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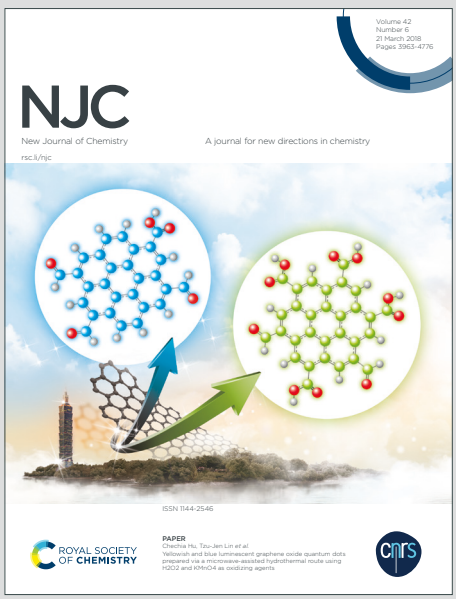

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ARTICLE

Current understanding of nanoparticle toxicity mechanisms and interactions with biological systems

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Mariana Garcés,[a, *] Lourdes Cáceres, [a] Diego Chiappetta, [c, d] Natalia Magnani, [a, b] and Pablo Evelson [a, b, †]

Nanotechnology is an emerging science involving the manipulation of matter at the nanometer scale. Nanoparticles (NP) are engineered structures with at least one dimension of 100 nm or less. These materials are progressively being used for commercial purposes and being incorporated into everyday manufactured articles at an increasing rate. These products include consumer items such as pharmaceuticals, cosmetics, food, food packaging, and household products, among others. The same unique physical and chemical properties that make NP so attractive may be associated with their potentially hazardous effects on cells and tissues. Despite the large benefit ensured from the application of nanotechnology, many issues related to NP behavior and adverse effects are not fully understood or should be examined anew. The traditional hypothesis that NP exhibit different or additional hazards due to their "nano" size has been challenged in recent years, and NP categorization according to their properties and toxicity mechanism has been proposed instead. Possible undesirable results of these capabilities are harmful interactions with biological systems and the environment, with the potential to generate toxicity. Both *in vivo* and *in vitro* studies have shown that NP are closely associated with toxicity by increasing intracellular reactive oxygen species (ROS) levels, and/or the levels of pro-inflammatory mediators. This review summarizes available data on NP toxicity in biological systems, with particular focus on oxidative stress and inflammation as the main mechanisms that lead to adverse health effects following NP exposure.

Background

Nanoparticles (NP) have been defined as nanomaterials with a natural, incidental, or manufactured origin, which have a diameter equal or smaller than 100 nm in at least one dimension, either in an unbound or an aggregate state[1,2]. The upper length limit has been established considering that 100 nm is the critical size where particles start presenting different properties from bulk materials[3]. The small size gives NP the feature of a large surface to volume ratio, which is the property that facilitates a myriad of applications[4]. The nanotechnology field has grown exponentially over the last decades with the production of NP that are used from electronics to cosmetics, food technology or biological sensors, among others[5,6,7]. Healthcare is one of the fields where nanotechnology promoted the greatest expansion. Moreover, interdisciplinary research in nanomedicine is in constant development due to the possibility of a tailored NP production for each specific use[8]. This growth is followed by an increased investment and creation of numerous research centers worldwide[9,10,11]. However, this new technology requires to be accompanied by a detailed analysis of the possible adverse effects due to NP exposure.

The same unique physical and chemical properties that make NP so attractive may be associated with their potentially detrimental effects on cells and tissues[12,13]. Reactive oxygen species (ROS) formation is one the most relevant toxic mechanisms elicited by NP, causing oxidative stress, inflammation and the consequent damage to proteins, cell membranes and DNA[14]. The redox imbalance induced by NP might be explained by different mechanisms, as oxidant species can be generated directly from the NP surface, by the transition metal present in NP that can participate in Fenton-type reactions, due to mitochondrial function alterations, or after the activation of inflammatory cells[14,15,16].

Nowadays, the harmful effects elicited by NP exposure in biological systems emerge as the relevant topic to focus on from the toxicological perspective[16,17]. Therefore, the aim of the present article is to summarize the current knowledge regarding the toxicological mechanisms triggered by NP exposure, pointing out on the possible redox and inflammatory pathways initiated upon interaction with biological systems.

[a.] *Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Química Analítica y Fisicoquímica, Cátedra de Química General e Inorgánica, Argentina*
[b.] *CONICET - Universidad de Buenos Aires, Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica, Argentina.*
[c.] *Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Tecnología Farmacéutica I, Buenos Aires, Argentina.*
[d.] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
* Mariana Garcés and Lourdes Cáceres share first authorship.
† Corresponding author. Universidad de Buenos Aires, Instituto de Bioquímica y Medicina Molecular (IBIMOL UBA-CONICET), Facultad de Farmacia y Bioquímica, Junín 954, C1113AD, Buenos Aires, Argentina. E-mail address: pevelson@ffyb.uba.ar
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NP classification

