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ASPECTS OF NANOPARTICLES TOXICITY ON THE HUMAN BODY

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Nanotechnologies are currently an emerging domain of biotechnologies with broad applications in biomedicine. It has developed the concept of "theranostic" (support for diagnosis and treatment), which is currently the forefront of research objectives in the field. Before any attempt to use nanoparticles as agents in diagnostic or treatment schemes, we must assure that any toxicity issues were exceeded. An important step in assessing the toxicity of nanoparticles is the detailed characterization of physico-chemical properties, including size, shape, composition and reactivity of surface. These parameters can guide us to select the route of nanoparticles administration, and may give us information about the potential effects on target organs. The dosage and time of administration are other responsible factors for assessing the toxicity of nanoparticles. It is very important to know if the dose usually used (in order to achieve biological response) is greater than real dose that causing toxic effects. In perspective, it requires the development of a regulatory framework to indicate maximum limits and optimal methods for exposure to nanoparticles. The purpose of this paper is to give the reader a short overview of the nanoparticles properties, with focus on hazards related to their toxicity, relevant to humans.

Keywords: toxicity, surface reactivity, endocytosis, oxidative stress.

INTRODUCTION

Nanomedicine is a new branch of biotechnology that has been developed as a result of increased interest in the synthesis of new materials, that have proven useful both in terms of prevention actions, and in the diagnosis and/or treatment. It has thus developed the concept of "**theranostic**" (support for diagnosis and treatment), which is currently the forefront of research objectives in the field.

Three features are defining for characterization and using of nanoparticles for biotechnology:

- small size of particles;
- special properties of nanoparticles;
- integrating nanoparticles in new technologies, devices and systems.

The Figure 1 represents the domain of applicability of nanoparticles in biomedicine, where the range of sizes makes them ideal candidates for interactions with biological molecules.

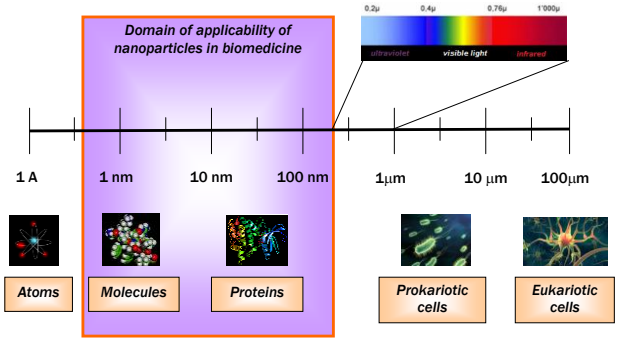

Figure 1. Domain of applicability of nanoparticles in biomedicine.

As a result of great benefits offered by nanoparticles applications in a wide variety of fields, their usage will significantly grow. Therefore, human body exposure to them will increase, rising the concerns about biosafety issues.

The purpose of this paper is to give the reader a short overview of the nanoparticles properties, with focus on hazards related to their toxicity, relevant to humans.

Historical context

The existence of nanometric structures (nanoparticles) dating back to the beginning of life on Earth. In nature, whenever a volcano erupts or a forest burns occurs the degradation of organic materials followed by the formation of nano-particles (carbon nanotubes, silica nanoparticles, fullerenes, etc.)[1].

Biological processes sometimes use nanoparticles. A good example is given by *Haliotis iris*, a mollusk popularly called abalone or ear-shell, that produces strong and hard shells, with very beautiful iridescence, produced by organic nanoparticles from lower layers that separate $CaCO_3$-platelets[2].

Over time, people have benefited from nanoparticles unaware of their existence. They used nanoparticles in the preparation of the dyes and paints, a definite proof being the traces of pigments found in the alveoli of the mummies of 5 000 years old[3]. The pottery was another human occupation that enjoyed of nanoparticles benefits, since ancient times. The technique of enameling of historical pottery, originally from the Islamic world, consists of covering the surface with a fine metal film containing nanoparticles of copper and silver dispersed in a glassy medium. The luster, obtained by baking the ceramics in the oven at 600°C, is visible as long as the film resists to the action of oxidizing atmosphere or because of other elements[4]. The wide variety of colors viewed in the stained glass windows of medieval cathedrals is due to the presence of metal nanoparticles, especially gold nanoparticles.

These, depending on the size of the particles, reflect light differently and exhibit a whole range of colors. The beauty of these works of art, as well as colors and their longevity has been appreciated for centuries.

