COMPREHENSIVE UNDERSTANDING OF BIO-NANO INTERACTIONS-A CHALLENGE FOR NANOTOXICOLOGICAL RESEARCH IN ALBANIA: A REVIEW

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ABSTRACT

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. Nanotechnology is an area of science that integrates, physics, electronics, mathematics and informatics. This new science area promises scientific advancement in many sectors such as medicine, consumer products, energy, materials and manufacturing. A new research area has evolved in the last 20 years. Nanotoxicology represents a new and growing research area in toxicology. It deals with the assessment of the toxicological properties of nanoparticles with the intention of determining whether (and to what extent) they pose an environmental or societal threat. Nano particles (NPs) and nanomaterials (NMs) exhibit unique

physicochemical properties, which make them interact with cells and tissues in an unpredictable manner. Being of comparable dimensions, fabricated nanoparticles, and cellular molecular organelles and macromolecules, a possible direct interaction in the nano-bio interface could be revealed. A holistic understanding of bio-nano interactions would be necessary for a safe and intelligent design and use of nanomaterials. The present paper reviews via *in vivo*, *in vitro*, and *in silico* methods and a battery of reliable, low-cost, and specific biomarkers of nanotoxicity effects to unearth the mechanisms of bio-nano interactions. Furthermore, potential opportunities and challenges in applying these biomarkers in the study of bio-nano interfaces are also provided.
Keywords: nanoparticles; bio-nano interactions; biomarkers; nanotoxicity

1. INTRODUCTION

Research in the field of eco-physio-toxicology for the effects of toxic chemicals on biological organisms at cell to organism levels has been made in the last twenty years in Albania. Information on the adverse effects of environmental and anthropogenic-borne toxicants on biota health could be found in (Aliko et al., 2021; Qyli *et al.* 2020; Sula et al., 2020,). Different animals from different taxa such as crustaceans, amphibians, and fish, in addition to the mechanisms of homeostatic perturbation, have been studied using a multi-biomarker approach. Reactive oxygen species (ROS) response, lysosomal membrane stability, erythron profile, histopathology of liver and kidney, behavior and development, and growth parameters were evaluated as endpoints (Figure 1). Considering the complexity of biological systems, our studies clearly pointed out that even small changes that occur in the subcellular level, if persist in long-term, can alter homeostasis equilibrium determining so the fate and functions of the organism by leading it to disease.

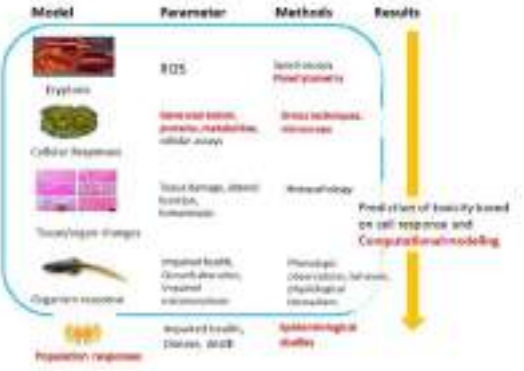

Fig. 1: General approach to evaluate toxic effects of NPs using cell and animal models, as well as a battery of reliable parameters/biomarkers.

The entire cell can be viewed as a "network of interlocking assembly of large protein machines" (Alberts, 1998), which, in turn, are often times of nanoscale dimensions (van den Heuvel and Dekker, 2007). Advancement in scientific research has made investigation on cells, tissues, and organs transformation and behavior as nanomaterials enter in contact with them unavoidable. Here, understanding the underlying mechanisms of material-biological system interaction is neccessary. This could provide therapeutic gain if some features of unwanted effects of a nanomaterial in the context of disease are proved useful. This is the case when ecotoxicology and physiology/medicine can be viewed as the yin-yang of nano-bio interactions (Bondarenko *et al*., 2021).

The present paper review summarizes the assessment approaches of toxicants' effects on model organisms and the perspective of nanotoxicity in the development of nanomedicine Furthermore, exploring interactions between engineered or incidental materials and biological systems at the nanoscale is a prerequisite for the safe and efficacious distribution of nanomaterials in patients (Shvedova *et al*., 2010; Wang *et al*., 2019).

