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Biological systems interact with Engineered NanoMaterials (ENMs): Possible environmental risks

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Summary. — There is a growing and controversial public debate on the potential risk of NanoMaterials (NMs) to living organisms, including humans. In particular, the processes of dispersion and bioaccumulation of Engineered NanoMaterials (ENMs) into the environment are poorly investigated. Biological systems interact with ENMs in a very complex dynamic way whose comprehension is still at its infancy. Thus the evaluation of the environmental impact of ENMs may be useful to minimize or eliminate ENMs toxicity and/or ecotoxicity, and to help authorities to draw directives and regulations for a safe production and use of ENMs. Here we briefly review biotoxicity and environmental risks of ENMs (like carbon- and metal-nanoparticles) reporting also our experience in the cytotoxicity of carbon (C) and silver (Ag) NanoParticles (NPs) on HeLa cells and nanoecotoxicity on *Paracentrotus lividus.*

PACS `92.40.kc` – Ground water quality.
PACS `87.19.xt` – Developmental diseases.
PACS `62.23.St` – Complex nanostructures, including patterned or assembled structures.

1. – Introduction

NanoMaterials (NMs), that have at least one dimension less than 100 nm, exhibit peculiar chemical, physical and optical properties dependent on their size. These properties are different from those present in the same material of conventional size, the so-called bulk material [1]. These NMs, like carbon nanostructures, metal oxides, liposomes, micelles and polymers, have a limitless range of applications, from biomedical imaging, drug delivery and therapeutics, to optics, electronics and quantum computing [2-6]. However, their unique physicochemical properties correspond to unique bioavailabilities and other characteristics that make them potentially toxic to humans. In fact, the tremendous

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(*) E-mail: `luciana.dini@unisalento.it` progress of nanotechnology has not been accompanied by sufficient studies of NMs toxicity even though their unique and completely new properties do not permit to extrapolate their toxicity from that of bulk materials nor from the toxicity of their constituents in ionic form. It is worth mentioning that the results of research on nanotoxicity have profound significance because the design of NMs used in the industry and consumer products should be based on the outcome of such research. If the industrial applications of NanoParticles (NPs) and nanostructured systems are increasing daily, it is not surprising that the possible risk of environmental adverse effects is only increasing [7]. Thus researchers are strongly motivated to better understand the NMs potential risk for human health and to open additional discussion on the possible environmental risk. The NMs can have a natural origin, such as those produced by natural combustion processes (volcanoes, spontaneous fires) or an anthropogenic origin, the so called Engineered NanoMaterials (ENMs) [8]. Among the NMs of natural source, refractory black carbon NPs impact human health, climate, and the carbon cycle. Eventually these particles enter aquatic environments, where they may affect the fate of other pollutants. Black carbon is a poorly characterized component of the freshwater environment. The black carbon derives from the incomplete combustion of vegetation (fires), fossil fuels, and petrogenic processes (mineral, graphite), and comprises a spectrum of related materials ranging from charred vegetation to refractory graphitic NPs [9]. ENMs can be done of carbon nanoparticles (C-NPs), in the form of hollow spheres, the buckyballs, or in the form of nanotubes. Metal NMs include quantum dots, nanogold, nanosilver and oxides of metals, such as titanium dioxide (TiO_2), etc. The NMs called dendrimers and nanosized polymers, due to the presence of internal cavities, are used for the selective delivery of drugs, markers and oligonucleotides. Composites NMs derive by the combination of materials of different nature reinforced with nanometer-sized particles. Some NMs could have the potential to become hazardous pollutants that may affect human health or the environment. However, the actual release of NMs into the environment is not under control. Indeed, the risk of a substance is determined by both effect and exposure and, for NMs, the knowledge of environmental exposure is quite low. It is necessary to assess these risks in relation to the types of NMs, their concentration in the environment and their toxicity to organisms, included humans. Indeed, the risk on human health depends, not only on direct exposition to NMs, but also on the complex ecological interaction between population communities and ecosystem. It is desirable that synthesis and assembly of NMs would benefit from the development of “clean” technologies, non-toxic and environmentally friendly procedures named “green chemistry” [10].

Herein, we focus on environmental risk of ENMs by reviewing in particular ecotoxicological data on different NPs, including carbon- and metal-based NPs. We address in particular the effects of NPs on the aquatic environment and the main weaknesses in econanotoxicological approaches.

2. – Nanocitoxicity

Since biological systems are continuously exposed to a wide array of ENMs, the assessment of health effects upon ENMs interaction is a very interesting field in nanotoxicology area. Nanocarrier systems can enter living organisms by three main routes, *i.e.*, skin, respiratory and gastrointestinal tract [11]. They can readily cross cell membranes, blood-brain barrier and blood-testis barrier, localizing in colon, lungs, bone marrow, liver, spleen, heart, kidneys and brain and affecting physiological mechanisms of these organs (as reviewed in [12]).