Why are nanoparticles so special?

The dimensions of nanoparticles are in the size range 1–100 nm. A nanometer is a millionth of a millimeter (1×10^{-9} m). To make it easier to understand and intuit such a scale, we can recall that: a hair has a thickness of 10 000 nm to 50 000 nm, a red blood cell has a diameter of 5 000–7 000 nm, viruses have generally size between 10 nm and 100 nm, and a DNA molecule having a diameter of 2 nm to 25 nm. In Figure 2 is schematically illustrated a parallel between the scale size of nanoparticles and the other structures.

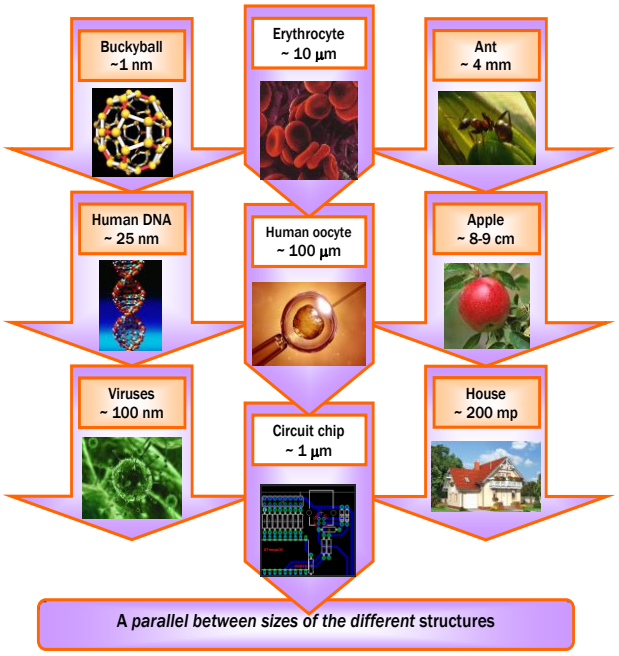

Figure 2. A parallel between sizes of the different structures.

What makes from nanoparticles a subject of study so attractive are their special properties.

Ordinary materials (carbon, silicon) changes its properties when are reduced to nanoscale. They express new and unexpected characteristics, such as: size, size distribution, morphology, chemical reactivity, very high electrical conductivity, or other properties that are not highlighted to macro or micro sizes.

We present below the most important properties exposed by nanometric particles.

SPECIFIC SURFACE AREA

Nanoparticles show a large surface-to-volume ratio, which gives them an increased surface energy and reactivity, the greater as the size of nanoparticles decreases. Therefore, the surface of contact with surrounding materials will increase. Large surface area of nanoparticles makes them to disperse easily in different liquids. With reducing of dimensions of nanoparticles increase the capacity of adsorption on different surfaces. These properties recommend them for use in paint industry, cosmetic industry, in medicine and for the manufacture of medicaments. Nanoparticles can be good catalysts, antimicrobials agents (colloidal nanosilver) or chemical sensors.

MAGNETICAL PROPERTIES

The magnetic properties of materials are determined primarily by the orbital motion of electrons (electron moving around the nucleus of an atom) and by the spin of electrons (electron rotation around its axis). Although the initial raw material may not have the magnetic properties, decrease in size may confer magnetic properties to nanoscale particles. Magnetic properties are useful tools in various medical applications: markers for biological fluids, drug delivery, contrast agents, etc. Thus, ZnO, TiO, CdS, CdSe, ZnSe and PbSe nanoparticles can be used in medical imaging, or for manipulation of genetic material.

OPTICAL PROPERTIES

Study of optical properties of nanoparticles represents a scientific field, where we talking about process such as: elastic light scattering, Raman scattering, reflectance, transmittance, second-harmonic generation, nonlinear optical properties, etc[5]. The nanoparticles have different optical properties, depending on the nature of the material they are made: insulator, semiconductor or metal[6]. Absorption and emission of certain wavelengths can be controlled through interventions on the size of nanoparticle, of interaction with various ligands or by external disruptions (doping process of the host material). Nanoparticles with optical properties are applied as labels in bioimaging (quantum dots, upconverting nanoparticles, gold nanoparticles, carbon nanoparticles, etc.).