The nanotechnology development brings a wide multiplicity of NP, and this versatility leads to several classification criteria based on NP shape, composition, functionality, mechanisms of action, among others[18,19]. Even though NP originated from human activities do not have a specific size, and their chemical characteristics are unknown, synthetic NP can be classified according to their composition[19,20]. As the present perspective article deals with toxicological endpoints, the nanomaterials composition results as the most suitable criteria to fully correlate their composition with certain biological mechanisms[21,22,23]. Within this category, they can be divided into four composition-based groups as carbon, inorganic, organic, and composite NP[8,24]. The first group consists of carbon-based NP, such as fullerenes, carbon nanotubes (CNT) and graphene. The carbon-carbon bonding confers unique strength and reactivity to the NP. Besides their stability, carbon-based NP can be loaded with relatively high amounts of a specific drug, which makes them especially attractive for nanomedicine. In addition, DNA molecules and small proteins, such as cytochrome c and streptavidin, may be absorbed on CNT[25,26]. Even though all NP types in this group are formed by carbon atoms, different configurations lead to differences in shapes[27]. For instance, we can encounter single wall carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) among the CNT family. The second composition-based group includes metal-based NP that are solid metallic nanoforms composed by Ag, Au, Ti, Si, Fe, Mn, Ni, or their corresponding oxides[28]. Currently, these materials can be synthesized and modified with several functional chemical groups that allow them to be conjugated with antibodies, ligands, and drugs of interest[29]. The so-called organic NP comprises liposomes, micelles, polymeric NP, protein/peptide-based NP, and dendrimers. While liposomes include spherical nanosystems made up of a lipid bilayer, micelles are colloidal aggregates of amphiphilic molecules, and polymeric NP are colloidal particles prepared by biocompatible polymres[30,31]. The main applications of liposomes are found in medicine and can be divided in two groups, multilamellar vesicles, and unilamellar vesicles, according to their main use into diagnostic and therapeutic[30]. Dendrimers are a particular type of polymeric NP: they are defined as macromolecules formed by a nucleus and multiple branches[32]. Today, dendrimers have several medicinal applications such as carriers for drug delivery and as contrast agents in magnetic resonance imaging[33]. Composite NP are advanced materials which have recently gained attention due to their scientific and technological importance, given that instead of developing a single novel material, they may be advantageous to develop carriers formed by multiple materials that are equipped with diverse functionalities such as polymer-matrix composites and microsphere composite NP[34,35]. Currently, multifunctional nanocarriers can be produced using a wide range of materials, with a high number of different molecules that can attach to the NP. Depending on the characteristics of the molecules that need to be delivered (proteins, antibiotics, or anticancer drugs), the type of nanocarrier varies from polymeric NP to dendrimers, metallic, or magnetic NP[36].

NP sources and routes of exposure in context

NP are abundant in nature, as they are produced in many natural processes, including photochemical reactions, volcanic eruptions, forest fires, and simple erosion. On the other hand, humans have contributed to the release of NP for centuries, as by-products of simple combustion during food cooking, and more recently, NP contributions has been expanded to other activities such as chemical manufacturing, welding, ore refining and smelting, combustion of coal and fuel oil for power generation, and for vehicle and airplane engines[19,24]. Engineered NP have been on the market for some time as they are commonly found in consumer goods[18]. Since synthetic NP are a potential occupational, environmental, and public hazard, the routes of exposure become relevant when assessing effects before their production and use. Humans can be exposed voluntarily or involuntary to NP mainly through organs that directly interact with the environment, such as the respiratory tract by inhalation, penetration through the skin, or the gastrointestinal tract via ingestion[18,37,38,39,40]. In addition, intravenous injections represent one of the most frequent protocols used in nanomedicine in order to reach target tissues.

Moreover, the development related to novel uses of NP in healthcare introduces new routes of exposure. For example, the visual system can be mentioned, since different ocular cell types can be reached by NP present in eye-drops[41]. Skin tissue exposure has been described after the use of NP in wound dressings. Studies demonstrate the ability of intact skin to protect itself against NP penetration beyond the surface layers[42,43]. However, regarding pharmaceutical products such as topical sunscreens with TiO_2-NP and ZnO-NP, the methods used to measure skin penetration are still evolving, and better standardization to improve validation is needed[42,44]. The use of NP as drug delivery agents in oral formulations is a new area in medical research. These agents are meant to be ingested and then translocate into circulation in order to reach target areas of the body[45,46]. Another important NP uptake route is the circulatory system following injection of NP-based drugs or drug delivery in diagnostic systems. The NP translocation depends on their size, coating, and site of injection: intravenously injected NP quickly spread throughout the circulatory system, with subsequent translocation mainly to the liver and spleen[1,47]. On the other hand, NP administered by intradermal injection accumulate in lymph nodes, and are subsequently localized in monocytes, macrophages, and myeloid dendritic cells, which constitutes a favorable aspect in vaccination designs[48,49]. A study where NP are administered via intramuscular injection shows the localization in the injection site, which can result beneficial in anti-tumoral therapies that require prolonged NP activity[50]. Another report using carbon dots (C-dots) shows that NP are rapidly cleared through the kidneys into urine, which affects tumor retention[51]. Regarding neuronal and lymphatic system, van Rooy and colleagues[52] showed that the use of NP-drug delivery enhances brain uptake, while

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Zhang and Lu[53] demonstrated that using nanospheres and nanocapsules for targeted drug delivery towards the lymphatic system is a promising approach in tumor metastasis therapy. Concerning NP inhalation, the ability of a particle to get deposited in the different respiratory tract regions depends on its size. Particles larger than 10 microns (10,000 nm) get trapped in the mouth, nose, and throat; only particles less than 10 microns enter the conductive airways (trachea and bronchi). Many of these particles are trapped by mucus and ultimately ingested. The NP small size allows them to be inhaled into the alveolar region of the lung where gas exchange occurs. Polymeric NP have gained attention in inhalation therapy and are being investigated to improve controlled drug delivery to the lung for severe pulmonary diseases treatment such as asthma, respiratory distress syndrome, among others[19,54,55]. Nanosuspensions, compared to conventional suspension aerosols inhalers, have a more effective drug dispersion in the propellant, and provide chemically and physically stable products with an increased number of particles per drop[56,57,58].

Everyday NP exposure through domestic and commercial products is also relevant. For instance, skin exposure to antibacterial textiles, or cosmetic products containing NP has become more frequent in recent years[59,60,61]. Moreover, the current applications of NP in food packaging and kitchen utensils could represent a route to incidental ingestion[62,63]. In addition, unintentional NP ingestion could take place, for instance, as a result of inhalation when mucus moves up the respiratory tract and is swallowed[64]. Several health and beauty care hygiene, and antibacterial products containing sprays with NP have entered our daily lives. Puisney and colleagues[40] reported how the increasing use of Ag-NP containing products could lead to adverse health effects. Ag-NP aerosol directly applied into the nasal or oral cavity are of great concern and the use of sprays such as deodorants, shoe sprays or disinfectant cleaning products could lead to accidental NP inhalation.

