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Editorial Open Access

The Involvement of Nano-Drug Delivery in Biosafety Issues

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OMICS Publishing Group is an Open Access publication model that enables the dissemination of research articles to the global community. The special features offered by OMICS Group Journals, such as Digital Articles, Audio Version, and Language Translation Social networking are of paramount importance to connect worldwide researchers within the nanomedical field. Hopefully, the Open Access Journal of Biosafety will contribute to improve the public health that depends on safety issues yielded from basic and applied research and on accurate interpretations made on regulatory guidances.

Nanotechnology encompasses a broad conjunct of techniques aimed to engineer, characterize and make use of structures of 1 (nanoplates), 2 (nanotubes) or 3 dimensions (nanoparticles) in the nanoscale, known as nano-objects. The upper limit of the nanoscale was fixed at 100 nm [1], but in the nanopharmaceutical field the nano-scale is accepted to rise up to 200-300 nm. Biosynthesized molecules (such as hormones, proteins, nucleic acids) and drugs, whose activity depends on a primary structure and not on new phenomena derived from its size in the nano-scale, do not fit into the definition of nano-object [2]. Also the lower limit of the nanoscale was fixed in 1 nm in order to exclude atoms [2]. Beyond these constraints, there is no restriction in chemical nature of nano-objects. Today, the global market of nanotechnological consumer product is gained by non biodegradable and mostly non-dispersive nano-objects. This is underscored by the raise from 212 to 1317 products (nearly 521%) between 2006 and 2011 [3]. On the other hand, Nanomedicine is the emerging discipline that employs nano-objects as tools to solve medical problems [4,5]. The volume market of Nanomedicine is expected to exceed $160 billion by 2015, according to a business report recently launched by the Global Industry Analysts Inc [6]. The main technological platform of Nanomedicine is nano-drug delivery, accounting for 78 % global sales and 58 % of patent filling worldwide [7,8] followed by development of nano-objects for *in vitro*/*in vivo* diagnosis [9] and tissue engineering [10]. The field is characterized by the advent of a different type of nano-objects, inherently dispersive or 'free'.

A survey of pre-clinical and clinical nanomedical developments allows identifying two groups of nano-objects. One group comprises nanoparticles made of metals (gold, silver, cupper), metallic oxides (titanium, zinc, cerium, iron), ceramics, semiconductors nanocrystals known as quantum dots (QD) (cadmiun selenide, cadmium sulphide, zinc sulphide, cadmium telluride, indium phosphide, and indium arsenide) and carbon-based nanotubes (CNT) and fullerens. These nano-objects typically differ from bulk material by manifesting changes in at least one of the following features: fluorescence (eg QD), color (localized surface plasmon resonance of Au and Ag nanoparticles); electronics, thermal and mechanics properties (metallicit/semiconducting; specific heat, thermal conductivity and thermo power; young modulus of carbon nanotubes), as well as chemical reactivity (metallic nanoparticles). Biodegradability is the breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo* [11]. Most of these nano-objects are non-biodegradable, biodurable and / or biopersistent. Some of them possess cores that dissolve, releasing intrinsically toxic ions when their capping and hydrosoluble envelope is destabilized. The second group comprises those prepared by self association of drug or lipids, or made of polymers such as poly(esters) polylactide, polyglycolide, polycaprolactone; poly(hydroxyalkanoate)s and their blocks copolymers; poly(ethylene glycol); starch; cellulose and chitosan. In this group, the presence of new physical or chemical phenomena because of their size in the nano-scale is almost absent. Most of them are biodegradable.

For a given mass of particles, as the diameter of the particles is reduced, the number of particles increases exponentially and the surface- to-volume ratio increases linearly. Because of this, nano-objects possess a large surface per unit mass. A typical example are the hollow mesoporous silica nanoparticles with an average pore diameter of about 2 nm and a surface area of 880 m^2/g. Nano-objects of the first group such as 10-50 nm diameter nanoparticles and nanotubes of high aspect ratio have maximal potential for surface phenomena such as redox catalysis and/or to establish attractive interactions. Excluding the dendrimers, these phenomena are minimal for the second group of nano-objects with sizes between 80-200 nm.

