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# Inorganic nanoparticles and biology

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**Resum**

Biocompatibilitat, biodistribució, biodegradació, inflamació i interferència amb el funcionament normal de les cèl·lules i teixits, entre d'altres, determinarà la toxicitat de les nanopartícules inorgàniques i nanoestructures de carbó, i per tant l'extensió del seu us. Exemples recents a la literatura científica mostren que les nanopartícules inorgàniques i els nanotubs de carboni no causen efectes tòxics aguts. De totes maneres, la interacció d'aquests materials amb organismes vius pot pertorbar la seva activitat normal induint funcionaments erronis i malalties. De fet, les interaccions entre nanopartícules i biologia que s'han observat, que poden ser usades per detectar i manipular estats biològics i contribuir a una millor diagnosis i teràpia, també podrien tenir un impacte negatiu en la salut i el medi ambient si s'alliberen incontroladament quantitats massives d'alguns tipus de nanopartícules, abans que es faci una avaluació exhaustiva dels riscos potencials d'aquests nous materials. La pregunta clau és si els riscos desconeguts de les nanopartícules, en particular el seu impacte en la salut i el medi ambient, supera els beneficis d'aquesta tecnologia en societat. Per això, pel futur desenvolupament de la nanotecnologia, l'avaluació de la seva potencial toxicitat es clau.

Paraules clau: nanopartícules · toxicologia · biodistribució

**Abstract**

Biocompatibility, biodistribution, biodegradation, inflammation and interference with the normal functioning of cells and tissues are some of the features that determine the toxicity of engineered inorganic nanoparticles and carbon nanostructures, and therefore the potential extent of their use. Recent examples in the literature show that engineered inorganic nanoparticles and carbon nanostructures do not normally cause acute toxic effects. However, their interaction with living organisms may disrupt normal activity leading to disordersand disease. Nanoparticle-organism interactions, which can be used to detect and manipulate biological states and to heal damaged organs in an environment controlled by specialists, as in clinical cases, could lead to environmental and human health hazards if nanoparticles are released prior to adequate risk assessment and without proper controls. The central question is whether the unknown risks of engineered nanoparticles, in particular their impact on health and the environment, outweigh their established benefits for society. Therefore, to accurately evaluate the utility of these materials it is necessary to assess their potential toxicity.

Keywords: nanoparticles · toxicology · biodistribution

## 1. Nanoparticles. New species in contact with biological systems

As society begins to use nanomaterials in greater quantities and in consumer products, interest in the broader implications of this emerging technology has grown together with unfounded "nanoeuphoria" and "nanoscares". The central question is whether the unknown risks of engineered nanoparticles (NP), in particular their impact on health and the environment, outweigh their established benefits for society [1]. Therefore, for any application and future developments, a key issue is to accurately evaluate the utility of these materials and it is necessary to assess their potential toxicity —whether due to their inherent chemical composition (e.g., reactive metals [2]), their physical size (e.g., Au55 attaching to DNA [3]), their large and accessible inorganic surfaces (e.g., TiO2 NP versus microparticles [4]) or as a consequence of their particular nanoscale characteristics (e.g., carbon nanotubes that have reached the lungs appear significantly more toxic than carbon black and graphite [5,6]). While there is a significant body of research on the effects of natural and incidental NP —those that occur as unintentional byproducts of other processes, such as combus-

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tion— only a few engineered nanomaterials have been studied in this way. In fact, some incidental NP are central to many natural processes, from marine aerosols [7] to volcanoes and forest fires [8-12], and they do not have a great effect on health [13]. Thus, it has been observed that nanomaterials, including fullerenes, are produced naturally in combustion processes, while burning paraffin and diesel produces carbon nanotubes (CNT) [14]. Nanomaterials can also be found perfectly integrated into biological structures. For example, biogenic magnetic NP occur naturally in many organisms ranging from bacteria through protozoa to animals [15,16]. A biological model of coated nanomaterials also found in humans is ferritin, which is an iron storage protein, approximately 12-nm in diameter, that contains 5- to 7-nm-sized hydrous ferric oxide inside a protective protein shell [17,18]. Obvious differences between natural, unintentional NP and intentional, anthropogenic NP are: i) the polydisperse and chemically complex nature of the former [19,20] in contrast to the monodisperse and precise chemically engineered characteristics of the latter, and ii) particle morphology (often a branched structure from combustion particles versus spherical forms of engineered NPs, although other shapes, such as tubes, wires, rings and disks, are also manufactured). Despite these differences, the same toxicological principles are likely to apply for both types of NP.