One last highly relevant NP exposure source is the occupational area; many studies and guidance documents have focused on inhalation and the dermal route as the primary routes of occupational exposure to NP[12,40,65,66,67,68]. Among the possible exposure scenarios, the workplaces where NP are produced, processed, used, and disposed, may pose specific challenges, facilitating the potential inhalation of aerosols, and cutaneous exposure to NP[69,70].

Extrapulmonary effects of NP exposure

It was extensively described that inhaled NP are able to deposit deep in the lungs and induce an oxidative stress and inflammatory response. It has been hypothesized that inflammatory mediators pass into the systemic circulation and indirectly influence the cardiovascular system. However, evidence of systemic inflammation following exposure could be also related with translocation of NP into the circulation and directly contribute to extrapulmonary alterations. It has been speculated that these processes may be accelerated by inflammation, through increased permeability of the alveolar wall or through assisted translocation within macrophages. Despite the mechanism by which NP exert extrapulmonary adverse effects numerous studies showed cardiovascular impairment and systemic alterations[71].

Cardiovascular impairment following CNT exposure was previously described. Ganguly and colleagues[72] showed that the extrapulmonary effects due to CNT inhalation were associated with indirect effects (particle-cell interactions in the lung) rather than direct effects (translocated CNT) within the first hours after exposure. Mercer and colleagues[73] showed that inhaled MWCNT, deposit in the lungs, and are later transported to the parietal pleura, the respiratory musculature, liver, kidney, heart, and brain in a singlet form, accumulating in time following exposure.

Kan and colleagues[74] showed that pulmonary exposure to engineered NP could initiate adverse effects on the heart by two pathophysiological phases in the cardiovascular system. The first involves neuron-regulated fast-onset and short-lasting cardiovascular reactions that include alterations in heart rate, blood pressure and cardiac contractility. The second, depending on the composition and structure of engineered NP, may involve inhaled engineered NP gradually and continuously crossing the air-blood barrel into the circulatory system or inducing an inflammation and oxidative stress response systemically in the circulation. Exposure to TiO_2-NP in the context of a realistic occupational exposure suggest that TiO_2-NP may translocate from the lung to extrapulmonary organs, such as liver and spleen, where they could possibly promote systemic health effects[75].

Exposure to ZnO-NP demonstrate cardiopulmonary impairments[76,77] after exposure via intratracheal instillation and inhalation. For example, instilled ZnO-NP showed increased levels of total cells, neutrophils, lactate dehydrogenase (LDH) and total protein in bronchoalveolar fluid (BALF) and 8-hydroxy-2'-deoxyguanosine in blood after 72 h. Moreover, inhalation of ZnO-NP caused an inflammatory cytological profile, cardiac inflammation, and the development of fibrosis after 7 days after exposure. Degeneration and necrosis of the myocardium were detected 30 days after exposure[76].

Relationship between NP harmful effects and their physicochemical properties

Although the small NP size is a positive and desirable characteristic regarding production versatility, detrimental effects might appear with the use of NP-containing products. Therefore, as the increased surface to volume ratio may change the NP behavior drastically, the toxicological potential of nanomaterials made nanotoxicology emerge a few years after nanotechnology started[78,79]. The main purpose in nanotoxicology studies is to elucidate if the unique NP properties represent a threat to the environment and to living things. At the beginning, this new discipline took the knowledge from air pollution toxicology as a starting point, considering that airborne particulate matter present in its composition particles of nanoscale size[1,80,81]. However, as NP can be engineered, new features are introduced to this research area, creating the necessity of detailed toxicity tests.

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In this sense, nanotoxicology moved forward to developing specific strategies to evaluate NP interactions with biological systems. Among different studies, Savage and colleagues[82] as well as Drasler et al[83], detailed the considerations for *in vitro* and *in vivo* testing to evaluate NP toxicity: viability, cytotoxicity, apoptosis, oxidative stress, inflammation, and endotoxin content are the recommended assays. Moreover, *in vivo* testing includes biodistribution, toxicokinetics and immune system response studies[82,83,84].

Since the physicochemical properties of NP play a critical role in toxicity, their complete characterization is also necessary to understand how NP interacts with cells and tissues. Many studies started showing that different NP may present dissimilar physicochemical properties such as composition, size, shape, charge, or coating surface, which affect their interaction with other materials, and also have an impact on their biodistribution and bonding with target molecules[23,85,86,87,88]. Therefore, nanotoxicology focuses its efforts in avoiding the unregulated NP production, use and the resulting release that might, in turn, cause hazardous effects to the environment and human health[40,89]. Accordingly, new procedures are required to elucidate whether NP presence may trigger biological effects after short-term exposure and the consequence of long-term treatments. In this sense, biokinetic parameters are relevant in order to establish the suitable NP dose and set appropriate safety regulations[90,91].

As previously mentioned, particle size and surface area are important material characteristics from a toxicological and health perspective. They determine the *in vivo* distribution, biological fate, toxicity and targeting ability[18]. As the size of a particle decreases, its surface area increases, which allows a greater proportion of its atoms or molecules to be displayed on its surface rather than within the interior of the material, potentially contributing to the development of adverse health effects[79,92,93]. For example, Au-NP smaller than 10 nm effectively enters the cell nucleus, whereas large NPs (10 or 16 nm) only penetrate through the cell membrane and are found only in the cytoplasm[94]. In addition, the NP size largely determines how the NP interacts with the different body compartments. *In vivo* experiments using NP smaller than 10 nm and larger NP (50-250 nm) suggested that large NP are recognized by the mononuclear phagocyte system, accumulating in the liver and spleen[95,96].

