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MAGNETITE (Fe_3O_4) NANOPARTICLES: ARE THEY REALLY SAFE?

NANO PARTÍCULAS MAGNÉTICAS (Fe_3O_4): ¿SON SEGURAS EN REALIDAD?

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Abstract

Iron oxide nanoparticles and in particular Fe3O4 Nanoparticles are new materials used in biotechnology and nanotechnology, Food and drugs administration has approved several coated and bare Iron oxide nanoparticless and they are actually used in biomedical applications as: Magnetic Resonance Imaging contrast agent, Thermotherapy agent, Drug carrier for cancer treatments, and so on. All this applications bring risk of: a single exposure, workplace exposure, incidental and environmental release and potential long life use. In this regard, iron oxide nanoparticles toxic effect, in particular neurotoxicity is not well known and it needs to be assessed. The aim of this review is to present several iron oxide nanoparticles in vitro and in vivo studies that reflex Fe3O4 actual an overview toxicological profile. ***Keywords***: IONP, toxic effect, risk, nanotechnology.

Resumen

Nano partículas de óxido de hierro y en particular las nano partículas Fe_3O_4 son nuevos materiales usados en biotecnología y nanotecnología, la Administración de Alimentos y Drogas ha aprobado varios nano partículas de óxido de hierro recubiertas y descubiertas que se están utilizando para aplicaciones de biomedicina tales como: agentes de contraste en Imágenes de Resonancia Magnética, agentes de Termoterapia, transportadores de fármacos para tratamientos de cáncer, entre otros. Todas estas aplicaciones implican riesgo de tipo: única exposición, exposición en el espacio laboral, descargas accidentales y ambientales y potencial uso prolongado. En este sentido, el efecto tóxico de las nano partículas de óxido de hierro, en particular de la toxicidad neuronal no son bien conocidas y necesitan ser estudiadas. El propósito de esta revisión es presentar varios estudios de nano partículas de óxido de hierro in vitro e in vivo que reflejan el perfil toxicológico de Fe_3O_4. ***Palabras claves***: IONP, efecto tóxico, riesgo, nanotecnología.

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1. Introducción

Nanotechnology is the science that deals with matter at the scale of 1 billionth of a meter (10^{-9} m = 1 nm), and is also the study of manipulating matter at the atomic and molecular levels. A nanoparticle is the fundamental component in the fabrication of a nanostructure, and is far smaller than the world of everyday objects that are described by Newton's laws of motion, but bigger than an atom or a simple molecule that are illustrated by quantum mechanics' laws (Horikoshi y Serpone, 2013).

Nanoparticles exhibit unconventional or enhanced physico-chemical properties, which are not encountered in the corresponding bulk materials (e.g., lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and specific magnetizations). Their physical and chemical properties depend not only on their composition, but also on the particle size, shape and aggregation (Jiang *et al.*, 2014).

Nowadays, nanoparticles have become trendsetter materials, every day we could find a new discovery or application development around nanoparticles. Actually, there is a catalogue of commercial nanoparticles for example: NanoGard^{TM} LL Zinc oxide, Zinc oxide NanoTek^{TM}, NanoArk^{TM} cerium oxide, NanoArk^{TM} Copper oxide, and so on. They are used as biosensors or iron nanoparticles against cancer and more, these are simple examples of their multifunctional uses. In general, biotechnology and biomedicine are two of the most highlight fields to apply nanoparticles technology.

2. Iron oxide nanoparticles (Magnetic nanoparticles)

Most materials found in Earth are generally thought of as being nonmagnetic, for example, either diamagnetic (repelled weakly from a magnetic field, as is water and almost any fatty substance) or paramagnetic (weakly attracted to a magnetic field, as is deoxyhemoglobin in blood cells). For these types of materials, the direct physical influence of the earth's magnetic field is extraordinarily weak (Kirschvink *et al.*, 1992). However, nanomaterial's magnetic property is based on its magnetic susceptibility, which is defined by the ratio of the induced magnetization to the applied magnetic field. The susceptibly of the material depends on their temperature, external magnetic field and atomic structure (Indira y Lakshmi, 2010).

The rich history of scientific interest in Iron Oxides Nanoparticles (IONPs) has been fuelled by valuable applications taking advantage of catalytic, electronic, and magnetic properties of these materials. At present, IONPs are part of nanomaterial's science and engineering and they, in particular, are developed based on their unique properties: colloidal stability, hydrodynamic diameter (HDD) and capacity to response to magnetic fields (superparamagnetism) (Zhang *et al.*, 2014). However, IONPs are actually in process of development in order to obtain new applications reducing their potential toxic effect.

IONPs are multifunctional, specifically for medical, biotechnological and pharmaceutics applications development. The materials in nanostructured form are the excellent candidates as probes because they can achieve high response to very small targets in practical conditions. Nanomaterials (e.g., nanoparticles, nanowires, nanotubes, and even Nanodevices) have been explored in many biomedical applications (e.g., biosensing, biological separation, molecular imaging, and anticancer therapy) because their novel properties and functions differ drastically from the bulk counterparts (Chi *et al.*, 2012).