Nanoparticles possess heterogeneous shape and size. In the lower limit of the nano-scale, dendrimers and quantum dots possess hydrodynamic diameters in the order of the globular proteins (4-7 nm). In the opposite side, targeted pegylated liposomes share diameters (between 100-200 nm) and structural complexity with virus [12]. In fact nano-objects and microorganisms are both particulate matter. This is why a number of containment measures for safe handling of nano-objects such as the use of HEPA filters, follows the practices of classical biosafety. However, biosafety is the discipline addressing the safe handling and containment of infectious micro organisms and hazardous biological materials, in contained laboratory settings to minimize risks to human health and the environment [13]. Its principles arose from Microbiology and were launched to impair the penetration of infectious/virulent agents across primary/secondary barriers after dermal/inhalatory exposition. According to that, infectious agents are divided in four risk groups that correlate with four biosafety levels. But nano-objects differ from micro organisms in being unable to replicate. Nano-objects are neither infectious agents nor fit the definition of biohazard. Nonetheless the exposition to certain nano-objects can be harmful. Different to classical biohazards, when evaluating the harmful effects of nano-objects, data on production method, structural features and material biodegradability/biopersistence come into play. Besides, their harmful effects has to be evaluated in the absence of the quantitative data needed to define an occupational exposition level (OEL) [14]. In such cases, the pharmaceutical industry assigns biological active entities, such as pharmaceuticals and

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infectious agents, into one of five occupational hazard bands using available toxicological information [15,16]. Similarly, according to their anticipated degree of hazard, a "control banding" approach is performed for a qualitative risk assessment of nano-objects. In general, control banding means a process in which a single control technology (such as general ventilation or containment) is applied to one range or band of exposures to a chemical (such as 1-10 mg/m^3) that falls within a given hazard group (such as skin and eye irritants, or severely irritating and corrosive) [14,17,18]. The Stoffenmanager Nano (version 1.0) is a recently published risk-banding tool developed for employers and employees to prioritize health risks occurring as a result of exposure to inhaled manufactured nano-objects [19] .

Undoubtedly scientists, private partners and governments from developed countries are leading the current nanomedical I+D+i worldwide scenario. In this context, the word nanotechnology often exclusively recalls on characteristics from the first group of nano-objects. However, less familiarized stakeholders from developing countries, could find challenging to cope with the welter of documentation on safety issues [20]. Moreover, most of the guidelines for biosafe handling of nano-objects in occupational settings do not reflect the sharp difference between the first and the second groups of nano-objects.

To properly conduct risk assessment on nano-objects, their harmful effects on living beings have to be determined [21,22]. Today a number of analytical tools can be used to quantify and correlate structural features of nano-objects with induced *in vitro* oxidative stress, type of cell death (necrosis/apoptosis), genotoxicity, and intracellular traffic [23], as well as pre-clinical studies of pharmacokinetics, biodistribution and dose-related toxicity [24]. However, the simple quantification of cytotoxicity/toxicity is useless to predict the risk of a non intentional exposition in work settings. The ability of nano-objects to penetrate / damage epithelial barriers of lungs and skin are the *in vitro* assays to be intended in first place for a realistic risk assessment. Remarkably, data has to be interpreted according to the following considerations:

1) The cytotoxicity/toxicity of nano-objects can not be extrapolated from toxicity data for the bulk material. Amongst the main differences with the bulk phase material, nanoparticles/nanotubes of the first group exhibit increased chemical reactivity (solubility, acidity), differ in surface chemistry, possess particular core chemistry, and present contaminating metals. Such differences become wider as the diameter of the nanoparticle falls below the 100 nm. Because of this, not only size distribution and shape, but number and exposed area of nano-objects per mass or volume, together with data on the features specified above, has to be informed to asses the effect of a given dose of the first group of nano-objects. On the contrary, doses of the second group of nano-objects can be suitably assessed on the bases of mass concentration plus size distribution.

2) Cells and nano-objects interact in a unique fashion, illustrated by the highly regulated endocytic mechanisms employed by cells to recognize and take up nano-objects [25,26]. This is the main reason why non-biodegradable but biocompatible (the ability of a material to perform with an appropriate host response in a specific application) bulk materials can become toxic and non-biocompatible when reduced to the nano scale. Bulk material can not be taken up by cells. However, if a bulk material is reduced to a 10 μm particle, it could be phagocytosed by accessible macrophages; if it is reduced to a particle of less than 200-300 nm, the resultant nano-object could be pinocytosed by most of the accessible cells. Afterwards, the intracellular pathway followed by the nano-object will mostly depend on its size, shape and surface nature. The outcome of the intracellular processing will depend on its biodegradability and the chemical nature of metabolites, leading to different degrees of toxicity. Nano-objects made of material containing C-C backbone, such as CNT are not biodegradable. There are other nanoparticles that are not biodegradable such as ceramics and quantum dots, but CNT are also biopersistent. Biopersistence is defined as the ability of a fiber to remain in the lung in spite of the lung's physiological clearance mechanisms. These defense mechanisms are a) transportation of entire particles by the mucociliary escalator and by alveolar macrophages, b) dissolution of fibers, and c) disintegration [27]. Bioerosion is defined as the conversion of a material that is insoluble in water into one that is water-soluble [28]. Biodurability includes only the removal of fibers from lungs by dissolution and disintegration [27]. Note that biodegradable/(bioderodible) and non biopersistent nano-objects are not excluded from being highly toxic, according to the route of exposition and the dose [4].