The characteristic shapes of NP are spheres, ellipsoids, cylinders, sheets, cubes, and rods. NP toxicity strongly depends on their shape: for example, spherical NP are more prone to endocytosis than nanotubes and nanofibers, since the uptake of a spherical particle requires a milder actin expansion array[97]. SWCNT have been found to more effectively block potassium channels compared to spherical fullerenes, which could be attributed to the "cork in a bottle" fitting that stops ion permeation[98].

Although the toxicity of NP strongly depends on their size and shape, the influence of other factors, such as the NP chemical composition, functionalization and, for certain NP, crystal structure, should not be disregarded. NP chemical composition is critical in determining its toxicity, since the reactive groups on a particle surface will certainly modify the biological effects[23,99,100,101]. Some metal ions present in NP, such as Ag and Cd, are in fact toxic and, therefore, cause damage to the cells. Other metal ions, such as Fe and Zn, are biologically relevant but when concentrations exceed the physiological levels, they might impair cellular pathways, enhancing toxicity[102]. On the other hand, CNT constitute a good example of NP structural diversity. They consist of rolled-up tubular shells of graphene sheets which are made up of benzene type hexagonal rings of carbon atoms[103,104]. The structure can be found as SWCNT or MWCNT. Although pure CNT are significantly unreactive, there are points in their structures which are more reactive than others, such as defects due to missing carbon atoms or residual impurities that may remain from its synthesis, such as metal, organic and support material[105,106]. Moreover, studies using MWCNT showed the distribution, persistence of pleural penetrations and fibrotic response following inhalation[107,108,109,110,111].

The NP surface charge plays an important role in stability, dispersion, and their toxicity, because it largely determines the interactions between NP and biological systems. The NP zeta potential is commonly used to characterize the surface charge, one of their most relevant physical properties. It reflects the electric potential of particles and is influenced by NP composition and the medium in which they are dispersed[79,99,112]. The undesired effects observed when positively charged NP interacts with biological systems is explained by their ability to easily pass-through membranes into the cells, in contrast to negatively charged and neutral NP[113]. Positively charged NP have an enhanced capacity for opsonization, by adsorption of proteins, facilitating phagocytosis, including antibodies and complement components, from blood and other biological fluids. The

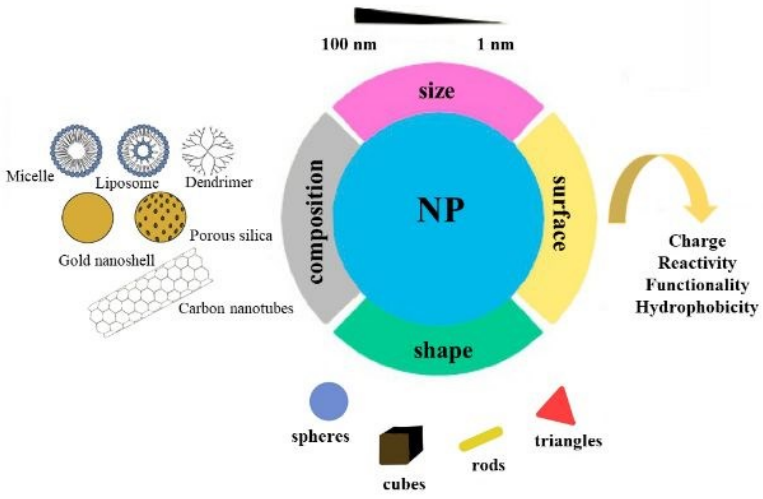

Figure 1. NP properties that must be considered when assessing hazard. Size, shape, composition, and surface characteristics comprises the properties that make NP unique and drive the current interest in the raised safety concerns.

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absorbed proteins, referred to as the protein crown, may affect the surface properties of NP[114,115]. **Figure 1** shows the main characteristics which define beneficial and toxicological effects of NP.

NP-induced toxic mechanisms

Toxicological effects elicited by NP are diverse and might include at least one of the following events: ROS production inside or outside the cell, alteration of cell membrane integrity by NP-produced ROS, particle dissolution affecting cellular function after NP internalization, mechanical damage to subcellular units, or dissolution of the NP outside the cell which can affect its integrity[86,]. The majority of these events lead to the occurrence of oxidative stress and the onset of an inflammatory response.

Role of oxidative stress

As the diversity in NP chemical composition along with different mechanisms regarding oxidants generation and redox signaling are expected to take place, the establishment of an oxidative stress situation is suggested as one of the major mechanisms of nanotoxicity[14]. Oxidative stress is currently defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage[116].

Therefore, it is important to characterize the oxidant species produced by NP *per se* or via the interaction with biological systems. In this sense, most studies postulate that ROS are the most relevant oxidants produced by these mechanisms. The accepted concept of ROS nowadays includes a variety of chemical entities such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), singlet oxygen ($^1O_2$) and, also organic peroxides (ROOH) and peroxynitrite ($ONOO^-$)[102]. All members of this group are molecules that present markedly different chemical and biological actions.

The antioxidant system is a complex and coordinated arrangement of enzymes and low molecular weight chemical species, such as superoxide dismutase (SOD), catalase (CAT), and glutathione expressed as reduced/oxidized ratio (GSH/GSSG) which maintains ROS levels at physiological levels[117]. An increase in oxidant production as a result of NP exposure can overwhelm the antioxidant system resulting in the occurrence of oxidative stress.

The key factors involved in NP-induced oxidants production include prooxidant functional groups on the reactive surface of NP, active redox cycling on the surface of NP due to transition metal-based NP, and particle-cell interactions. Most metal-based NP elicit free radical mediated toxicity via the Haber-Weiss reaction, where $O_2^{\bullet-}$ reacts with H_2O_2 in the presence of transition metals such as Fe or Cu leading to the formation of $HO^{\bullet}$ (1)[118].