More and more *in vitro* studies have been performed to understand the ENMs cytotoxicity in mammalian cells. The cellular uptake of ENMs depends on their properties, such as shape, size and surface [13]. Cellular toxicity may depend on: 1) direct toxicity related to chemical composition and surface reactivity; 2) ROS generation; 3) release of ions and impurities in the cell; 4) cell functions alteration related to small size and hydrophobicity degree. On the other hand, NPs cytotoxicity depends on a) stabilizing coating agents; b) physicochemical parameters of NPs (diameter, surface charge, surface topography and area); c) incubation conditions (time and concentration); d) types of cell used (as reviewed in [14]).

Silver nanoparticles (Ag-NPs) have a wide range of applications and consequently a highest degree of commercialization and their human health effects, by using different cell lines, have been extensively studied [15].

In this context, we demonstrated the toxic effects of different amounts of two glycans-capped Ag-NPs, *i.e.*, β-D glucose (AgNPs-G) and β-D glucose-sucrose (AgNPs-GS), on human epitheloid cervix carcinoma HeLa cells [16]. Particularly, we observed a direct dose- and time-response relationship, *i.e.*, cytotoxicity increases with the Ag-NPs/cell number and incubation time. Moreover, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide (MTT) assay demonstrated that AgNPs-G are more toxic than AgNPs-GS. In fact, 2000 NPs/cell produce 50% of cell viability decrement at 24 h for AgNPs-G *vs.* 10000 NPs/cell at 48 h for AgNPs-GS. Morphological evaluation confirms the data: HeLa cells undergo cell death detaching from substrate [16].

3. – Environmental impact of NMs

As above reported, the wide and quick advances in ENMs design and application ignite in scientists the debate about not only the benefits of nanotechnologies for humans but also about the risks linked to their use to human health and environment [17]. The term nano-pollution is generically referred to all waste, in the form of NPs, generated during the NMs fabrication or erosion consequent to the life use. Moreover, NMs can also be used in bioremediation involving their deliberate release into the environment [18]. At present, very few studies of the hazards of nanotechnology to non-humans and the environment there are. The released NPs are able to accumulate in soil and/or water and, consequently, in vegetables and finally they can be assimilated by animals, causing unknown effects. The transportation in soil and water depends on particles characteristics, such as size, charge, solubility, aggregation, agglomeration, diffusion and deposition [19]. In particular, aggregation and agglomeration play a key role in the transport of the material in the environment [18], where NPs can be biodegrade and bioavailable [19].

Som and coworkers [20] indicate five criteria to determine NMs environmental fate and effects: the indication of hazardous effects at realistic exposure concentrations, the tendency to dissolve in water to form metal ions, the tendency to agglomerate or sediment under natural conditions, the fate in waste water facilities and the stability during incineration.

Since it is well known that the industrial and urban wastes end up in water (rivers, lakes, sea), nanoscale products and by-products inevitably pollute the aquatic environment.

Also, in various aquatic environments the suspended sediment particles can sequester and transport chemical pollutants over significant distances implying a diffusion of nano-pollution. Aquatic organisms can take up NPs by direct ingestion or through gills,

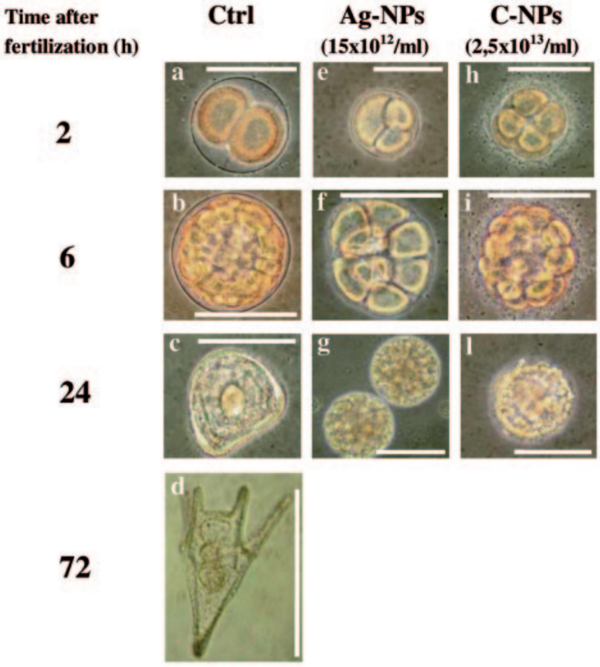

Fig. 1. – Light microscope images taken by using the inverted microscope Eclipse TS100 (Nikon, Kawasaki, Kanagawa Prefecture, Japan) of the development stages of *P. lividus* after fertilization in the absence (a-d) or in the presence of Ag-NPs (15×10^{12} NPs/mL of sea water) (e-g) or C-NPs (2.5×10^{13} NPs/mL of sea water) (h-l). a: Normal two blastomers stage; b: normal blastula stage; c: normal gastrula stage; d: normal adult pluteus; e, h) altered two blastomers stage; f, i) disorganized blastulae; g, l) died gastrulae. Ctrl = untreated embryos; C-/Ag- NPs = carbon or silver nanoparticles.

olfactory organs and body well and their dangerous effects are related to concentration of the particles, as widely demonstrated for Ag-NPs, ZnO-NPs and TiO_2-NPs.

