relationship. (Reproduced with permission from Schulte et al. 2018, © Elsevier Inc.)

nanomaterials exists and is highlighted in Fig. 1.8. However, hurdles such as in vivo behavior of the nanomaterials and the ability to create additional functionality through sophisticated fabrication methods are warranted (Cheng et al. 2012). Regulatory bodies, consumer, and society expectations about their safety are increasing, and as with all developing technologies, it is pertinent to identify possible hazards and develop risk assessment and management approaches (Drasler et al. 2017; Meldrum et al. 2017). Hansen (2010) and Breggin and coworkers (2009) sufficiently provide for an overview of national and international initiatives to regulate nanomaterials.

## 1.6 Conclusion

Nanomaterials have been extensively utilized for their enhanced biomedical properties. Information and data on the safety of these materials in the physiological environment is however essential prior to the use in the treatment or prevention of any physiological conditions. This review has provided an extensive account on the studies that have been undertaken on gold, silver, copper, iron, zinc, and titanium as well as on silica and nanoclay nanomaterials with focus given on their therapeutic

safety profiles as well as the cytotoxic effects due to varying particle size and concentration. All evaluated nanomaterials have been noted to be inherently cytotoxic; however, research into these respective materials has aimed to increase their biocompatibility through modification of particle size, shape, and charge as well as through the use of metal derivatives with increased safety profiles. The alternatives to cytotoxicity and in vivo studies with the aim to minimize the use of animal models in the therapeutic safety studies have also been provided to highlight the advancements in therapeutic safety analyses. Therapeutic safety analysis of nanomaterials is therefore a vital tool to ensure the effective use of potentially toxic materials in the treatment and prevention of physiological conditions.

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