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^- + HO^{\bullet} \qquad (1)$$

This is a two-step reaction which begins when an oxidized metal ($metal^{n+1}$) is reduced by $O_2^{\bullet-}$ (2).

$$metal^{n+1} + O_2^{\bullet-} \rightarrow metal^{n+} + O_2 \qquad (2)$$

Subsequently, the reduced metal ($metal^{n+}$) reacts with H_2O_2 to yield $HO^{\bullet}$, in what is known as Fenton reaction (3)

$$metal^{n+} + H_2O_2 \rightarrow metal^{n+1} + HO^{\bullet} + OH^- \qquad (3)$$

The $HO^{\bullet}$ radical is the most reactive of O_2-derived free radicals, with a half-life of 10^{-9} s, and a high standard reduction potential (E°=2.00 V)[119,120]. Given its extreme reactivity, $HO^{\bullet}$ radical can rapidly induce oxidative damage to carbohydrates, amino acids, phospholipids, DNA, and organic acids, which could lead to inactivation of enzymes among other relevant biological processes. Certain metal-based NP can react directly with cellular molecules to generate free radicals or induce cell signaling pathways. Although these NP are able to generate $HO^{\bullet}$, the efficiencies at which they produce this radical vary greatly. Reduction potential of the ion adsorbed to the NP, or the NP core itself, depending on the engineering of the nanomaterial, is an important thermodynamic property that allows the prediction of the course of free radical generation[121].

Metallic NP led to increased ROS production that triggers the occurrence of oxidative stress via Fenton and Haber-Weiss reactions. For example, NP containing Cu and Fe, increase ROS generation via Fenton reactions, while the ones containing Cr, Co and V can catalyze both Fenton and Haber-Weiss reactions[16].

Apart from the intrinsic oxidative properties of metal-based NP, a plethora of events regarding oxidative metabolism are triggered when NP interact with cell systems. The most relevant sources of oxidative mediators in physiological conditions are the NADPH oxidases (NOXes) family and the mitochondria, which also have implications in the crosstalk between oxidative stress and inflammation[122].

Role of inflammation

The innate immune system constitutes the first line of defense against foreign agents and materials that come in contact with the body. It involves multiple cell types which are primarily localized in tissues that serve as an interface with the environment, such as skin, respiratory and gastrointestinal mucosa. These cell types include mostly phagocytic cells, such as polymorphonuclear leukocytes (PMN), tissue-resident macrophages and systemic circulation-derived monocytes. Apart from their particle uptake capabilities, these cells are involved in the secretion of soluble mediators, namely cytokines and chemokines, that modulate the onset of the inflammatory response[123].

After inhalation, NP ranging between 10 nm and 100 nm can reach the alveolar space, regardless of their density[91]. Typically, resident macrophages will internalize NP to clear the alveolar space from the foreign material. However, if NP deposition persists and overwhelms the local phagocytic defense, epithelial injury will take place, leading to the recruitment of PMN cells and circulating monocytes. In a previously published study where mice were exposed to $Ni(OH)_2$-NP in whole-body inhalation chambers, an increase in mRNA expression of chemokine C-X-C motif ligand 2 (CXCL2), chemokine C-C motif ligand-2 (CCL2), interleukin 1 alpha (IL-1α), and tumor necrosis factor alpha (TNF-α) in lavage lung tissue was observed, and the response was dependent on $Ni(OH)_2$-NP dosage and length of exposure[124]. In a similar approach, Mo and collegueas[125] conclude that the length of exposure to Ni-NP, but also the surface characteristics of the NP, have an impact in the extent of the inflammatory response and lung injury, reported as

increased levels of chemokine C-X-C motif ligand 1 (CXCL1) and oxidative damage to macromolecules. Moreover, current knowledge on metal-NP interactions with biological systems highlight their potential to exacerbate, rather than induce, respiratory and dermal allergy[126].

In dermal exposure models using porcine skin, Ag-NP have been reported to exacerbate the release of interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), and TNF-α in vitro, as well as intracellular and intercellular epidermal edema with focal dermal inflammation revealed by ultrastructural alterations[127].

Toxicological effects of inorganic and carbon-based NP

Taking into consideration the widespread applications of inorganic and carbon-based NP, and their ability to induce oxidative damage and inflammation in biological systems, we hereby focus on metal-based NP and CNT toxicity mechanisms.

Metal/metal oxide NP

The toxic effects of metal-based NP have been investigated over the past decades. In that sense, we hereby summarize the main mechanisms by which different inorganic NP may exert toxicological effects due to oxidative stress, discussing the most relevant original studies concerning metal-coated NP.

The toxicity of metallic and metal oxide NP including Fe, Mn, Cu, Ti, Zn, Cd, Al_2O_3, CuO, NiO, TiO_2, ZnO, and VO_2 have been characterized by their ability to increase ROS generation, leading to oxidative stress and, in some models, triggering apoptosis[128,129,130]. *In vitro* studies in *E. coli* exposed to ZnO-NP and TiO_2-NP revealed ROS-mediated genotoxicity characterized by DNA damage[115]. Similar results were obtained in human lung carcinoma cells (A549) incubated with CuO-NP and Au-NP[132,133,134]. Mice i.p. exposed to Cu, Fe, Ti metal oxide-NP and Ag-NP showed signs of oxidative DNA damage and micronuclei formation[135]. In addition, it has been reported that oxidative stress takes place in pulmonary-derived rat cells exposed to ZnO-NP and SiO-NP, with different extents of inflammation[136]. Moreover, SiO_2, TiO_2 and Fe_3O_4 stimulate inflammatory reaction in brain microglia and damage neurons *in vitro*[137]. Regarding VO_2-NP, *in vitro* studies using A549 cell line exposed to this type of NP showed increased ROS generation, mitochondrial damage, cell membrane leakage, the occurrence of apoptosis, and the establishment of an inflammatory response after short-term exposure[138]. Similar results were found using VO_2-NP and titanium dioxide-coated VO_2-NP in A549 and human bronchial epithelial (BEAS-2B) cell lines[139].

