Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Toxicology

Engineered nanomaterials and oxidative stress: Current understanding and future challenges

Ryan P. Mendoza and Jared M. Brown

Abstract

Engineered nanomaterials (ENMs) are being incorporated at an unprecedented rate into consumer and biomedical products. This increased usage will ultimately lead to increased human exposure; therefore, understanding ENM safety is an important concern to the public. Although ENMs may exert toxicity through multiple mechanisms, one common mechanism of toxicity recognized across a range of ENMs with varying physicochemical properties is oxidative stress. Further, it is recognized that several key physicochemical properties of ENMs including size, material composition, surface chemistry, band gap, and level of ionic dissolution for example contribute to ENM driven oxidative stress. While it has been shown that exposure of cells to ENMs at high acute doses produce reactive oxygen species at a toxic level often leading to cytotoxicity, there is little research looking at oxidative stress caused by ENM exposure at more relevant low or non-toxic doses. Although the former can lead to apoptosis, genotoxicity, and inflammation, the latter can potentially be damaging as chronic changes to the intracellular redox state leads to cellular reprogramming, resulting in disease initiation and progression among other systemic damage. This current opinions article will review the physicochemical properties and mechanisms associated with ENM-driven oxidative stress and will discuss the need for research investigating effects on the redox proteome that may lead to cellular dysfunction at low or chronic doses of ENMs.

Addresses

Colorado Center for Nanomedicine and Nanosafety, Department of Pharmaceutical Sciences, Skaggs School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Corresponding author: Brown, Jared M. (jared.brown@ucdenver.edu)

Current Opinion in Toxicology 2019, **13**:74–80

This review comes from a themed issue on **Oxidative Toxicology: From molecules, to cells, to tissues**

Edited by **Dimitrios Kouretas, James R. Roede** and **Aristidis M. Tsatsakis**

Available online 7 September 2018

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cotox.2018.09.001

Keywords

Nanoparticle, Nanotoxicity, Nanotoxicology, Oxidative stress, Redox proteome.

1. Introduction

Engineered nanomaterials (ENMs) have a diverse range of applications due to their unique properties at the nanoscale. They have shown potential benefits for medical advancements in drug delivery, imaging, vaccinations, and sterile wound treatment among others [1]. With the incorporation of ENMs into biomedical and consumer products rapidly expanding, their health and safety remains of utmost concern to sustainable use of these products. A major challenge in understanding ENM toxicity lies in their diversity. ENMs are defined as any man-made material with at least one dimension less than 100 nm; however, this classification includes a large range of varying physicochemical properties such as material composition, hydrodynamic size, shape, and surface chemistry/charge as examples. Due to the wide diversity of these physicochemical properties, ENMs can have beneficial applications but present a major challenge to understanding their safety.

A common mechanism of toxicity recognized across ENM types is their ability to impact the oxidative state of the cell. It has been clearly recognized that many ENMs promote direct damage through reactive oxygen species (ROS) production and an increase in cellular oxidative stress often leading to cytotoxicity [2–4]. It is also important to recognize that ENMs, particularly at low or non-cytotoxic doses, may lead to alterations in the intracellular redox state, which could impact redox signaling and control that is essential to cell function. These cellular alterations may not lead to overt toxicity, but may shift cell function and signaling as well as impact disease initiation and progression. This redox signaling homeostasis within the human body occurs via oxidation and reduction of protein thiols and has been termed the *redox proteome* [5]. The redox proteome consists of all proteins in the cell regulated by the redox state. Thiol groups, particularly those present on cysteine amino acids, tend to be specifically sensitive to redox influence. These cysteine thiols, also known as sulfur switches, are heavily incorporated into redox sensing and signaling because they are stable over a wide range of redox potentials and can be ionized to different oxidative states over the normal physiological pH range, including a negative thiolate form ($-RS^-$), fully reduced form (-SH), and higher oxidized forms ($-RSO_2^-$). Regulatory redox