If nanomaterials have received enormous attention it is because of their potential interaction with living systems [21,22]. This gives rise to potential applications in biology and medicine, due to their ability to detect the state of biological systems and living organisms optically, electrically and magnetically, thanks to recent developments in materials physics and chemistry [23]. Thus, NP can be designed with different properties, such as fluorescence or possessing a magnetic moment [24,25], and these properties can be harnessed and used as local nanoprobes or nano-manipulators in biological and medical applications (e.g.: fluorescence labeling of cellular compartments [24]; the use of fluorescent or magnetic particles as contrast agents; magnetic separation [26]; and targeted drug delivery [27]). Derivatization of NP with biological molecules has successfully been applied in materials science and biological research in recent years. Conjugates of NP biopolymers (like proteins or DNA) show great promise in both fields: biological diagnostics, where NP can provide unique detection signatures; and nanotechnology, where the information content of biomolecules can be harnessed for the spatial patterning of NP. There are many strategies available for bioconjugation of NP, including attachment to elastin [28], antisenses [29], biotin-avidin [24], antigen-antibodies [30], peptides [31], proteins [32], etc.

Thus, the characteristic biokinetic behavior of NP promises applications in diagnostic and therapeutic devices, and in tools to investigate and understand molecular processes and structures in living cells [33-36]. However, precisely this unique biokinetic behavior of NPs (cellular endocytosis, transcytosis, neuronal, lymphatic and circulatory translocation and distribution, etc.) which makes them so attractive for medical applications, may be associated with potential toxicity. Not only bacteria, viruses and parasites, but also inorganic foreign bodies can be the cause of various pathologies: silicosis, asbestosis and inflammatory reactions to the debris from worn out prostheses or related to diesel exhaust fumes [37] are only a few among many possible examples. Thus, for example, NP-facilitated drug delivery to the central nervous system (CNS) raises the question of the fate of the NP after their translocation to specific cell types or to sub-cellular structures in the brain.

## 2. Nanoparticles before nanotechnology

Although humans have been exposed to airborne NP of natural origin throughout our evolution, such exposure has increased dramatically over the last century due to anthropogenic sources, such as internal combustion engines, power plants, and many other sources of thermodegradation. Similarly, we have been in contact with man-made inorganic NP for some time. A well-known case is the use of NP as pigments since ancient Roman times, as in the Lycurgus cup, or in cosmetic ointments. It has been claimedthat lead-based chemistry involving the combination of naturally available minerals with oils, various creams, or water, which was initiated in Egypt more than 4000 years ago, could have resulted in the synthesis of lead sulfide (PbS) NP with a diameter of about 5 nm. These crystals appear quite similar to PbS quantum dots synthesized in modern materials science techniques [38]. Another case is the observation of TiO2 NP in the lungs (in the alveoli) of the Oetzi man (5,400 years old) [39]. It is believed that TiO2 was used as white pigment in tattoos. Similarly, researches from the *Centre de recherche et de restauration des musées de France* together with L'Oréal Research, as well as Argonne National Laboratory, showed that an ancient dyeing process for blacking hair is a remarkable illustration of synthetic nanoscale biomineralization. And nowadays, nanotechnology is likely to become yet another source of potential contamination by unprecedentedly small-sized inorganic particles.