In vivo studies in rats exposed to VO_2-NP through inhalation suggest the toxic potential of these NP as indicated by the changes in the levels of oxidative stress markers. In a similar study, histopathological and immune-histopathology analysis of lung tissue showed augmented damage and inflammatory response in VO_2-NP-exposed animals[140].

Exposure to Ag-NP assessed in human lung fibroblast cells (IMR-90), human glioblastoma cells (U251), and A549 cell lines induced increased ROS production, mitochondrial damage, and apoptosis[141,142]. Meanwhile, *in vivo* exposure to Ag-NP and Au-NP has been associated to inflammatory response, pulmonary injury, mitochondrial content release, oxidative stress, together with liver and kidney toxicity[143,144]. In a recently published study, our group found that Ag-NP intranasal instillation caused NP lung deposition, increased lung tissue O_2 consumption and NOX activity, as well as the contribution of antioxidant defenses in Balb/c mice. Moreover, A549 cells incubated with Ag-NP showed altered mitochondrial function, contributing to oxidative damage, while a 3D mucociliary tissue model (EpiAirway) revealed alterations in the lung barrier integrity, after Ag-NP exposure[145]. The toxicological effects upon exposure to Ag-NP could be related with their properties as antibacterial, antifungals and antivirals[146]. Mechanisms behind Ag-NP activity against different organisms are adhesion onto the cell wall surface and membrane, penetration into the cell, and intracellular structures and biomolecules destabilization. Ag-NP effects rely on their size, in fact particles with a size range between 10 and 15 nm exhibits enhanced antimicrobial activity, and even smaller particles (1 to 10 nm) have been found to display even better activity. Since smaller particles present a larger surface area, the number of atoms increases, as consequence, enhancing interaction with biological systems[147,148].

It is well established that uncontrolled generation of ROS triggers a proinflammatory response including the release of cytokines and mediators such as TNF-α, IL-1β, interleukin 2 (IL-2), IL-6, platelet-derived growth factor subunit B (PDGF-β) and CXCL2. Several studies, including coexposure of metal oxide NP with a bacterial endotoxin, demonstrated exaggerated lung inflammation and pulmonary edema, and a ROS-mediated inflammatory response[126,149,150,151]. **Table 1** shows a list of studies describing the toxicity mechanism of metal-based NP.

Table 1. Summary of studies describing the toxicity mechanisms of inorganic NP

Study	Nanoparticle	Model	Samples evaluated	Effects observed	References
In vitro	TiO_2-NP	Human mesangial cells (IP15) Human Kidney epithelial cells (HK-2)		↑ ROS production	128

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	ZnO-NP CdS-NP		↑ ROS production ↓ GSH/GSSG ratio Nuclear Factor (NF) -κB nuclear translocation	
In vitro	Al_2O_3-NP Mn_3O_4-NP SiO_2-NP SnO_2-NP	*S. cerevisiae*	Cell membrane damage ↓ Cell viability Apoptotic hallmarks	129
In vitro	VO_2-NP	Human lung carcinoma cells (A549)	NP exposure length dependent effects: Cell growth inhibition ↓ Cell viability ↑ ROS production Mitochondrial damage Inflammation	138
In vitro	VO_2-NP T-VO_2-NP	A549 Human bronchial epithelial cells (BEAS-2B)	Cytotoxicity Apoptosis Cell proliferation inhibition ↑ ROS production ↓ GSH	139
In vitro	TiO_2-NP ZnO-NP	E. Coli	DNA damage ↓ GSH ↑ Hydroperoxide ions, ↑ Lipid peroxidation	131
In vitro	CuO-NP	A549	↓ GSH ↑ Lipid peroxidation ↑ CAT activity ↑ SOD activity Genotoxicity	132
			↑ ROS production DNA damage	133
In vitro	Ag-NP	3D mucociliary tissue model (EpiAirway)	Lung barrier alterations	145
		A549	↑ NOX activity ↓ ATP production rate ↑ H_2O_2 production rate Oxidative damage	
In vitro	Ag-NP	A549	↑ ROS production ↓ Mitochondrial inner membrane potential Apoptosis Cell cycle arrest	141
In vitro	Ag-NP	Human lung fibroblast cells (IMR-90) Human glioblastoma cells (U251)	↑ ROS production ↓ ATP content DNA damage Cell cycle arrest	142
In vitro	Ag-NP	Anaerobic bacteria Gram negative bacteria	NP-size dependent bactericidal effects	147 148

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In vitro	Au-NP	A549		↓ GSH content Transient DNA damage	134
In vitro	TiO_2-NP CeO_2-NP	BEAS-2B		↑ Gene expression: IL-6, IL-1, PDGF-β, leukemia inhibitory factor	149
	TiO_2-NP SiO_2-NP Fe_3O_4-NP			↑ inducible nitric oxide synthase (iNOS) expression ↑ CCL2, chemokine C-C motif ligand 3 (CCL3) expression ↑ NF-κB activation ↑ TNF- α, IL-1β, IL-6 secretion	137
In vitro	Crystalline Si-NP	Primary rat alveolar macrophages (AM)		CXCL2 secretion IL-6 secretion	136
	ZnO-NP	Immortalized rat lung epithelial cells (L2)		Cytotoxicity	
		AM+L2 (coculture)		IL-6 secretion	
In vivo	CuO-NP Fe_3O_4-NP Fe_2O_3-NP TiO_2-NP Ag-NP	Mice	Liver Blood Urine	Reticulocyte micronuclei formation Oxidative DNA damage	135
In vivo	NiO-NP	Mice	Lung	Surface area dependent inflammation and lung injury: ↑ TNF- α, IL-1β, IL-6 in BALF ↑ neutrophils ↑ NP internalization in macrophages Asthma model: Surface area dependent Th response Size dependent lung function alterations and eosinophil burden	126
In vivo	Ag-NP	Mice	Lung	Pulmonary injury Mitochondrial content release Neutrophil recruitment	143
				Lung deposition ↑ lung tissue O_2 consumption ↑ NOX activity ↑ SOD activity ↑ CAT activity ↓ GSH/GSSG ratio	145
In vivo	Ag-NP Au-NP	Mice	Blood	↑ ROS production ↓ Antioxidant status ↑ Inflammatory markers	144
			Brain Liver Kidney Spleen	↑ ROS production Liver and kidney toxicity DNA damage	
In vivo	TiO_2-NP	Mice	Lung	Shape and length dependent lung injury and inflammation: ↑ Fibrosis	151

