Nanoparticles: nanotoxicity aspects

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Abstract: The giant steps towards Nanosciences dictate the need to gain a broad knowledge about not only beneficial but also noxious properties of Nanomaterials. Apart from the remarkable advantages of Nanoparticles (NPs) in medicine and industry, there have been raised plenty of concerns about their potential adverse effects in living organisms and ecosystems as well. Without a doubt, it is of critical importance to ensure that NPs medical and industrial applications are accompanied by the essential safety so that the balance will be tilted in favor of the profits that society will earn.

However, the evaluation of NPs toxic effects remains a great challenge for the scientific community due to the wealth of factors that Nanotoxicity depends on. Size, surface area, dosing, shape, surface coating and charge and bulk material are the basic parameters under investigation to assess the risk involved in NPs usage. Our purpose is to highlight NPs physical and chemical properties responsible for induced toxicity, describe the mechanisms that take place in their interaction with cells and organs and finally report the potential harmful consequences that may result from the innovative applications of Nanomaterials.

1. Introduction to nanotoxicity
Over the last decade, a plethora of scientists deals with the estimation of Nanoparticles (NPs) adverse effects. Nanotoxicology is referred as the science that investigates the mechanisms of interaction between NPs and humans, as well as the possible caused pathogenies. The basic obstacle in this specific field is the great variation in NPs characteristics and properties. Numerous \textit{in vivo}, \textit{in vitro} and \textit{in situ} models have been used in experiments attempting nanotoxicity levels estimation. The dependence of NPs biodistribution and uptake upon their physicochemical properties such as size, surface chemistry, dosing, shape, surface coating and charge and bulk material, demonstrates the difficulties lying in nanotoxicity precise determination. Our purpose is to highlight the potential mechanisms of nanotoxicity, the entrances of NPs to human organisms along with the nano-characteristics that are responsible for any adverse effects.

2. Mechanism of NPs toxicity
NPs owe their tremendous popularity to their small size. This specific feature increases the possibility for NPs to enter the organisms and cells without being trapped under the defense mechanisms of human body (e.g. skin mucosa, pulmonary epithelium, macrophages etc). Without a doubt, the presence and severity of any toxic effect apart from each NPs properties, strongly depends on cells and organisms characteristics and a variety of environmental conditions as well.

However, the most common effect arising from NPs – cells interaction is the overproduction of Reactive Oxygen Species (ROS), such as Hydroxyl radical (\(\cdot\)OH), Singlet oxygen (\(\cdot\)O), Superoxide anion (\(\cdot\)O\(_2\)), Hydrogen peroxide (H\(_2\)O\(_2\)), Hypoclorious acid (HOCI) etc [1]. It should be noted that ROS generation is a physiological procedure resulting from normal cell functions or organisms’ exposure to radiation, drugs, air pollution etc. Under normal circumstances, humans’ defense mechanisms activate a variety of antioxidants to catalyze the oxidative explosion that occurs (superoxide dismutase, catalase, glutathione Peroxidase etc).

The activation of macrophages and neutrophils once they recognize NPs as a potential threat [2] and mitochondrial disruption [3] are strongly related to ROS outburst. Still, there are various procedures underlying ROS over – production depending on NPs surface characteristics [4, 5], or their material (e.g. Fenton type and Haber – Weiss reactions in case of metal NPs [5]). Along with ROS, the oxidative stress generated subsequently has been proved to be severely noxious for cells and organs. Human and rodents cell apoptosis, lung and cardiovascular inflammation and DNA damages are only some of the adverse effects of different NPs attributed to ROS production [6].

3. Nanoparticles uptake

The manifold results of NPs usage in medicine are based on their ability to skip the human defense system and accumulate in specific cells and organs. Depending on the route of administration and their physicochemical properties, NPs can overpass several human protective barriers, such as the dermal stratum corneum, the bronchial epithelium, lung and gastric mucociliary system etc. Assuming that macrophages are not able to destroy or subject to clearance nano structures that invade human body, absorption from any tissues and organs is likely to happen. As a matter of fact, liver and spleen due to their permeable blood vessels seem quite appealing targets to NPs. Jani et al. report a spleen, blood, liver and bone marrow accumulation of a 7% and 4% of 50 nm and 100 nm polystyrene particles respectively in rats experiments. Increasing size beyond 100 nm prevented bone marrow absorption whereas NPs larger than 300 nm have not been found in blood [7].