ELECTRICAL PROPERTIES

The electrical conductivity of the nanoparticles can be determined by the individual properties of the nanoparticles. Specific surface area, the size and chemical structure of nanoparticles may control the dielectric and ferroelectric properties, influencing also the ionic potential or the electronic affinity of nanoparticles. In biomedical applications, the ceramic nanoparticles, such as metal oxides, can be used to offer a special electromagnetic protection for different devices.

TOXICITY OF NANOPARTICLES

After the initial boom and subsequent development of nanotechnologies, introducing of nanoparticles in many fields and activities has generated the problem of toxicity. The same properties that make them so desirable and so intense applied are responsible for the toxic effect.

Conventionally, when we speak about the toxicity we refer to key parameters like dimensions, dosage and time of application[7]. The relationship exposure-dose-response is not a linear process in case of nanoparticles usage, currently being many discussions related to critical dose, especially for applications in the medical field.

FACTORS RESPONSIBLE FOR NANOPARTICLES TOXICITY

The **size** of nanoparticles plays an important role to determine the toxicity, because can influence the charge, the chemical surface reactivity and the mobility of particles. Even small dimensional differences can influence the body distribution and the biological actions of nanoparticles[8].

Toxicological studies have demonstrated that small nanoparticles (< 100 nm) may penetrate between cells, pass into cells, penetrate in subcellular components, or translocate in other parts of the body, resulting in toxic effects in different organs[9–12]. The small dimensions increase the nanoparticles mobility that makes them easier to airborne and inhale. Therefore, they can cause respiratory health problems, typically causing more inflammation than larger particles made from the same material[13,14].

Nanoparticles have many **shapes** depending on the conditions of synthesis, and chemical structure of the nanoparticles: spherical, cylindrical (needle-like, tubes, rods), cubical, prismatic, *etc*. The shape of the NP has influence on the absorption and deposition of NPs in various organ systems[15]. Long-aspect-ratio nanoparticles (rods, fibers, needle-like) are more toxic than spherical, process closely related to their mechanical properties and ability of macrophages to phagocytize them (nanotubes are more toxic than the fullerenes)[16,17].

Reducing the size of nanoparticles is accompanied by increasing of **surface** relative to the volume and therefore increasing of chemical reactivity. This can be an advantage because nanoparticles may be used as chemical catalysts or biosensors, but also it is the main source of inconvenience due to increased toxicity. Nanoparticles, especially those with sizes smaller than 100 nm, show a strong tendency to aggregate and agglomerate in the presence of the biological fluids (saline solutions with pH and concentrations similar to biological environment)[18]. Therefore, most of studies about toxicity are focused rather on the characteristics of surfaces, than on the core material. A measure to prevent surface reactivity is the protective coating with a shell made from a biocompatible material.

In the environmental context and especially in biological medium we shall never find simple nanoparticles[19]. In the biological environment, the nanoparticles can attach different element on the surface, especially proteins, but and traces of polymers or lipids have been identified. The process is called "**corona**" **effect** and can be strongly (for interaction between nanoparticles and proteins) or weaker (for interaction between attached protein and new molecules)[20,7]. Given these considerations it is necessary that in assessment of nanotoxicity to taking into account characteristics of the nanoparticles (size, shape, surface), and also the environment where nanoparticles will be used[21].

The **dosage** and **time of administration** are other responsible factors for assessing the toxicity of nanoparticles. Real problems arise from evaluate correct dose of administration for avoiding overdosing or using of too low doses. Administration of high doses is easily identifiable, because the effects are obvious. Long-term expose to low doses rises the difficulty to identify and to asses the effects.

ENTRY ROUTES OF NANOPARTICLES IN BODY SYSTEMS

The entry routes of nanoparticles into the body are represented by inhalation, ingestion, instillation, absorption through the skin or digestive tract[9,22], but also voluntary administration by injection and drug delivery[23]. There is also, the possibility to translocation of the nanoparticles in secondary organs after primary uptake[24,25].

The main entry routes of nanoparticles into the body are schematically represented in Figure 3.

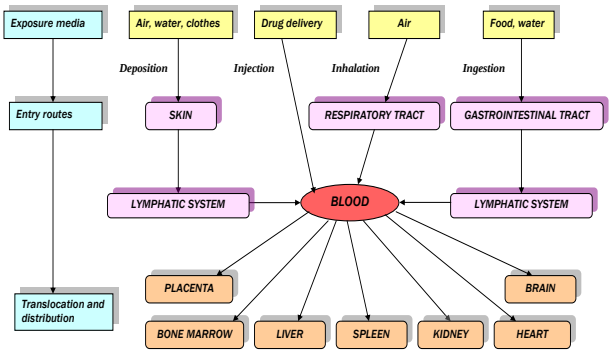

Figure 3. Routes of exposure to (Modified after Oberdorster, G., E. Oberdorster, and J. Oberdorster, Environ Health Perspect, 2005. 113(7): 823–39 [26]).