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				↑ Macrophage deposition ↑ PMN ↑ Albumin ↑ LDH	
In vivo	TiO_2-NP	Mice	Lung	↑ Neutrophil influx ↑ Protein levels in bronchoalveolar lavage fluid ↑ ROS ↑ Inflammatory mediators and signaling molecules Synergistic effects with lipopolysaccharides (LPS)	150
In vivo	Crystalline Si-NP ZnO-NP	Rat	Lung	Inflammation Cytotoxicity Reversible inflammation	136
In vivo	V_2O_5-NP VO_2-NP	Rat	Lung	Cytotoxicity ↑ malondialdehyde (MDA) ↓ GSH Tissue damage and inflammation Carcinogenic potential	140

Carbon nanotubes (CNT)

The toxicity induced by CNT has been described both *in vitro* and *in vivo*. The occurrence of oxidative stress, as a consequence of an impaired antioxidant defense and enhanced free radical production, together with the onset of an inflammatory response, constitute the primary mechanisms of cytotoxicity due to CNT exposure[152,153,154,155,156]. In addition, apoptosis and cell cycle inhibition have been described *in vitro*[157,158]. A common explanation for the observed effects of CNT on cells is their ability to penetrate the cell membrane, which depends on their physicochemical properties[159].

Regarding pulmonary effects of CNT exposure, reports show the development of granulomas, peri-bronchial and interstitial signs of inflammation, fibrosis, and collagen deposition[160,161,162]. Katsuhide and collegues[163] reported that the pulmonary toxicity of SWCNT is closely associated with the size of the bundles, since SWCNT formed as relatively thin bundles with short linear shapes elicited delayed pulmonary inflammation with slower recovery, in contrast with SWCNT with a relatively thick bundle and long linear shapes. Altogether, pulmonary alterations could lead to functional respiratory deficiencies and decreased bacterial clearance[152].

In vitro exposure to MWCNT has been associated with cytotoxicity and inflammasome activation[164], as well as mitochondrial dysfunction [165], while *in vivo* studies show signs of pulmonary inflammation, fibrosis, and even the ability to induce neoplastic progression in initiated cells [151,164,166,167,168].

CNT exposure has also been proposed as a risk factor in the development of atherosclerosis, based on the hypothesis that inhaled NP causes local oxidative stress and inflammation in the lung, and that this response leads to detrimental cardiovascular effects[169,170]. Among other potential consequences of CNT exposure are reproductive and developmental toxicity, however, these fields remain largely understudied. **Table 2** shows a list of studies describing the toxicity mechanism of CNT.

Table 2. Summary of studies describing the toxicity mechanisms of carbon-based NP

Study	Nanoparticle	Model	Samples Evaluated	Effects	References
In vitro	CNT	Rat alveolar macrophages (NR8383), A549		↑ ROS production	153
In vitro	Ground MWCNT	Rat peritoneal macrophages		Inflammation	154
In vitro	CNTs	Human aortic endothelial cells (HAEC)			155

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In vitro	Pristine MWCNT and oxidized MWCNT	T lymphocytes		Apoptosis	158
In vitro	MWCNT	Human epidermal keratinocytes (HEK)		Cell cycle inhibition	157
		Mice monocytes/macrophages (J774.1)		Cell membrane penetration	159
		Human bronchial epithelial cells (BEC)		↑ Mitochondrial gene expression ↓ Oxygen consumption rate ↓ mitochondrial biomass Mitophagy	165
		Mice-derived alveolar macrophages Human acute monocytic leukemia cell line (THP-1)		Cytotoxicity IL-1 ß release	164
In vitro	SWCNT	NR8383		Cell membrane penetration Size-dependant cell growth inhibition ↑ ROS production ↑ CCL3 expression	163
In vivo	SWCNT	Mice	Lung	Peri-bronchial and interstitial inflammation	160
		Rat	Lung	Size-dependant inflammatory response and recovery, ROS production, and gene expression	163
		Mice	Lung	↓ Bacterial clearance	152
		Mice	Lung Aorta Heart	↑ hemeoxygenase-1 (HO-1) Mitochondrial (mt)DNA damage ↑ Protein oxidation ↓ GSH/GSSG ratio	169
		ApoE$^{-/-}$ mice	Aorta	Accelerated plaque formation mtDNA damage	
In vivo	MWCNT	Rat	Lung	Fibrosis and collagen deposition granulomas	161 162
			Liver	↑ aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities. ↓ SOD and glutathione S-transferase (GST) activities ↓ GSH ↑ CAT and glutathione peroxidase (GPx) activities ↑ H_2O_2 ↑ Lipid peroxidation ↑ Micronucleated	156

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				polychromaticerythrocytes (MNPCE) ↑ cyclooxygenase-2 (COX-2) and iNOS protein expressions	
			Blood	↑ IL-1ß, IL-6, TNF-a	
In vivo	MWCNT	Mice	Lung	Pulmonary inflammation Fibrosis	166 168
				Growth and neoplastic progression of initiated cells	167
				Pulmonary inflammation Bronciolocentric inflammation Bronchiolar epithelial hyperplasia and hypertrophy Fibrosis Vascular changes Rare pleural penetration	151
				Inflammation ↑ PMN ↑ IL-18 ↑ Cathepsin B ↑ LDH	164

NP toxicity in biological systems

Oxidative stress and inflammation interplay caused by NP exposure

Following NP exposure, the activation of oxidative stress and inflammatory pathways largely account for NP toxicological effects. The establishment of an oxidative stress situation might elicit several biological stress responses such as redox signaling cascade activation, mitochondrial dysfunction, inflammation, cell cycle arrest and apoptosis[171,172].