sensing proteins, such as thioredoxin (TRX) and peroxiredoxin (PRX), can manipulate cysteine thiols by reduction/oxidation or physical binding. Furthermore, there are a number of cysteine thiol covalent modifications, such as S-glutathionylation or S-cysteinylation, which can modify redox sensitive proteins. Any of these changes to a protein's thiols can impact activity levels, modify binding ability, or completely change function of the protein [6]. Redox-mediated modulation of these proteins can affect downstream pathways including many important cellular homeostatic pathways that, when disrupted, can lead to cardiovascular diseases, cancer, and neurodegeneration as examples [7]. Certain ENMs have shown the ability to manipulate thiol sulfur switches but only very preliminary research has been done in these regards. Furthermore, potential toxicity from ENM manipulation of the intracellular redox state and any resulting damage represents a challenge in fully understanding ENM safety particularly at low dose and/or chronic exposure. In this current opinions review, we will discuss 1) the impact of ENM physicochemical properties on redox balance; and 2) mechanisms by which ENMs may impact the cellular redox balance. Importantly, we will identify areas where more research is needed in understanding the impact of ENM exposure, particularly at low and/or chronic dose levels, on the redox proteome, oxidative stress and critical cellular functions.

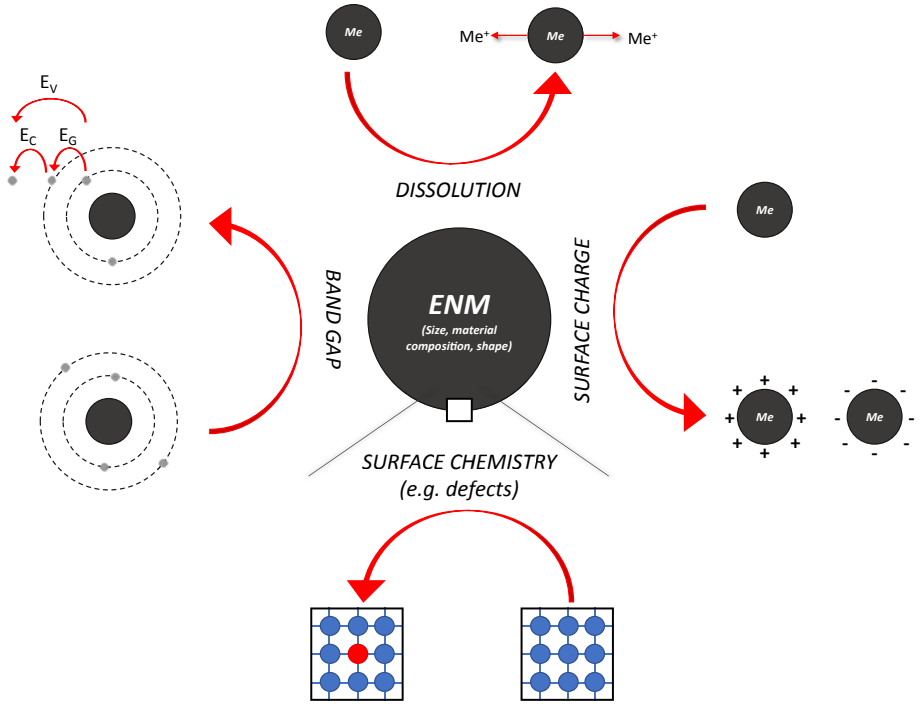
2. Contribution of ENM physicochemical properties to redox dyshomeostasis

ENMs have the potential to manipulate the intracellular oxidation state through several different mechanisms which are dependent upon their physicochemical properties. ENMs can be reactive based on such physicochemical properties as band gap, surface defects, surface charge, and level of ionic dissolution (Fig. 1). This ability of ENMs to shift the redox proteome and reprogram cells to promote disease initiation and progression is a central toxicological concern for the development and implementation of ENMs into biomedical and consumer products.

2.1. Band gap

The band gap, also known as an energy gap (E_G), represents the energy needed to advance a valence band electron to the conduction band within any material's electron orbital. From this, both valence energy (E_V) and conduction energy (E_C) can be calculated, which are the energies needed to exchange electrons between the respective bands and the surrounding environment. E_C is an important property because electron transfer from the conduction band to the surrounding environment requires significantly less energy than from the valence band and is therefore the most likely electron transfer to occur. Zhang et al. explored the E_C of 24 metal-based ENM and its relation to the intracellular redox

Figure 1



An overview of several key physicochemical properties that affect ENM ability to exchange electrons with the surrounding environment and be reactive within the oxidative realm. Figure includes visual representations of ionic dissolution, surface change, surface chemistry (chemical defect), and band gap. Size, material composition, and shape are also physicochemical properties that affect ENM reactivity (Me = metal, E_V = valence energy, E_C = conduction energy, E_G = gap energy or band gap).