## 3. Assessing the risk

In order to gauge the engineered NP toxicity risk, it is important to analyze existing nanoparticles, and how man and biological systems are exposed to extremely fine inorganic matter. Scientific literature is available on cytotoxicity and immunotoxicology of metal salts, and the controlled use of their cytotoxicity (as in the case of the antineoplastic drug CisPlatin). There is also literature on the toxicity of micrometric particles (which induce granulomatosis). However, little attention has been paid to the response of cells to engineered NP despite the fact that now we can investigate the subtle interactions and biological relevance of the interplay between inorganic NP and biology. For example, it was recently shown that minute clay particles induced order in random coil peptides when attached to the NP [[40]. They assumed the form of a helix when connected with the negatively charged silica sphere. The complex took on the properties of a catalyst, a function similar to that of enzymes in living cells [40]. Similarly, it was shown that Au NP could conform polypeptide structures into artificial proteins [41]. Summa-

rizing, the potential cytotoxicity of NP has been attributed to their size [42], shape (e.g., needle-like carbon nanotubes [5,6]), chemical composition (e.g., heavy metals [43,44]), or surface interaction with cells (e.g., the interaction of CdSe/ZnS particles with cells [45]). Finding information about nanotoxicology is complicated by the fact that NP may be called ultrafine particles by toxicologists [46], Aitken mode and nucleation mode particles by atmospheric scientists [47,48], and engineered nanostructured materials by materials scientists

## 4. Nanotechnology today

Despite all the above, nanospheres are already used in people. Last year, the manufacturing industry spent $30 billion on nanotechnology —this is expected to rise to $2.6 trillion by 2014. Today, there are almost 400 manufacturer-identified nanotechnology-based consumer products on the market, including computer chips, automobile parts, clothing, cosmetics, dietary supplements, wound dressings, dental-bonding agents, fuel cells, tires, optics and electronics [50]. In some of these products (such as skin creams and toothpastes) nanoparticles are in direct contact with the human body, and they can continually enter the environment by washing off consumer products [51]. The National Science Foundation estimates that by 2015 the nanotechnology sector will employ more than 2 million workers. What has been unclear, or ignored, so far, is that these foreign bodies below a certain size can enter animal organisms, mainly through ingestion or respiration. They may then interact with the gastrointestinal wall, the skin or the pulmonary alveoli, and be carried by blood or lymph traveling quite freely through tissues. Alternatively, they may settle in tissue they encounter during migration and thus enter the food chain. Funding for risk-focused research is a small -but increasing- fraction of what is spent on commercial applications of nanotechnology [52].

Therefore, a number of questions need to be addressed: To what extent does the nanoform of a substance have enhanced dermal penetration or increased systemic uptake via the lungs or gastrointestinal tract? What determines the proportion of NP entering into systemic circulation that will be distributed throughout the body, reach the bone marrow, cross the blood—brain barrier, cross the placenta and affect fetal development, or be sequestered effectively in the liver? Do nanoparticles released into the environment affect species that are important in food chain dynamics? What are the long-term consequences of exposure to NP? What are the water solubility, reactivity (oxidation, agglomeration, corrosion), environment persistence and environment dispersion of NP?