At present, Fe_3O_4 iron oxide nanoparticles (Fe_3O_4-NPs) are involved in biomedical and biotechnological application, it is due to their good tolerance showed *in vivo* and the evident lower toxic effect compared with Fe_2O_3 iron oxide nanoparticles (Wang *et al.*, 2011; Pisanic *et al.*, 2007). In particular, the excellent properties of Fe_3O_4-NPs give rise to numerous multitask applications including Magnetic Resonance Imaging (MRI) contrast agents, multimodal imaging, ferrofluid technology for thermotherapy, targeted drug delivery, cancer tumor detection via magnetometry, gene therapy, biomolecular separation, *in vivo* biomolecular detection, and tissue repair (Deng *et al.*, 2014; Wu *et al.*, 2013; Singh *et al.*, 2010).

The surface engineered Fe_3O_4-NPs (e.g. with targeting ligand/ molecules attached to their surfaces) used together with the aid of an external magnetic field is recognized as a modern technology to introduce particles to the desired site where the drug is released locally represent a great advantage

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to Fe_3O_4-NPs *per se*. Engineered Fe_3O_4-NPs, such a system, has the potential to minimize the side effects and the required dosage of the drugs (Kim *et al.*, 2012). However, once the engineered Fe_3O_4-NPs are inside the cells, the coating is likely digested leaving bare particles exposed to cellular components and organelles thereby potentially influencing the overall integrity of the cells. Fe_3O_4-NPs with appropriate surface chemistry exhibit many interesting properties that can be exploited in a variety of biomedical applications such as MRI contrast enhancement, tissue repair, hyperthermia, drug delivery and in cell separation (Mahdavi *et al.*, 2013; Singh *et al.*, 2010).

3. Iron oxide nanoparticles toxicity

Nowadays, IONPs remain the only magnetic nanoparticles that have been approved for clinical use and commercially available approved by Food and Drugs Administration (FDA) (Busquets *et al.*, 2014; Mahmoudi *et al.*, 2009). However, accumulating evidence indicates that exposure to IONPs causes apoptosis and alters the functionality of macrophages. In fact, exposure of the murine macrophage cell line J774 to IONPs resulted in an increased production of intracellular Reactive Oxygen Species (ROS), with subsequent cell injury and apoptosis (Shen *et al.*, 2011). Experimental studies have shown that metal and metal oxide nanoparticles induced DNA damage and apoptosis through ROS generation and oxidative stress (Ahamed *et al.*, 2011). Fe_3O_4-NPs, in particular, could produce; oxidative stress following a direct route through Fe^{+2} and Fe^{+3} ions liberation from nanoparticles themself (Sun *et al.*, 2013), by a surface catalytic action over H_2O_2 (Lu *et al.*, 2007) producing OH and OH^- those are highly reactive species (Luo *et al.*, 2014); and producing inflammation reactions (Ahamed *et al.*, 2011; Choi *et al.*, 2009).

The direct route to produce ROS by Fenton, Fenton-like and Heber-Weiss reactions is described below. The following equation illustrated reaction models and subsequent products (Luo *et al.*, 2014; Yan *et al.*, 2013). Equations (1), (2) and (3) show the reactions mediated by Fe^{+2}

$$Fe(s) + H_2O_2 + 2H^+ \longrightarrow Fe^{+2} + 2H_2O \quad (1)$$

$$Fe_{+2} + O_2 \longrightarrow Fe^{+3} + \cdot O_2 \quad (2)$$

$$Fe^{+2} + \cdot O_2^- + 2H^+ \longrightarrow Fe^{+3} + H_2O_2 \quad (3)$$

The presence of iron Fe^{+3} ions at the end of the reactions chain produce new reactions involved in ROS production described by Yah and co-workers (2012). In this case, this reaction could reintroduce iron Fe^{+2} ions to the reaction chain and increase the ROS amounts. In the reaction (5) we could see, at the end of the reaction, the presences of Fe^{+2} ions that might enter in (2), (3) reaction, brining an virtual endless circle of reactions.

$$Fe^{+3} + H_2O_2 \longrightarrow FeOOH^{+2} + H^+ \quad (4)$$

$$FeOOH^{+2} \longrightarrow Fe^{+2} + \cdot O_2H \quad (5)$$

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^- + \cdot OH \quad (6)$$

Both, inflammation route and the surface catalytic capacity are not described well at cellular level. Over the toxicology point of view, Fe_3O_4-NPs are good tolerated *in vivo* showing no toxic effect (Wu *et al.*, 2013; Wang *et al.*, 2011; Weissleder *et al.*, 1989). In contrast, others *in vivo* studies show a fairy toxic effect over different tissue and cell, i.e. Shen and co-workers (2011) have reported Fe_3O_4-NPs induced a differential effect on antigen-specific cytokine expression by T cells. In addition, the suppressive effect of Fe_3O_4-NPs on interferon-γ was closely associated with the diminishment of glutathione. However, further information about toxicity of Fe_3O_4-NPs is missing.