3) Nano-objects may exhibit potential interference issues with standard cytotoxicity assays [29]. For instance dendrimers were reported to interfere with endotoxin test (Limulus Amebocyte Lysate) causing false positive results [30]. The large surface per unit mass *of* fullerens and CNT is responsible for their high adsorption capacity of proteins, and/or of contaminant metals (predominantly Fe, Ni, Co). Consequently their effective size, charge and behavior will vary according to the set of adsorbed material. Hence, these nano-objects could confound cytotoxicity data by inducing indirect effects through the adsorption of nutrients and growth factors from culture media. The excess surface energy of CNT, metal oxide, and silica nano-objects, which is size-dependent, enhances their catalytic activity. Hence, redox-active nano-objects such as TiO_2, ZnO and single wall CNT may cause false signals in assays based on substrate oxidation. Metallic nano-objects, QD or nanoshells, can absorb and emit light of different wavelengths, and might distort the signal intensity in assays with an optical readout, which is the case for most of the commonly used cytotoxicity method. Superparamagnetism of Fe_2O_3 generates strong, local magnetic fields which lead to the production of free radicals that in turn may interfere with cytotoxicity methods based on redox reactions. Metallic nano-objects that dissolve in aqueous solutions, will release metal ions or trace metals when introduced into biological media. Cytotoxicity assays that are sensitive to metal ions may therefore be perturbed in the presence of dissolving nanoparticles. None of these interferences are manifested by most of the lipid/polymer based biodegradable nano-objects. On the contrary, testing the cytotoxicity of metallic/non-biodegradable nano-objects may require of novel technologies different from classical MTT production and LDH release assays [31].

4) The toxicity of nano-objects depends on the route of exposition. Once released into air, nano-objects remain airborne for considerable periods of time. By inhalation (the primary exposure route to the human body for nano-objects) [32], volatile nanoparticles/nanotubes gain access to the deep alveolar epithelium region of the lungs, an extremely thin barrier (<0.5 μm) of vast surface area (> 100 m^2) [33]. Hence *in vitro* realistic assays for risk assessment would be performed on polarized epithelial cell lines, preferably avoiding the use of suspension exposures [34]. For instance, triple cell co-culture model (human bronquial epithelial cell line 16HBE14o, human blood monocyte-derived macrophages and dendritic cells), that mimics the airway epithelial barrier is preferred over conventional monocultures [35]. In second place, non intended exposition can occur by skin contact or swallowing after inhalation and in order to test penetration reconstituted tridimensional skin models (such as EpiDerm), or

enterocyte-like Caco-2 and mucus-secreting MTX-E12 could be used, respectively [36].

On the other hand, it is only after intravenous administration that nano-objects come in contact with blood cells and plasma proteins [37,38]. In this environment, cells can recognize specific surface features of nano-objects, in a manner similar to pathogenic microorganisms. This can lead to acute reactivity such as complement activation [39,40]. This phenomenon however, can take place after intravenous administration at determined dosages but not in occupational settings after inhalation or skin contact [41,42].