NanoToxicology Approaches: advanced *in vitro* and *in vivo* models

Nanoparticles and nanomaterials are thought to behave differently from the bulk substance of the same chemical compound. Due to their tiny size and large surface area, the interaction of NPs with cells is far more powerful than their bulk counterparts (Tirumala *et al*., 2021). Besides their beneficial biological effects, most often nanoparticles can cause adverse effects on biological systems, even though this is a very debatable argument. Figuring out any nanoparticles or nanomaterials toxic effects produced upon living cells or organisms is essential to understand mechanisms associated with nanotoxicity outcomes.

Generally, three approaches are used by our laboratory to evaluate the toxicity of NPs, cell-based *in vitro*, organism-based *in-vivo* assays, and *in silico* methods (Figure 2).

Cell-based *in vitro* assays

Cell-based tests, well-known as *in vitro* assays, are commonly used to assess the safety and toxicity of xenobiotics, replacing testing in animals. To reduce the increased number of animals used each year in toxicity tests, using non-mammalian red blood cells, i.e., amphibians and fish offers a great system to investigate the possible effects of toxicants in toxicology studies. Substituting experiments animals with cells go in respect with the principle of 3Rs (reduction, refinement, and replacement) proposed by Charles Hume and William Russell in 1957. According to this principle, the number of animals

sacrificed is reduced and the stress upon animals during handling and operational procedures is also minimized.

Fig. 2: Schematic presentation of scientific approaches employed for nanoparticle toxicity assessment.

Laboratory investigation about fish and frog erythrocytes was carried out to evaluate the toxic effects of different environmental toxicants such as copper nanoparticles (Aliko *et al*., 2015), different pharmaceuticals such as fluoxetine and ibuprofen (Aliko *et al*., 2021), and pesticides and PCBs (Sula *et al*., 2020). In vitro toxicity is usually evaluated using cellular viability, apoptosis, oxidative stress enzymes, and genotoxicity as endpoints. Studies have shown a significant increase in the frequency of cellular and nuclear abnormalities in erythrocytes of *Bufo bufo* tadpoles exposed for seven days to two environmentally realistic concentrations of pharmaceuticals fluoxetine, ibuprofen, and their mixture, which proves the pharmaceuticals' role as erythrocyte apoptosis-inducers (Aliko *et al*., 2021).

Non-mammalian erythrocyte cell seems to be a very good model cell in toxicity assessments, due to its morphological characteristics, long lifespan, and maturation during circulation, traits that make it susceptible to xenotoxines (Podsiedlik *et al*., 2020). Following exposure to xenobiotics, erythrocytes undergo different morphological changes like shape changes due to disruption of membrane integrity. Usually, there is a dose-dependent shape alteration of fish erythrocytes exposed to environmental pollution, with red blood cells shifting from ellipsoidal to a circular shape, as a way to compensate for the reduction in hemoglobin concentration provoked by stress (Sula *et al*., 2020). Thus, measuring erythrocyte rotundity, as a biomarker of cell resilience and membrane stability, can help in assessing the cytotoxic effect of xenobiotics on freshwater biota.

Red blood cells represent the main cells in circulations and they are responsible for oxygen transport towards body tissues and cells; any factor that perturbates this process could be lethal. Possessing a nucleus and being covered by a multi-component plasma membrane, the non-mammalian

erythrocyte has a characteristic ellipsoid shape, relatively high flexibility, elasticity, and deformability. Any alteration of this structure makes red blood cells suffer and compromises their function until it decides to undertake apoptosis. So, programmed cell death seems a clear sign of erythrocyte response to injury; this makes erythrocyte an excellent reliable biomarker of cell toxicity (Farang and Alagawany, 2018; Aliko *et al*.,2021).

Erythrocytes are capable of explaining antioxidant capacity and producing reactive oxygen species (ROS) as a response to various toxicants due to the antioxidative enzymes they contain. By measuring the altered concentration of these enzymes in the blood, one can assess tissues' response to stressors. The generation of ROS in blood impairs the antioxidant defense system to eliminate oxidative stress, altering so, the normal body homeostasis. Our findings revealed that acute exposure of goldfish to manganese induced a significant increase in superoxide dismutase (SOD), catalase (CAT), and glutathion-S-transferase (GST) antioxidant enzymes in the blood, strongly suggesting that Mn^{2+} exposure caused generalized oxidative stress, which leads to activation of protective mechanisms necessary for scavenging of produced O_2-radicals in erythrocytes (Aliko *et al*., 2018).