potential, which is normally maintained at a range of −4.14eV to −4.84eV. The authors hypothesized that if the E_C overlaps with cell potential, it will freely exchange electrons and be reactive in the oxidative realm. Out of the 24 ENM tested, they identified six with overlapping potentials (Co_3O_4, Cr_2O_4, Ni_2O_3, Mn_2O_3, CoO, and TiO_2). Out of those six ENMs, five were the most toxic of all 24 tested both *in vitro* demonstrated by mitochondrial damage and ROS production and *in vivo* shown through increased inflammation and oxidative stress markers [8]. This demonstrated a correlation between band gap and potential oxidative toxicity. In addition to this, several ENMs outside the band gap range have properties that cause intracellular oxidative misbalance through different mechanisms.

2.2. Metal-based reactive elements

Cerium dioxide (CeO_2) exists in two valent states, Ce^{3+} and a Ce^{4+}, with a conduction energy that falls out of the intracellular redox potential range (−3.80 eV) [8]. Despite this, CeO_2 at the Ce^{4+} state has the ability to interact with oxidative species such as nitric oxide (NO) significantly more than CeO_2 at the Ce^{3+} state, leading to cardiovascular dysfunction and changing redox kinetics within the cell [9]. CeO_2 could be one of many ENMs with the ability to do this but is one of very few whose toxicity is extensively researched. Similarly, Zhang et al. above identified two ENMs that fell out of the overlapping E_C range (CuO and ZnO) but still caused serious oxidative damage through ionic dissolution [8].

In addition to the elemental state, certain metal-based ENMs have the potential to release metal ions that are reactive with the redox environment around them [10,11]. The likelihood that these ENMs shed ions and the ion's potential toxicity is based on different physicochemical properties. For example, it has been shown that silver nanoparticles (AgNPs) are prone to release reactive ions compared to other metals and, importantly, AgNPs are more likely to undergo dissolution as the surface area is decreased [12]. Dissolution can be a major source of oxidative stress within the cell as well as extracellularly. There is a large gap in the current literature as to whether observed nanotoxicity is caused by the ENM itself or the metal ions it releases. In this regard, new tools are being developed, such as single-particle inductively coupled plasma mass spectrometry (ICP-MS), that may further define the contribution of ions vs. ENM to oxidative stress and should improve our understanding of these mechanisms.

2.3. Surface chemistry

Another physicochemical property that contributes to ENM toxicity and oxidative stress is variation in surface chemistry such as chemical defects in the surface of the ENM. Surface defects are a common occurrence during mass production of ENMs that includes both physical defects, where the ENM lattice structure is compromised, or chemical defects, where an unintended chemical dopant is introduced into the lattice for example. These defects will affect the ability of ENM to exchange electrons impacting its reactivity with the redox proteome. One example is titanium dioxide nanoparticles (TiO_2) which are not normally oxidatively reactive but can absorb photons to create an oxidizing energy gap in the electron orbital. As mentioned before, certain energy gaps can cause ENMs to be more reactive. TiO_2 nanoparticles that are manipulated into this band gap will specifically produce hydroxyl radicals and malondialdehyde (MDA), a reactive byproduct of lipid peroxidation. The nanoparticle's level of photon absorbance and resultant oxidative stress can change depending on the specific chemical surface defects introduced during production, such as implementation of silica and aluminum dopants [13]. Since introduction of these defects can significantly affect ENM reactivity in the oxidative realm, they must be explored and defined by type and prevalence during production along with all physicochemical properties that can potentially lead to redox state manipulation.

3. Mechanisms of cellular ENM-mediated redox state modulation

As discussed above, ENMs possess unique physicochemical properties to promote oxidative stress. Importantly, many ENMs are readily internalized by cells through various mechanisms that can further promote intracellular oxidative stress. Once localized within the cell, ENMs can lead to dysregulation of several normal cellular functions including the intracellular redox proteome. There is sufficient evidence showing that ENMs can be taken up into a variety of cell types, including macrophages, endothelial cells, and lung alveolar cells as examples [14–16]. The uptake of ENMs is driven by physicochemical properties including size, shape, charge, and surface coating. In addition to the physicochemical identity of ENMs, nanoparticles entering a biological milleau often attract an assortment of proteins to their surface, termed a 'protein corona' [17]. The presence and composition of the protein corona can change the rate of ENM cellular uptake highlighting the importance of understanding these critical physicochemical properties including protein corona formation, which constitutes the 'biological identity' of an ENM [18].