Respiratory Route

The main route of airborne nanoparticles to get into the body is the respiratory system. At the level of upper respiratory tract, a lot of inspired nanoparticles are trapped in the protective mucus layer from mucociliary escalator, that perform the clearance[14,27]. On the olfactory route, the nanoparticles may penetrate the olfactory bulb and from there to reach the brain, where they are responsible for the neurotoxic effects[28]. At the lower respiratory tract can be performed the clearance of the nanoparticles through macrophage phagocytosis[29]. When the lung is subjected to prolonged exposure, nanoparticles may lodged in lungs and after the diffusion through epithelial cells can be translocated (by blood or lymphatic system) to other body organs: liver, spleen, heart, brain, bone marrow[24,30].

Dermal Route

The skin is the largest organ of the body, with the leading role to form a barrier against harmful environmental factors. The structure of skin includes three layers: epidermis, dermis and hypodermis (subcutaneous layer). The outer layer of the epidermis, the *stratum corneum*, with a thickness of about 10 μm, covers most of the body. It consists of keratinized cells and dead corneocytes embedded within lipid regions[31], which makes it impermeable to most compounds, especially for

water-soluble molecules. The dermis layer is composed by connective tissue that contains numerous blood vessel, sensory nerve endings, lymph vessels, sebaceous glands, excretory canals of sweat glands and hair follicles. It is a layer permeable to solutes. The skin has an irregular surface because is crossed by pores for opening to the outside of the excretion channels of sweat glands, sebaceous glands and hair follicles.

The nanoparticles (ZnO, nanosilver, TiO_2, etc.) reach the surface of the skin in large quantities especially through cosmetic products and in particular through sunscreen lotions[32]. Their translocation in body systems is inconclusive and needs more studies. A diffusion process can take place through the lipid layers of the stratum corneum[33]. Other particles (fullerene[34], quantum dots[35]) are able to penetrate the dermis. Some of the studies have shown that, after penetrate the dermis the nanoparticles can pass in lymph macrophages, lymph or blood vessels or may reach the regional lymph nodes and accumulate here.

Four pathways of penetration across the skin have been identified: intercellular, transcellular and two transappendageal, through hair follicles and sweat glands[36,37].

Besides nanoparticle characteristics (size, shape, composition, dose, etc.), there are a number of factors that favor the penetration of nanoparticles to the skin: the skin barrier integrity (wound and lesions)[7], the contaminate surface, the anatomical side, skin diseases (allergic dermatitis, atopic eczema, psoriasis)[37], mechanical flexions[34,38], hair follicles[39], irritant detergents and various chemicals.

Gastrointestinal tract route

Another major way to increase nanoparticles penetration in the body is through ingestion. They can enter in the gastrointestinal tract together with water, food, drugs, drug delivery devices, cosmetics (toothpaste and lipstick), dental prosthesis debris, or by secondary ingestion of inhaled particles[9,40,41]. Each area of the gastrointestinal tract encompasses digestive, absorptive, secretory, and protective functions[40]. Once inside the gut, most of nanoparticles passed through the digestive tract and are eliminated rapidly through feces[42], without being absorbed. However, some of them penetrate the mucus barrier[43], are absorbed in the small intestine and is distributed by the lymphatic system and by systemic circulation to the body organs: liver, spleen, kidney and brain[42].

Drug delivery

Drug delivery is one of the best routes to use nanoparticles for biomedical applications. For drug delivery biocompatible and biodegradable nanoparticle are needed, as it is the intention to transport and release the drug[23]. There are many applications of nanoparticles in drug delivery: disease diagnosis, imaging, and the treatment of various disease conditions[44], especially cancer therapy. Quantum dots, upconverting nanoparticles, magnetic nanoparticles, iron oxide nanoparticles and gold nanoparticles show more beneficial effects in imaging and in the treatment of cancer cells[15]. Another promising nanoparticle is cerium oxide, which are being used as antioxidants to treat various medical conditions[15].