Some studies suggest that NP mediated ROS can either induce or mediate the activation of the mitogen-activated protein kinase (MAPK) pathway. The growth factor-regulated extracellular signal-related kinases (ERKs), and the stress-activated MAPKs, c-jun NH2-terminal kinases (JNKs), and p38 MAPKs are involved in the signal transduction mechanism[141,174,175,176,177]. The MAPK pathway regulates a diverse range of cellular responses, ranging from cell proliferation and differentiation to cell death, mainly by triggering apoptosis.

Altered mitochondrial respiration together with enhanced NOX activity might also play a central role after NP exposure. In this sense, NOX activation by inflammatory pathways seems to account for increased ROS production, while mitochondrial dysfunction may also contribute to ROS release leading to oxidative damage and tissue dysfunction[173].

Moreover, an interplay between specific ROS sources has been recognized, where the consequences of the redox crosstalk, mainly between mitochondria and NOX, might enhance ROS production. In this scenario, various ROS sources interaction stimulates each other in a positive feedback fashion, resulting in a complex oxidative stress and redox signaling network[118].

Interestingly, oxidative stress also results in the release of pro-inflammatory mediators through the principal cascades such as the Nuclear Factor-Kb (NF-κB), MAPK and phosphoinositide 3-kinase (PI3-K) pathways, suggesting that oxidative stress is linked to inflammation reciprocally[87]. Both *in vitro* and *in vivo* studies showed

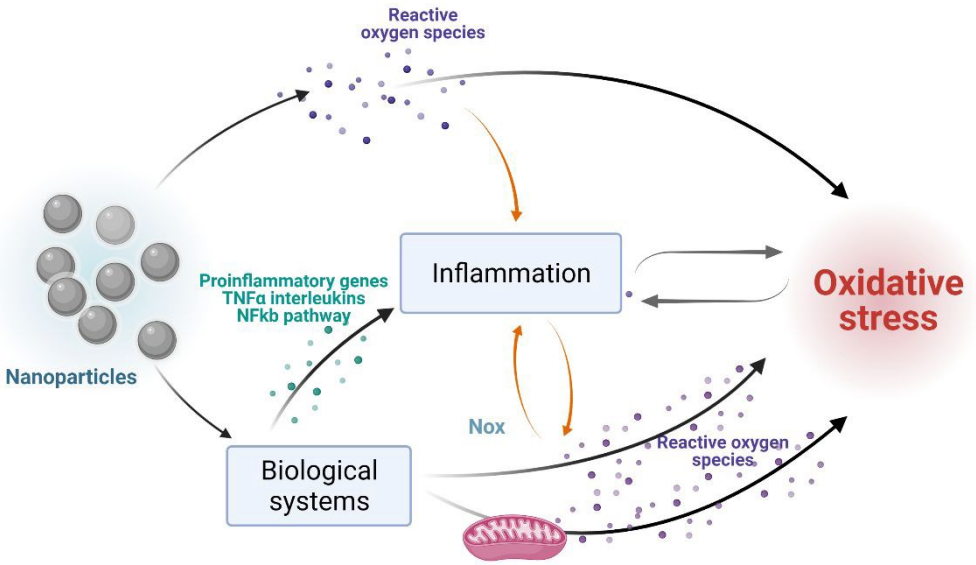

Figure 2. Main factors responsible for oxidative stress induced by NP. The mechanism of NP-induced ROS generation involves NP-related factors and the interaction with cellular components. After being internalized into cells, NP generate ROS upon interaction with mitochondria and NADPH oxidase (NOX). In addition, interaction of NP with cell enhances an inflammatory response through the synthesis of inflammatory mediators such as IL-1ß, IL-6 and TNF-α.

that NP-induced lung injury and pulmonary fibrosis lead to the ROS-mediated activation of NF-κB and production of pro-inflammatory mediators such as TNF-α, IL-8, IL-2, and IL-6, as a consequence of NP recognition by Toll-like receptors. These sequential molecular and cellular events are known to cause oxidative stress, followed by severe cellular genotoxicity, and then programmed cell death[14,86].

In summary, several metal oxide NP, along with SWCNT and MWCNT are known to induce an inflammatory response dependent on the NF-κB pathway, resulting in an elevated secretion of inflammatory mediators. This type of response is often linked to the onset of oxidative stress, leading to DNA damage, cell cycle arrest, and apoptosis. **Figure 2** illustrates the interplay between oxidative stress and inflammation due to NP exposure.

Conclusions

Nanotoxicity has become a growing concern of nanotechnology. Numerous *in vitro* and *in vivo* studies have consistently demonstrated that the redox state is disturbed by ROS production in response to NP, and/or by increasing the levels of pro-inflammatory mediators, resulting in oxidative stress. Taken together, it is important to be able to characterize NP not only in their structures and composition, but also in terms of their interaction with biological systems in order to achieve a better understanding and develop more and better systems taking biosafety into account. For this purpose, it is essential to promote the advancement in the nanotoxicology field, and therefore, limit the associated potential risks to human health that the exposure to NP might cause.

Author Contributions

Mariana Garcés: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Visualization. Lourdes Cáceres: Conceptualization, Writing - Original Draft, Writing - Review & Editing, Visualization. Diego Chiappetta: Writing - Review & Editing, Supervision. Natalia Magnani: Conceptualization, Writing - Review & Editing, Supervision. Pablo Evelson: Conceptualization, Writing - Review & Editing, Project administration.

Conflicts of interest

There are no conflicts to declare.

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