There are multiple mechanisms through which cells can uptake ENMs including receptor mediated endocytosis, passive diffusion, and active phagocytosis [19–21]. Cells also have the ability to uptake metal ions shed from ENMs through different mechanisms including the divalent metal transporter (DMT1) [22]. Additionally, when ENMs are taken up into cells they are even more likely the undergo dissolution because of the change to more acidic intracellular pH levels, particularly in lysosomal compartments [23]. Dissolution of metal-based ENMs is

potentially occurring at both an extracellular and intracellular level, contributing to oxidative damage. ENMs can cause oxidative stress through several other intracellular mechanisms, including modulating those that are innate and essential to cellular function.

3.1. ENM driven NADPH oxidase and mitochondrial damage

Once ENMs enter the cell, they can manipulate cellular pathways to cause overproduction of ROS. One example by which this occurs is NADPH oxidase, which is highly expressed in phagocytizing immune cells such as macrophages and neutrophils. This is of utmost relevance because these cells readily uptake ENMs. This enzyme is part of these cells' normal defense system and will produce ROS to kill any pathogens it phagocytizes. Once the pathogen is cleared, ROS production stops. It has been observed that ENMs can constitutively turn on this system as the cell recognizes the nanoparticle as foreign but is unable to kill or clear the ENM through ROS production. Again, ENM physicochemical properties can dictate the level of NADPH oxidase activation. For example, Sun et al. compared spherical nanoparticles (carbon and silver) to large aspect ratio particles (multiwalled carbon nanotubes [MWCNT] and silver nanowires) to analyze the effect of ENM shape and material composition in activating NADPH oxidase. It was found that large aspect nanoparticles (e.g. MWCNT) caused more NADPH oxidase based oxidative damage than spherical particles. Furthermore, they linked NADPH oxidase-dependent inflammasome recruitment to MWCNT-mediated lung fibrosis [24]. This demonstrates a direct connection between ENM-mediated oxidative stress and disease.

Another source of intracellular redox manipulation is ENM-mediated mitochondrial damage. Whereas the mitochondria normally leak low levels of ROS that help maintain intracellular homeostatic redox kinetics, ENMs can damage or change mitochondrial function to shift this redox state. Gold nanoparticles (AuNP) were shown to cause mitochondrial damage including caspase activation and decreased respiration *in vitro*. This damage was reversed with introduction of antioxidants showing that ROS was central to the toxicity [25]. It was not addressed in this study whether this oxidative stress was through direct interaction with the mitochondria or indirect damage, but there is evidence that ENM can enter the mitochondrial compartment [26]. Further, physicochemical properties, like surface charge, also affect ENM interaction with the mitochondria. Hussain et al. explored how modulating the surface charge of AuNP can change oxidative reactivity of the ENM. They showed that ROS production was similar between charged and neutral AuNP, but that charged ENMs induced further mitochondrial stress compared to neutral particles through loss of mitochondrial membrane potential and efflux of Ca^{2+} ions into the cytosol [27]. ENM-mediated manipulation of intracellular redox state through modulation of inherent cellular pathways can be both a source of acute direct ROS damage and of chronic redox state changes leading to cellular reprogramming at a systemic level.