3.1 Skin absorption

The dermal application of substances that contain NPs such as drugs, creams, cosmetics, toothpastes, sunscreens etc, makes skin a major entrance of NPs into living organisms. Damaged skin, hair follicles and direct absorption from corneocytes are potential ways of NPs transport into deeper skin layers. Irritated skin, allergies or other dermal pathogenies are among possible effects arising from NPs absorption. However, the severity of adverse effects as well as the depth of NPs penetration in skin is a matter of NPs dosage, physicochemical properties, time of exposure etc [8]. It seems that NPs smaller than 4 nm are able to penetrate and permeate unharmed skin, while larger ones can only penetrate into damaged skin, leaving NPs larger than 45 nm unable to penetrate either on damaged or undamaged skin [9].

3.2 Lung uptake

Inhalation is the major process through which NPs could enter human organisms. The toxic effects of airborne NPs are normally controlled by the bronchial epithelium and the mucociliary system which is apparent in lungs. However, in favor of their small size, NPs could defeat the security respiratory guards and enter the systemic circulation. In this case, depending on their concentration and physicochemical properties, NPs could cause various lung diseases such as asthma, emphysema or even lung cancer. The translocation of NPs from lung in other organs such as bone marrow, lymph node, brain or heart is likely to happen [10], a phenomenon which is responsible for more severe diseases including Alzheimer, Parkinson or cardiac malignances [11].
3.3 Gastrointestinal route
Clearance of inhaled NPs through lung mucosa, direct ingestion of nanopolluted food and water, drugs and hand to mouth contact are the potential routes for NPs to access gastrointestinal tract. NPs could be found in stomach and surrounding tissues as a result of epithelium cells and the gastric mucosa overcome [10]. Oxidative stress induced by TiO$_2$ ingestion, appears to lead in digestive gland cell membrane injuries [12]. Apart from epithelium, gland cell membrane, liver and kidney injuries, Crohn’s disease and colon cancer are also associated with gastrointestinal absorption.

4. Factors affecting nanotoxicity
The wide variety in experimental protocols and simulations in literature conclude in the same basic finding: similarly to NPs kinetics and clearance, nanotoxicity levels strongly depend on a mixture of factors. It is impossible to conclude in safe results, as the comparison of studies could not be accurate since there are altered parameters in each one. Here follow some of the different aspects of NPs nature that are under investigation in scientific community.

4.1 Size, shape and surface area characteristics
The innovation in NPs usage in medical applications is basically attributed in their extra small size. Undoubtedly, their ability to overcome the human defense mechanisms and accumulate to particular structures of interest is their most appealing property promising enhanced results in medical imaging and therapeutical applications. However, it is the same property that leads to increased deleterious effects, since smaller size implies greater surface area and greater area to volume ratio per given mass. Therefore, smaller NPs are accompanied with increased biological reactivity as a result of the increased number of bulk material molecules they consist of. Indeed, size dependent cytotoxicity of Ag NPs in vitro [13] and in vivo as well [14] has been experimentally verified. Different sized TiO$_2$ NPs have been shown to pose different levels of toxicity in rats pulmonary system [15], while ultrafine inhaled NPs which mainly accumulate in the alveoli region than the upper lung airways, seem more deleterious than larger ones due to their clearance complexity [16].

Numerous in vitro and in vivo studies in literature report the impact of NPs shape in cellular uptake[17]. It seems that cells are more accessible to rod - like NPs compared to cylindrical [18] as well as needle - like NPs [19] compared to other forms of NPs while ZnO nanorods have been found to induce increased toxicity in lung epithelium compared to spherical [20].

NPs biokinetics, cell absorption and consequently nanotoxicity too are closely associated with NPs coating agent [21]. Generation of ROS [22] and surface radicals [23] are coating dependent and so is the induced toxicity. It is suggested that uncoated Ag NPs induce greater toxicity than coated ones [24], while the same applies for carboxyl coated Iron NPs compared to amine coated [25].

Surface charge has been confirmed to affect NPs toxicity as well. The negative charged plasma membrane demonstrates the enhanced probability of cells with positive charged NPs. In various experiments, toxic effects of positive charged NPs have been proved greater than these of negative or neutral [21].

4.2 NPs material
Although the majority of NPs is linked to toxic effects, it is difficult to come up with a quantitative evaluation, as experiments through literature use NPs of different properties, sizes and concentrations. In terms of comparison, experiments in embryonic zebrafish and bacteria respectively have reported a descending order of toxicity evoked by CuO - ZnO - CO$_2$O$_4$ - TiO$_2$ NPs [26] and CuO - ZnO - NiO - Sn$_2$O$_3$ [27]. Silver (Ag) NPs appear more toxic than gold (Au) [28] and the same stands for Single Walled Carbon Nanotubes (SWCNT) compared to Multi Walled (MWCNT) [29] and Carbon Black NPs [30].
4.2.1 Non-metallic NPs. Over the last years, various biocompatible polymeric coatings such as BSA, chitosan and PLGA have been developed in order to achieve safe cancerous cells nano - targeting with minimized toxic effects [31]. Indeed polymeric based nanosystems have been observed to be the least toxic choice in nanomedical applications [32].