## 5. Evaluating nanotoxicity

Finally, the numerous studies appearing on NP cytotoxicity, such as the study of cell viability after cell cultures are exposed to NP, should not be confused with fully-fledged toxicological risk assessment. Risk assessments take into account exposure rates, uptake mechanisms, transport within the body, inflammation [53], memory and much more. Furthermore, the fact that NP are non-toxic for a cell culture within the parameters of a standard cytotoxicity experiment does not mean that the NP are non-toxic: they may affect a tissue, disrupting normal functioning, without significantly or rapidly killing the cells. Cytotoxicity studies are usually used as indicators of whether more extensive toxicological studies are needed. However, as nanotechnology is a new discipline (and due to its interdisciplinary character) the currently available data can be confusing. This is often due to the lack of experimental protocols, as in the case of optical interference between the nanomaterials and cell viability assays. Recently, it was shown how standard cell-viability tests may result in “fake” toxicity from carbon nanotubes [54], and explain why some studies have concluded that carbon nanotubes —which are studied for their potential to improve building materials, drug-delivery systems, and electronics, to name a few applications— are dangerous to human health while others have not. The same happens with Au NP: Au NP absorb MMT or XTT wavelengths strongly, so the optical density measured at these wavelengths will be affected by Au NP absorption [55] and therefore may lead to false results [56]. In the simplest case, samples containing NP will show more cellular activity than control samples, which may mask toxic effects or suggest proliferation powers of the NP. The way that NP are prepared, their surface state, the solvent and solutions which carry them, their agglomerated state, etc, may all modify results [57,58]. We have also observed that chemically identical Au NP - peptide conjugates with different surface structures may or may not trigger an immune response and inflammation. Furthermore, despite continuing encouragement for interdisciplinary collaboration, biological applications of objects designed using nanotechnology still suffer from a lack of interdisciplinary coordination. Chemists, physicists, and engineers create new advanced materials with sophisticated functionalities on a daily basis, but their understanding of biology is usually limited. This leads to straightforward uptake of nanoparticles being studied, while whether the incorporated particles are stuck in endosomal/lysosomal structures or free in the cytoplasm is ignored. Biologists typically study the uptake of NP by cells using relatively undefined nanoparticles with large polydispersity, limited colloidal stability, unknown surface chemistry, etc. And finally, the social aspect of toxicity makes it difficult to extract conclusions, and results (even the more technical ones) are often biased towards toxicity or non-toxicity depending on the context of the report, e.g., presenting a new medical device versus evaluating the toxicity of a material.

## 6. Regulation and social impact of nanotechnology

Regulation and social impact are normally overlooked by scientists, but the world does not change because of the introduction of a new technology on its own. Technology gets introduced into social networks and therefore the applications that develop are a mix of social and technological forces. If society

embraces and finds uses for a technology then it survives, if we do not, then no matter how good the technology is, it will die. Nowadays, some may believe that engineered NP are so risky that they call for a precautionary halt in NP-related research. However, the precautionary principle should not be used to stop research related to nanotechnology and NP, but uncontrolled industrial uses. A major environmental or health and safety problem—whether real or not—involving a product or application labeled as 'nanotechnology'—whether it actually is nanotechnology or not—could dampen public confidence and investment in nanotechnology. It may even lead to unwise regulation. At this point, adequate governmental regulation is difficult given the lack of data on engineered NP and risk assessment. Efforts are being made by the American National Standards Institute (ANSI) and by the International Council on Nanotechnology (ICON, a coalition of academic, industrial, governmental and //civil society organizations), as well as the International Organization for Standardization (ISO, Geneva, Switzerland). These associations are collecting data to aid understanding and regulation of nanotechnology. In parallel, international non-governmental ecological, environmental, labor and biocentric associations have very recently teamed up to issue a list of recommendations to avoid nanotechnology poisoning [59].Their concerns are not only about consumer and worker health and safety but also about the social and ethical implications of socio-economic issues (related to weak economies in developing countries and patents). We hope that in the near future, governmental and non-governmental organizations will work together to assess risks and introduce nanotechnology into society. We also hope that these concerns regarding health and the environment will be extended to other human activities, leading to responsible and rational use, and sustainability, not just of nanotechnology which is in fashion.

Apart from these governmental, academic and civil associations, products and companies are evolving rapidly, increasing the number of products (such as nanokeratine) and their nanotechnological quality.