3.2. Impact of ENM exposure on redox regulated cell signaling

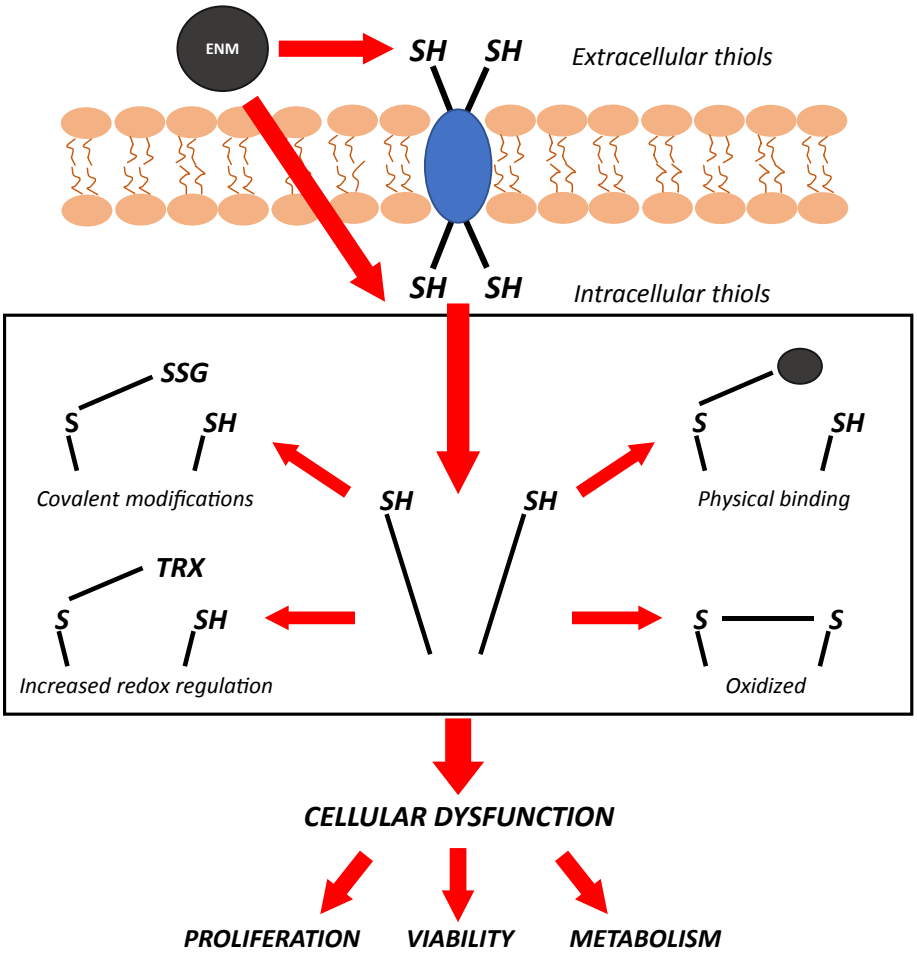
ENM-mediated changes to redox-sensitive cysteine thiol sulfur switches can have detrimental effects to the cell (Fig. 2). Currently, few studies have examined the ability of ENMs to modulate redox signaling effects within the cell despite the importance of these thiol switches in regulation of many cellular functions. Duan et al. has shown an increase in S-glutathionylation to cysteine thiols caused by ENMs, and correlated levels of this specific thiol manipulation with more oxidatively damaging particles ($CoO > Fe_3O_4 > SiO_2$). Some functional pathways they found to be significantly affected by these ENM-driven thiol modifications include PI3K/AKT, Nrf2-Keap1, ERK/MAPK, and mTOR signaling which are all associated with cellular metabolism and homeostasis [28]. Chronic reprogramming of any of these critical pathways can lead to serious cellular viability and functional consequences. ENMs of varying physicochemical properties have also been demonstrated to induce increased levels of metallothionein, which are cysteine-rich proteins known to bind to and sequester metals [29,30]. This provides another mechanism through which ENM can manipulate thiols, potentially leading to changes in redox signaling and control. So far, only few specific modifications of sulfur switches by ENMs have been recognized; however, there are many more modifications that can affect thiols and the ability of ENMs to modulate the changes should be further investigated.

It has been estimated that over 80% of Cys residues in the redox proteome are correlated to functional pathways [7]. These functional changes are concerning because they can accumulate over time and lead to disease and systemic damage. For example, there are redox sensitive switches that control p53, NF-kB, and PTEN pathways which are correlated with proliferation/cancer and inflammation [7]. Any manipulation of these pathways, such as with chronic, low-dose exposure to ENMs, needs to be examined. Additionally, while ROS production can lead to shifts in these pathways, it is the small redox changes over time that can potentially reprogram cells. Furthermore, modification of the redox proteome will likely vary across cell and animal models due to genetic differences that have not been explored as well.