Dose dependent toxic effects from Si NPs applications have been reported in literature attributed to ROS and oxidative stress increase [1]. In fact, recent studies emphasize the influence of Si NPs dosage and size to the toxicity caused in different cell lines [33, 34].

Concerning Carbon NPs, SWCNT and MWCNT seem to induce ROS and oxidative stress controlled by their size [1] and concentration [6]. Alveoli macrophage cell apoptosis and necrosis which could lead to lung cancer, asbestosis [35] and interstitial granuloma [36] have been experimentally confirmed. Surface chemistry dependent cell death has been also been reported [37]. The translocation of CNPs on central nervous system [38] in addition to reported keratinocytes cytotoxicity [39] are some issues that should be thoroughly investigated in terms of toxicity, as proper CNPs functionalization could probably minimize the potential adverse effects caused by their handling and applications.

4.2.2 Metallic NPs. The biocompatibility of Au NPs, their image contrast properties along with the effectiveness in tumor targeting make them the most appealing particles in targeted cancer therapy and radiotherapy enhancement. Although in vitro results from experiments using gold nanospheres of different characteristics in leukemia and dendritic cell lines [40], keratinocytes [41] and macrophages [42], report no significant toxicity, it is insecure to predict the same results for in vivo experimental models. Indeed, Au NPs dosage [32], size [43], or even route of administration [44] seem to play a vital role in Au toxicity assessment. In vivo experiments by Wan-Seob Cho et al. demonstrate inflammation and apoptosis on liver caused by PEG Au NPs [45], while citrate capped of 8 – 37 nm Au NPs had severe results in mice tested in contradiction to HeLa cell lines, where no toxic effect was reported [46].

DNA damage, oxidative stress, mitochondrial dysfunction, and cell death are some of the reported side effects from Ag NPs application. Size and shape seem to play a vital role in mitochondrial damages and ROS levels [47]. A study on pig skin cells exposed to Ag NPs in a concentration of 34 mg/ml reveals focal inflammation and edema [48] while human skin carcinoma cell apoptosis has been observed too [49]. Brain accumulation of inhaled Ag NPs in rats has been also confirmed [50]. It should be underlined, that even Ag NPs exist on various commercially available products, no toxic effect seem to be caused due to our daily contact with them [51].

4.2.3 Metal Oxide NPs. Metal oxide NPs possible applications in medicine and the prospective toxicity are under detailed research. Increased ROS levels [52] and DNA damages [53] are among TiO$_2$ NPs adverse effects. In vivo experiments in mice with inhaled TiO$_2$ NPs in sizes ranging from 2 – 5 nm report a dose dependent lung inflammation [54], while a 3 months intragastric administration seems to cause damages to mice reproductive system [55]. Even a single dose of intratracheal injected TiO$_2$ NPs appears to cause pulmonary disorders in mice [56] and rats too [57].

ZnO NPs cytotoxicity [58] and genotoxicity [59] have been confirmed in vitro, while liver, kidney and lung inflammation have also been observed in mice experiments [59]. A qualitative and quantitative effect on Wistar rats sperm has been also experimentally observed [60] and additionally it seems that ZnO NPs could lead to ultimate human and rodent cell death, depending on the administrated dosage [61].

Superparamagnetic NPs (SPIONS) toxicity is strongly related to their coating [62]. Berry et al. have observed cell death when uncoated or dextran – coated NPs have been applied in contrary to albumin ones [63], while applying polyvinyl (PVA) coating could possibly reduce the caused toxicity [64]. Inhaled Iron Oxide NPs, apart from lungs they have been found to accumulate in liver, spleen and cross the BBB [65] and intravenous SPIONS injection in mice appears to induce liver and kidney damages [66].
5. Conclusions

It is clear from literature studies that almost all NPs accordingly to their properties and concentration are linked to toxic effects of different extent. The non uniform experimental practices result in conflicting conclusions, a fact that complicates the categorization of the exact mechanisms and NPs features and materials that are responsible for obtained toxicities. Yet, the scientific community should dedicate attention to establish and validate well designed and reproductive protocols for toxicity examination, in order to accurately determine the potential threats arising from NPs applications.

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