CELLULAR UPTAKE OF NANOPARTICLES

Once they penetrated inside the body, depending on the entrance route, nanoparticles have pass across numerous biological barriers to reach the final target. For intravascular delivery, nanoparticles must overcome first the reticuloendothelial system comprising the liver and spleen, after that must penetrate the endothelial wall of blood vessels. At the third level, nanoparticles must pervade the extracellular matrix, and after that it must enter into the cell[45].

Once inside in the circulation, nanoparticles have access to the cells. There are several ways to penetrate the cell: endocytosis (phagocytosis, pinocytosis), passive diffusion through transmembrane channels or membranes penetration[9,46]. Endocytosis is the most incriminated mechanism for cellular uptake of nanoparticles. The process consists in integrating nanoparticles in vesicles drawn from the cellular plasmalema and formation of the early endosomes, late endosomes and transferring them eventually to lysosomes. Nanoparticles generally end up in lysosomes where the cell tries to either digest or excrete those[47]. If nanoparticles are able to escape from these vesicles, they can diffuse free in the cytoplasm and enter into lipid vesicles, mitocondria or into nucleus (if their diameter is less than 9 nm[48]). After cellular uptake, they can damage the

organelles or the DNA, or cause cell death. Entering of nanoparticles in the cell and their behavior are dependent on physico-chemical characteristics (size, shape, surface reactivity, concentration, etc.), but also on the type of cell and parameters of biological environment.

MECHANISM OF ACTION OF NANOPARTICLES

Nanoparticles can exert various toxic effects on cells: oxidative stress, inflammation, genetic damage, cell division inhibition, apoptosis and even cellular death[15,49,50].

OXIDATIVE STRESS – ROS PRODUCTION

Oxidative stress is the main mechanism by which nanoparticles manifest their toxicity. There are two ways of producing oxidative stress: depletion of antioxidants and increased production of ROS (reactive oxygen species) and RNS (reactive nitrogen species)[15]. Studies show that the most common mechanism by which nanoparticles induce oxidative stress is the production of ROS. ROS consists in a large quantity of oxidative species, including: superoxide anion ($O_2^{-\bullet}$), peroxide ($O_2^{-2\bullet}$), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$)[15,49]. In the body, ROS is a byproduct of oxygen metabolism and has important roles in homeostasis and cellular signaling[51]. Large amounts of ROS can produce degradation of proteins, lipids peroxidation, damage of DNA. ROS may activate signaling networks associated with loss of cell growth, fibrosis, and carcinogenesis, and can be even cause of cell death. They have an important effect on cell membranes, including plasmalema, membrane of mitochondria or membrane of other cellular organelles[14,15,49]. There are many studies showing the production of ROS by nanoparticles as TiO_2, ZnO[52], carbon nanotubes[53], SiO_2, silver nanoparticles, etc.

INFLAMMATORY EFFECT

The inflammatory response is a protective mechanism of the organism that appears due to penetration of foreign agents in the body. When the inflammation increases beyond a certain point, causes different diseases[15]. Numerous studies have demonstrated the inflammatory effect of carbon nanotubes[54], fullerenes[55], silver nanoparticles, TiO_2, nanoparticles[56], demonstrated by appearence of allergic reactions or the development of granulomas in target tissues.

GENOTOXICITY AND CARCINOGENESIS

Cytotoxic and genotoxic effects of nanoparticles can be produced either by direct mechanism on cellular compounds (by the production of oxidative stress), or through an indirect mechanism as a result of inflammation. Small nanoparticles can penetrate the membrane of mitochondria or of the nucleus, being responsible for the occurrence of mutations, followed by the subsequent development of different granulomas or tumors[14,15,49]. ZnO, TiO_2 and other nanoparticles from sunscreen lotions can damage the DNA and histones structure and can activate p53 or similar oncogene proteins[15].

CONCLUSIONS

Nanotechnologies are currently an emerging domain of biotechnologies with broad applications in biomedicine. However, toxicity issues must be addressed before any attempt to use nanoparticles as agents in diagnostic or treatment schemes. An important step in assessing the toxicity of nanoparticles is the detailed characterization of physicochemical properties, including size, shape, composition and reactivity of surface. Another key point for using the nanoparticles in biomedical applications is that to assess whether high dose used in order to achieve biological response is greater than real dose that causing toxic effects.

In perspective, it requires the development of a regulatory framework to indicate maximum limits and optimal methods for exposure to nanoparticles.

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