3.3. Possible contribution of extracellular thiol groups to ENM toxicity

The manipulation of the oxidative state extracellularly by ENMs can also lead to a variety of negative effects within the cell. This occurs through many mechanisms including

Figure 2



Possible modifications of redox-sensitive thiol groups by ENMs. ENMs can potentially affect both extracellular and intracellular thiols through several different mechanisms including covalent modification, physical binding, increased redox regulation, and oxidation. These manipulations can be chronic and lead to cell reprogramming and dysfunction including pathways involved in proliferation, viability, and metabolism. This reprogramming of the cell can lead to disease initiation or progression among other systemic damage.

lipid peroxidation, which produces reactive species like malondialdehyde (MDA) and 4-hydroxynonenal (HNE) that can cause direct damage to the cell [8,31]. Redox-sensitive proteins that are modified in the extracellular space can be taken up into cells, potentially leading to endoplasmic reticulum (ER) stress from the unfolded protein response or can further shift intracellular redox state [32,33]. However, these are more likely targets of oxidative damage from reactive oxygen species formed from acute, high doses of ENMs. More relevant chronic, low doses are likely to induce modulation of extracellular thiol residues without causing direct damage.

There are extracellular sulfur switches present on the apical side of the membrane that can signal intracellularly, including epidermal growth factor receptor (EGFR) which is strongly related to cell homeostasis and has upregulated activity in cancer cells [34]. These thiols can regulate important pathways in the ER, cytoplasm, nucleus and mitochondria such as the unfolded protein response, Nrf2-Keap1, and ASK-1 [7]. Extracellular thiols have direct exposure to the surrounding environment which can explain why they control such important intracellular function. Changes to these thiols can be chronic and lead to cellular reprogramming and disease [35]. ENMs have the potential to affect these thiols through direct interaction, but also by changing the extracellular oxidation state. An oxidizing extracellular state leads to a more oxidized state of extracellular thiols thereby resulting in activation of NF-kB as well as increased mitochondrial ROS production [36,37]. Interestingly, all of these events could occur without uptake of the ENM into the cell, which has yet to be explored. There is a large gap in the literature looking at how ENMs can cause damage through association with the membrane and even less

looking at their possible activation of extracellular thiols. It will be important to understand if extracellular thiols are affected by ENMs, what intracellular pathways they modulate, and what phenotypic changes may eventually occur in various cell types.

4. Concluding remarks

ENMs have enormous potential in many facets of society; despite this, it is important to understand safety of these materials and one common toxicological outcome across many nanoparticle types is the induction of oxidative stress. It has been demonstrated that ENM driven oxidative stress can lead to inflammation, cell apoptosis, and genotoxicity [38–40] but many of these studies have utilized acute high-dose exposures that may not accurately represent exposure levels through consumer products or nanotherapeutics. In contrast, chronic, low-dose exposures may lead to more subtle changes to the redox proteome to promote inappropriate cell signaling resulting in pathophysiological outcomes. These redox proteome changes are currently overlooked in the nanotoxicology field and should be examined to ensure safe implementation of these promising materials. For example, future nanotoxicology studies need to focus on how chronic changes to the redox proteome can be caused by low or non-toxic doses of ENMs (e.g. thiol modifications), and implications of such changes on disease initiation or progression. These studies will be important to further understand how key ENM physicochemical properties such as band gap, surface defects, and propensity for dissolution impact the redox proteome.

Acknowledgements

This work was supported by the National Institute of Environmental Health Sciences grant R01 ES019311.

Conflict of interest

The authors declare no conflict of interest.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Bogart LK, Pourroy G, Murphy CJ, Puntes V, Pellegrino T, Rosenblum D, Peer D, Levy R: **Nanoparticles for imaging, sensing, and therapeutic intervention**. *ACS Nano* 2014, **8**: 3107–3122.

2. Castiglioni S, Cazzaniga A, Perrotta C, Maier JA: **Silver nanoparticles-induced cytotoxicity requires ERK activation in human bladder carcinoma cells**. *Toxicol Lett* 2015, **237**: 237–243.

3. Schrand AM, Lin JB, Hussain SM: **Assessment of cytotoxicity of carbon nanoparticles using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetr azolium (MTS) cell viability assay**. *Methods Mol Biol* 2012, **906**:395–402.

4. Li S, Yang S, Chen G, Li X, Chen J, Ma Y, Ma Y: **Mechanism of cellular uptake, localization and cytotoxicity of organic nanoparticles**. *J Nanosci Nanotechnol* 2014, **14**:3292–3298.

5. ** Go YM, Jones DP: **The redox proteome**. *J Biol Chem* 2013, **288**: 26512–26520.

6. Jones DP: **Radical-free biology of oxidative stress**. *Am J Physiol Cell Physiol* 2008, **295**:C849–C868.

7. * Go YM, Chandler JD, Jones DP: **The cysteine proteome**. *Free Radic Biol Med* 2015, **84**:227–245.