5) The production method and the size/ physical aggregation state play a major role when evaluating potential toxicities of nano-objects [43]. Although nanoparticles are readily collected by HEPA filters [17], the most penetrating particle size for respirators equipped with commonly used electrostatic filter media remains in the range of 30–100 nm [43]. Because of this nanoparticles/nanotubes produced by techniques involving massive dispersion in the gas phase, or nanostructured powders, in volatile liquid formulations and dusty dry formulations requires of contention measures to impair their inhalation, and in a lesser extent, swallowing or skin contact. For instance laser pyrolisis (gas phase) synthesized supermagnetic iron oxide nanoparticles (SPION), used as contrast agents for magnetic resonance imaging, but are classified as biocompatible and biodegradable, showing no severe toxic effects *in vitro* or *in vivo* [44]. On the other hand, air dispersed, intrinsically toxic and biopersistent nano-objects maximize their chances of penetration across epithelial barriers in unwanted contact and their potential hazard increases during manufacture. Chemical aerosol flow [45] / laser pyrolysis synthesized QD, can expose their toxic core after mechanical or physical stress destabilization. They can be hazardous during manufacture, but later non intended contact with QD is poorly harmful if the commercial presentation is dispersed in aqueous buffer. Besides, after skin contact, QD are trapped within the stratum corneum, and are removed without entering the dermis [46]. On the other hand, CNT can be synthesized by electric arc discharge, laser evaporation or chemical vapor deposition. CNT are hollow structures, insistently presented as suitable alternative to well established drug nano-carriers such as the biodegradable nanovesicles of the second group. However, today there is no consensus on the CNT toxicity. The absence of data confirming their safety and improved therapeutic efficacy over liposomes for instance, hampers its full acceptance by the nano-drug delivery field [47,48]. Moreover, available data suggests that CNT pose threats for general manipulation and non intended exposition. Effectively, unpurified CNTs can cross the stratum corneum and after accessing the mice skin dermis, cause oxidative stress, depletion of glutathione, increased dermal cell number, localized alopecia and skin thickening [49,50]. Once taken up by cells, CNT can cause oxidative stress, chronic inflammation, and apoptotic death [51]. In general terms, oxidative stress induced by exposure to biopersistent nano-objects of the first group may stimulate an increase of the cytosolic calcium concentration [52] or may cause the translocation of transcription factors (e.g., NF- B) to the nucleus, which regulate pro-inflammatory genes, such as TNF-α and iNOS [53]. These nano-objects may exert pro inflammatory effects and induce the cell's apoptosis through a reactive oxygen species mediated mechanism, often mediated by glutathione depletion [52,54]. Alternatively, exceeding oxidative stress may also modify proteins, lipids and nucleic acids, which further stimulates the anti-oxidant defense system or even leads to cell death [55]. CNT toxicity may be dependent upon the metal (particularly iron) content. Metals may interact with the skin, initiate oxidative stress, and induce redox sensitive transcription factors thereby affecting/leading to inflammation. However pristine, non-functionalized CNT are biopersistent and exert pronounced pathogenic effects in animal models, with induction of oxidative stress, inflammation, fibrosis and mutagenic effects. CNT present the phenomena of nanopenetration, an energy-independent passive process, where the nanotubes diffuse across the cellular membrane [56]. Nanopenetration enables the uncontrolled distribution of CNT within the body. If inhaled, CNT are not cleared by the mucocilliary escalator; only a few will be removed by alveolar macrophages and most of them will be taken up by the alveolar epithelium [57]. After that, their translocation or displacement to organs distant from the point of penetration, can take place. Translocation occurs along weeks and months, and it has been reported only for biopersistent material. In general inhalation of CNT or of similar biopersistent nano-objects of the first group can affect places distant from the respiratory system, such as the cardiovascular and/or immune systems, to potentially accumulate in the nervous system. In sum, *in vitro* and pre-clinical tests on the first group of nano-objects suggest the appearance of health risks in intentional exposition. Their industrial manufacture has to be under close security measures regulated by the Toxic Substances Control Act (TSCA), enforced by the Environmental Protection Agency (EPA). Further exposition is supervised by the Department of Labor through the Occupational Safety and Health Administration (OSHA), and the National Institute for Occupational Safety and Health (NIOSH). NIOSH is the leading federal agency providing guidance and conducting research on the occupational safety and health implications and applications of nanotechnology in USA. Currently, excepting the dendrimers, the second group of nano-objects is not under surveillance of TSCA/NIOSH.

On the other hand, nano-objects from the second group, namely nanoparticles and nano-vesicles such as liposomes and niosomes; solid lipid nanoparticles, nanocapsules and nanospheres are already accepted by the cosmetic industry [58,59]. More importantly, these plus plain, targeted, sterically stabilized vesicles, micelles and polymeric micelles (co-polymer based micelles, e.g. pluronic F127), polymer nanoparticles (chitosan-based), nanocrystals (made of sirolimus, aprepitant, fenofibrate, megestrol acetate) are entering the pharmaceutical industry to Increase dissolution velocity and saturation solubility, to modify bioavailability, pharmacokinetics, biodistribution and intracellular traffic of drugs loaded to their structure. Together with a good therapeutic/cosmetic performance, biodegradable and poorly biopersistent nano-objects of the second group reduce consistently the potential harmful in intended expositions. Their scaling up is done on the bases on aqueous suspensions. None of them cross primary barriers of contention such as splash shields, face protection, gloves, and lab coats. When in contact with skin or if inhaled, they remain at the site of contact (epithelial barriers) and do not traslocate. Therefore, the manufacture and further handling of these nano-objects would not pose a threat for workers, excepting if loaded with hazardous (mostly anti-neoplasic) drugs. Nonetheless, the absence of risks in the work place does not exclude deadly acute toxicities during therapeutic use.

The reasons why the pharmacy and cosmetic industry have already accepted the second group of nano-objects have been outlined here. Different to the first one, the second group is under regulation of the governmental body Food and Drug Administration (FDA) through the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER), and the Center for Devices and Radiological Health (CDRH) en EUA. Up to now, current advanced pre-clinical and clinical trials [60] suggest that first group of

nano-objects is far for being under the regulatory acceptances of the drug delivery field [61-64].

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