Among different aquatic organisms, invertebrates, such as mussels (*Mytilus edulis*), cockles, sea urchins (*Paracentrotus lividus*), water flea (*Daphnia magna*), etc., and vertebrates, such as zebrafish (*Danio rerio*), are widely used as laboratory model to study nanoecotoxicology.

In particular, the embryonic development of these species has been used to study potential hazardous effects of different materials.

Several data in the literature demonstrate a direct relationship between NPs presence and altered development of organisms. Some studies report that TiO2-NPs present in water may accumulate in *Danio rerio* and *Daphnia magna* altering their development in relation to bioconcentration and ROS generation [21, 22].

Recently, Zhu [23] demonstrated the effect of Fe_2O_3-NPs, used in biomedical areas, in environmental remediation, and in various industrial applications, on early life stages of the *Danio rerio*. In particular, Fe_2O_3-NPs aggregates cause a serious delay in embryo hatching, malformation in embryos and larvae, and eventually mortality.

In this context, we demonstrated that also C-NPs and Ag-NPs affect development of *P. lividus*. An early alteration of life stages, soon after 2 h from egg fertilization, was observed. In particular, the presence of Ag-NPs affects the synchronism of segmentation process; conversely, C-NPs affects the speed of segmentation, slowing it up (fig. 1;

Table I. – *Percentages of normal and modified embryos found in the early stage life of P. lividus after their fertilization when they were incubated with C- or Ag-NPs (T0). The embryos scoring was done by counting at least* 500 *embryos in at least* 10 *optical fields chosen at random by using the inverted microscope Eclipse TS100 (Nikon, Kawasaki, Kanagawa Prefecture, Japan). The errors measured as SD never exceeded the* 3%*. Ctrl = untreated embryos; C-/Ag- NPs = carbon or silver nanoparticles; h = hours.*

Time after fertilization (h)	Ctrl		Ag-NPs (15×10^{12}/mL)		C-NPs (2.5×10^{12}/mL)	
	Normal	Modified	Normal	Modified	Normal	Modified
2	85 ± 3%	15 ± 2%	35 ± 3%	65 ± 2%	77 ± 3%	23 ± 1%
6	85 ± 2%	15 ± 3%	–	100 ± 1%	60 ± 2%	40 ± 3%
24	85 ± 3%	15 ± 1%	–	100 ± 2%	60 ± 1%	40 ± 2%
48	85 ± 1%	15 ± 2%	–	100 ± 3%	–	100 ± 3%
72	85 ± 3%	15 ± 3%	–	100 ± 2%	–	100 ± 3%

table I). Moreover, both Ag-NPs and C-NPs stop the sea urchin development after 24 h; in fact a normal adult larva pluteus was only observed in the absence of NPs during egg fertilization. Figure 1 shows representative images of different life stages of *P. lividus* whose eggs have been fertilized in the presence of C-NPs or Ag-NPs.

Manno and coworkers [24] demonstrated that small C-NPs (13 nm diameter) induced alteration to *P. lividus* plutei when administered directly to the larval stage. The toxic effect is proportional to the amount of C-NPs in sea water. Moreover, C-NPs interfere with biomineralization process in *P. lividus* plutei altering in a dose-independent manner the cyclophilin gene (Sp-Cyc-1) expression, involved in skeletogenesis. Moreover, the presence of C-NPs induces the *P. lividus* to elaborate a defense mechanism ending in the formation of new material inside the organisms similar to aragonite.

4. – Conclusions

It is becoming increasingly necessary to assess the real risks of ENMs, in relation to the types, concentration in the environment and toxicity on livings, including humans. Indeed, the risk on human health depends not only on direct exposition to ENMs but also on the ecological disorders that ENMs may determine into the ecosystem. In fact, the risk of human contamination through the food chain is very high: it is like a snake eating its own tail, since the problem of NMs released in the environment, would relapse on humans.

Further studies should be performed in order to answer to these questions. i) How and in what quantities, natural and synthetic NMs are released into our environment? ii) What is the toxic level of contamination? iii) What are the most appropriate analytical methods to investigate the toxicological effects to the environment? iv) What effects cause the NMs on fish, insects, bacteria, plants and other organisms? By answering all these questions new safe ENMs for the humans and environment will be developed, and thus the limitless potential of the ENMs will be fully exploited.

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