8. ** Wang Y, Zi XY, Su J, Zhang HX, Zhang XR, Zhu HY, Li JX, Yin M, Yang F, Hu YP: **Cuprous oxide nanoparticles selectively induce apoptosis of tumor cells**. *Int J Nanomed* 2012, **7**: 2641–2652.

9. Minarchick VC, Stapleton PA, Fix NR, Leonard SS, Sabolsky EM, Nurkiewicz TR: **Intravenous and gastric cerium dioxide nanoparticle exposure disrupts microvascular smooth muscle signaling**. *Toxicol Sci* 2015, **144**:77–89.

10. Pratsinis A, Hervella P, Leroux JC, Pratsinis SE, Sotiriou GA: **Toxicity of silver nanoparticles in macrophages**. *Small* 2013, **9**:2576–2584.

11. Bernstein D, Castranova V, Donaldson K, Fubini B, Hadley J, Hesterberg T, Kane A, Lai D, McConnell EE, Muhle H, *et al.*: **Testing of fibrous particles: short-term assays and strategies**. *Inhal Toxicol* 2005, **17**:497–537.

12. Hamilton RF, Buckingham S, Holian A: **The effect of size on Ag nanosphere toxicity in macrophage cell models and lung epithelial cell lines is dependent on particle dissolution**. *Int J Mol Sci* 2014, **15**:6815–6830.

13. Tang Y, Cai R, Cao D, Kong X, Lu Y: **Photocatalytic production of hydroxyl radicals by commercial TiO2 nanoparticles and phototoxic hazard identification**. *Toxicology* 2018, **406–407**:1–8.

14. Fowler R, Vllasaliu D, Trillo FF, Garnett M, Alexander C, Horsley H, Smith B, Whitcombe I, Eaton M, Stolnik S: **Nanoparticle transport in epithelial cells: pathway switching through bioconjugation**. *Small* 2013, **9**:3282–3294.

15. Ruenraroengsak P, Chen S, Hu S, Melbourne J, Sweeney S, Thorley AJ, Skepper JN, Shaffer MS, Tetley TD, Porter AE: **Translocation of functionalized multi-walled carbon nanotubes across human pulmonary alveolar epithelium: dominant role of epithelial type 1 cells**. *ACS Nano* 2016, **10**: 5070–5085.

16. Shannahan JH, Sowrirajan H, Persaud I, Podila R, Brown JM: **Impact of silver and iron nanoparticle exposure on cholesterol uptake by macrophages**. *J Nanomater* 2015, **2015**.

17. Cheng X, Tian X, Wu A, Li J, Tian J, Chong Y, Chai Z, Zhao Y, Chen C, Ge C: **Protein corona influences cellular uptake of gold nanoparticles by phagocytic and nonphagocytic cells in a size-dependent manner**. *ACS Appl Mater Interfaces* 2015, **7**: 20568–20575.

18. Shannahan JH, Podila R, Aldossari AA, Emerson H, Powell BA, Ke PC, Rao AM, Brown JM: **Formation of a protein corona on silver nanoparticles mediates cellular toxicity via scavenger receptors**. *Toxicol Sci* 2015, **143**:136–146.

19. Shannahan JH, Bai W, Brown JM: **Implications of scavenger receptors in the safe development of nanotherapeutics**. *Recept Clin Investig* 2015, **2**:e811.

20. Wang X, Reece SP, Brown JM: **Immunotoxicological impact of engineered nanomaterial exposure: mechanisms of immune cell modulation**. *Toxicol Mech Meth* 2013, **23**:168–177.

21. Lee KJ, Nallathamby PD, Browning LM, Osgood CJ, Xu XH: **In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos**. *ACS Nano* 2007, **1**:133–143.

22. Moriya M, Ho YH, Grana A, Nguyen L, Alvarez A, Jamil R, Ackland ML, Michalczyk A, Hamer P, Ramos D, *et al.*: **Copper is taken up efficiently from albumin and alpha2-macroglobulin by cultured human cells by more than one mechanism**. *Am J Physiol Cell Physiol* 2008, **295**:C708–C721.

23. Liu J, Wang Z, Liu FD, Kane AB, Hurt RH: **Chemical transformations of nanosilver in biological environments**. *ACS Nano* 2012, **6**:9887–9899.