## 7. Nanosilver: a case study

A paradigmatic case of recent regulation is *nanosilver*. The uncontrolled use of nanosilver is being banned in the US and it will soon also be banned in Europe and probably the rest of the world. The US Environmental Protection Agency (EPA) took its first step to regulate a nanomaterial, *nanosilver*, silver NP being used as a pesticide [60]. Nanosilver is found as a pesticide in several dozen products, including socks and shoes, and plastic storage containers. It prevents fungal and bacterial growth. However, the EPA was targeting a washing machine[yes I am], built by a major manufacturer, which releases nano-engineered silver ions into the wash cycle to kill bacteria. The silver would clearly flow into the environment. It is clear that the uncontrolled dispersion of persistent bactericide NPs in the environment or in the body presents a risk. Colloidal silver is not deactivated after killing the bacteria (as penicillin would be, for example). It is a catalyst, and therefore highly persistent and active until it agglomerates or is subsumed into a body that results in its deactivation. Nanosilver attaches to the cell membrane creating lethal pores and producing bacteria lysis. Silver in its macroscopic form is already known to damage aquatic organisms.

## 8. Conclusions

Apart from industrial uses of materials and commodities, which can be delayed until full risk assessment is carried out, the special interaction of NP with organisms means that inorganic engineered NP are also at the heart of the development of nanobiomedical robots. As mentioned, the most important impact of the new nanometric revolution will not come from materials science but from life science [61]. Therefore, if we are to start introducing tiny foreign bodies into people for diagnosis and therapy, knowledge of the specific interactions with the system has to be deep and extensive. A significant effort must be made in the coming years to resolve such questions, since interaction with living organisms is difficult and often unpredictable. The balance of positive and negative consequences of the interaction of NP with people can be illustrated by the example of iron oxide. It has been suggested that it is useful as a hyperthermia agent for cancer treatment in places, such as the brain, where surgery is not an option, or as an MRI contrast agent. It is also used as a drug for iron administration in cases of anemia. Recently iron oxide was found to be concomitant with Alzheimer's disease [62,63] with size and composition which excludes its ferritine origin. In addition, it was recently reported that iron dextran administrated to mice was dissolved in the liver and transformed into other iron deposits showing the metabolism of iron oxide NP [64,65]. In fact, the toxicity of superparamagnetic iron oxide NP has been the focus of research for a long time [66-68] but reports of potential medical benefits continue to appear constantly, together, of course, with those concerning its toxicity. Thus, while some find new promise for nerve cell regeneration [69], others detect the toxic effect of this material on neuronal cells [70] and so on. In the end, both the potential toxic and potential beneficial effects will coexist. Therefore, looking at these data, one will have to carefully decide about a prolonged treatment of young patients for anemia with iron NP which could alter iron metabolism in the brain and, in the future, lead to neurodegeneration. While if the patient, of any age, has a terminal cancer, should he/she care about Parkinson's or Alzheimer's disease decades later compared to surviving the coming months?

Nowadays, many people believe nanotechnology will be the key to solving many of the world's most pressing medical problems, while others believe it could lead to a potential disaster [71]. There is, therefore, a profusion of opinions, including those in prestigious scientific journals [52,72-74] where a rational approach based on common sense is called for. It is clear that we can cope [75] and that the approach to risk assessment has to be comprehensive, including dose, time, memory, damage, cancer, etc. Fortunately, inorganic NP normally have signatures which allow them to be monitored in organic/biological environments. Thus, it is likely that at the same

time that we see NP and carbon nanostructures used in controlled medical applications, restrictive regulations will appear to control the dispersion of these *new* chemicals. What we can already say is that each NP is different (size, shape, composition, surface, etc.) and therefore so is its toxicity, and it will be difficult to define generic nanotoxicity.

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*Authors work at The Inorganic Nanoparticles group at the ICN-CIN2 on the synthesis, characterization and bio-applications of engineered inorganic nanoparticles (NP), mixing expertise from physics, chemistry and biochemistry on the design of inorganic nanoparticles conjugates for their controlled interaction with biological systems.Understanding that the inorganic NPs during thier synthetic process or when exposed to the working environment are coated with organic molecules. This fact can be exploited to control the size, structure and shape of the inorganic core, its stability and the minimal interparticle distance upon collapse. On the other side, the fact of linking an active molecule to an inorganic surface allows to modify the molecule activity. Therefore, the design of NP - organic molecule conjugates pretends to take advantage of both, controlled properties of the inorganic core and controlled properties of the coating molecules.*