Organism-based *in vivo* assays: fish and amphibians

To evaluate *in vivo* toxicity of nanomaterials and nanoparticles, model organisms must be exposed either to acute or chronic stressors and multiple biomarkers, such as biochemical, hematological, histopathological, immunological, and behavioral, must be measured. Fish and amphibians are the most investigated model organisms in our laboratory. Both species inhabit aquatic ecosystems, with fishes living entirely in water, while amphibians exhibit an amphibious life, with larval phase in water and adults sharing both areas, land, and water. The use of non-mammalian animal models such as *Carassius auratus* (Aliko *et al*., 2018), *Carassius carassius* (Sula *et al*., 2020), *Bufo bufo* (Aliko *et al*., 2020), *Carcinus aestuarii* (Qyli *et al*., 2020) represents an ideal strategy to overcome the ethical problems related to the traditional animal models used in the safety assessment of NPs.

The fishes were exposed to copper nanoparticles (CuONPs), at two environmentally relevant doses of 0.5 and 1 µg/L for 96h for the toxicity test. In addition, ROS enzymes, tissue histopathology, biochemical and physiological parameters were also evaluated. All analyzed biomarkers showed significant differences between exposed groups and in relation to the control group (unpublished data). Copper nanoparticles at 1 µg/L seemed to enter the cell, destabilizing the erythrocyte membrane, causing cytotoxicity and genotoxicity by leading the cell toward apoptosis. Another investigation involving the goldfish exposed to an environmentally realistic dose of manganese proves these results (Aliko *et al*., 2018). However, the evaluation

of chronic toxicity and carcinogenic potential of nanoparticles and nanomaterial requires long-term studies of 12-24 months.

***In silico assays*: modeling tadpole growth and cytotoxicity**

In silico modeling represents a relatively new tool that combines experimental approaches, providing a powerful technique to help in understanding molecular mechanisms between NPs and biological macromolecules at the atomic level (Huang *et al*., 2021). Even though nanomaterials are designed to be used successfully in the medical field, due to some of their properties they can create toxic effects in organisms. This is because they enter into cells, react with cellular components, and most probably interact with DNA causing breaks, altered basis, and chromosomal damage. This affects DNA function and causes a clastogenic effect producing micronuclei (Azqueta and Dusinska, 2015; Aliko *et al*., 2021).

One of the promising approaches we are using to study the genotoxic effects of copper nanoparticles is molecular docking. This tool is based on computational simulations and evaluation of copper (II) oxide-DNA molecule interactions based on 3D structural knowledge. The molecular docking study showed that CuONPs, despite their low molecular weight, binds to the minor groove of DNA in an energetically favorable (-2.13 kcal mol^{-1}) manner, suggesting a direct interaction with the genetic material, supporting the data from micronucleus assay (unpublished study).

Toad embryos and tadpoles are used for the assessment of the developmental toxicity. These life phases are very sensitive to teratogenic insults and also cover almost all morphogenic processes such as neurulation, limb bud formation, cardiac looping. Acute exposure of *Bufo viridis* tadpoles at Gosner stage 21 to different copper concentrations (0.01, 0.05, and 0.1mg/L) for 120 hours, caused poor larval development and growth (Aliko *et al*., 2015).

Future perspectives

The present review aims at uncovering the molecular mechanisms of nano-bio interactions as the use of nanomaterials in biomedicine holds great promise, especially in diseased related with radical oxygen species-ROS. In the near future, nano-bio interface research has to be focused on: i) a full understanding of nanomaterial redox species-induction; ii) research nano-bio interactions considering the complex environment *in vivo* and, iii) applying computational simulation in order to deeply and accurately investigate the nano-bio interactions. In this case, *in silico* methods combined with experimental data offer great possibilities in evaluating the toxicological behavior of NPs and NMs. Understanding the risk produced by nanoparticles

and nanomaterials is of great concern for the safety and development of nanotechnology and nanomedicine.

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