24. * Sun B, Wang X, Ji Z, Wang M, Liao YP, Chang CH, Li R, Zhang H, Nel AE, Xia T: **NADPH oxidase-dependent NLRP3 inflammasome activation and its important role in lung fibrosis by multiwalled carbon nanotubes**. *Small* 2015, **11**: 2087–2097.

25. Pan Y, Leifert A, Ruau D, Neuss S, Bornemann J, Schmid G, Brandau W, Simon U, Jahnen-Dechent W: **Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage**. *Small* 2009, **5**:2067–2076.

26. Ma X, Wang X, Zhou M, Fei H: **A mitochondria-targeting gold-peptide nanoassembly for enhanced cancer-cell killing**. *Adv Healthcare Mater* 2013, **2**:1638–1643.

27. ** Schaeublin NM, Braydich-Stolle LK, Schrand AM, Miller JM, Hutchison J, Schlager JJ, Hussain SM: **Surface charge of gold nanoparticles mediates mechanism of toxicity**. *Nanoscale* 2011, **3**:410–420.

28. Duan J, Kodali VK, Gaffrey MJ, Guo J, Chu RK, Camp DG, Smith RD, Thrall BD, Qian WJ: **Quantitative profiling of protein S-glutathionylation reveals redox-dependent regulation of macrophage function during nanoparticle-induced oxidative stress**. *ACS Nano* 2016, **10**:524–538.

29. Bulcke F, Dringen R: **Copper oxide nanoparticles stimulate glycolytic flux and increase the cellular contents of glutathione and metallothioneins in cultured astrocytes**. *Neurochem Res* 2015, **40**:15–26.

30. Zhang H, Wang X, Wang M, Li L, Chang CH, Ji Z, Xia T, Nel AE: **Mammalian cells exhibit a range of sensitivities to silver nanoparticles that are partially explicable by variations in antioxidant defense and metallothionein expression**. *Small* 2015, **11**:3797–3805.

31. Kumari M, Singh SP, Chinde S, Rahman MF, Mahboob M, Grover P: **Toxicity study of cerium oxide nanoparticles in human neuroblastoma cells**. *Int J Toxicol* 2014, **33**:86–97.

32. Park EJ, Choi DH, Kim Y, Lee EW, Song J, Cho MH, Kim JH, Kim SW: **Magnetic iron oxide nanoparticles induce autophagy preceding apoptosis through mitochondrial damage and ER stress in RAW264.7 cells**. *Toxicol In Vitro* 2014, **28**:1402–1412.

33. Simard JC, Durocher I, Girard D: **Silver nanoparticles induce irremediable endoplasmic reticulum stress leading to unfolded protein response dependent apoptosis in breast cancer cells**. *Apoptosis* 2016, **21**:1279–1290.

34. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ, Hung MC: **Survival of cancer cells is maintained by EGFR independent of its kinase activity**. *Canc Cell* 2008, **13**: 385–393.

35. Go YM, Jones DP: **Cysteine/cystine redox signaling in cardiovascular disease**. *Free Radic Biol Med* 2011, **50**:495–509.

36. Go YM, Jones DP: **Redox clamp model for study of extracellular thiols and disulfides in redox signaling**. *Meth Enzymol* 2010, **474**:165–179.

37. Go YM, Park H, Koval M, Orr M, Reed M, Liang Y, Smith D, Pohl J, Jones DP: **A key role for mitochondria in endothelial signaling by plasma cysteine/cystine redox potential**. *Free Radic Biol Med* 2010, **48**:275–283.

38. Di Bucchianico S, Gliga AR, Akerlund E, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL: **Calcium-dependent cyto- and genotoxicity of nickel metal and nickel oxide nanoparticles in human lung cells**. *Part Fibre Toxicol* 2018, **15**:32.

39. Golbamaki A, Golbamaki N, Sizochenko N, Rasulev B, Leszczynski J, Benfenati E: **Genotoxicity induced by metal oxide nanoparticles: a weight of evidence study and effect of particle surface and electronic properties**. *Nanotoxicology* 2018:1–17.

40. Ho CC, Lee HL, Chen CY, Luo YH, Tsai MH, Tsai HT, Lin P: **Involvement of the cytokine-Ido1-AhR loop in zinc oxide nanoparticle-induced acute pulmonary inflammation**. *Nanotoxicology* 2017, **11**:360–370.