4. Iron oxide nanoparticles neurotoxicity

A better understanding of how properties of nanoparticles define their interactions with cells, tissues, organs and systems in animals and humans is a scientific challenge, but one that must be addressed to ascertain the feasibility of using nanobiotechnologies in biomedical applications (Cengelli *et al.*, 2006). In general, IONPs represent a risk

because they may release iron ions, this might produce a disruption of normal iron metabolism in the brain that is a characteristic of several neurodegenerative disorders such as: Alzheimer's disease (AD), Parkinson's disease and progressive supranuclear palsy (Bajaj *et al.*, 2009). For example, excess iron accumulation is known to occur in AD patients, particularly in AD plaques and total iron levels are elevated in the hippocampus, amygdala and the cerebral cortex (Hautot *et al.*, 2003; Dobson, 2001). However, functionalized Fe_3O_4-NPs are active investigated as a T1 and T2 MRI agent, this characteristic are one of the principal strategies for develop an early AD diagnosis tool (Busquets *et al.*, 2014). Based on, detection or identification of amyloid plaques, using IONPs as negative contrast agents.

Schafer and co-workers (2000) affirm that various experiments have also demonstrated that iron-oxygen complexes may be even more effective catalysts for free-radical damage in brain tissue than the Fenton reaction, so the potential deleterious effect of magnetite is possibly even more significant. As described above, the normal brain contains approximately 60 mg of non-heme iron distributed in the parenchyma and an increase in this value could represent a risk. Because of the relative accessibility of blood, liver and bone marrow, there have been many more direct studies of iron metabolism in these organs than of that in the brain and, the great deal is known of the role of iron in the physiology and pathology of tissues outside the Central Nervous system (CNS) and their diseases related with an increase of iron oxides amounts (Schenck y Zimmerman, 2004).

The striatum and hippocampus are important structures in the brain and they are associated with the development of Parkinson's and Alzheimer's diseases (Wu *et al.*, 2013; Dobson, 2001). *In vitro* studies have demonstrated that, bare Fe_3O_4-NPs may decrease neuron viability, trigger oxidative stress, and activate JNK- and p53-mediated pathways to regulate the cell cycle and apoptosis. These results suggest that environmental exposure to Fe_3O_4-NPs may play a role in development of neurodegenerative diseases. Over PC 12 cells Xue and co-workers (2012) have conducted a study over four different Nanoparticles (SiO2-NPs, TiO2-NPs, Hydroxyapaptite (HPA)-NPs and Fe3O4-NPs) and they have showed variably enhanced secretion of cytokines by microglia. Several of these soluble factors produced by NP-treated microglia affected dopamine synthesis through the suppression of Th expression and also caused cytotoxicity to PC12 cells. This study provides important evidence into the potentially adverse effects on neurons via microglia exposed *in vitro*

Neuro-cytotoxicity and coating relationships must be assessed, with toxic effect of IONPs need to be considered. According to Deng and co-workers (2014) after exposure to different concentration of Silicon Fiber (SF)- Fe_3O_4-NPs, the reactive oxygen species generation in PC12 cells were reduced compared with uncoated Fe_3O_4-NPs. 1 to 5 days of treatment with SF- Fe_3O_4-NPs did not destroy cell membrane and cyto-skeleton, and could improve the neurons extension in a dose-dependent manner at lower concentration ($6.25 - 50$ g/mL), because SF peptide coating could delay the release of iron ions and the increase of surface crystal defects of Fe_3O_4-NPs. Intact mitochondria in a neurons indicate the extension activity of neurons of cells treated with SF-NPs.

5. Conclusions

At present, there is a considerable lack of information about general toxicity and neurotoxicity of Fe_3O_4-NPs, this lack makes imperative to assess its risk. The potential multifunctional application and new developments in biomedical application based on Fe_3O_4-NPs increase the risk of exposure. Emerging industries bring multiple work-place risk of exposure and, disposition after use of Fe_3O_4-NPs also brings an environmental risk of human exposure or ecosystems release. At the moment, there is no information about limits of exposure or referential values that can use as reference in any exposure scenery. In this regard and considering the translocation of Fe_3O_4-NPs, potential applications, approved used and incidental exposure, their toxicity must be estimated.

Iron oxide nanoparticles are widely used in the biomedical fields such as: MRI, drug and gene carriers, hyperthermia treatment agent, and magnetic separation. It is of significant meaning to assess the potential risks of IONPs considering their extensive applications. IONPs have become increasingly evident a factor, that might contribute to the development of neurodegenerative diseases, such as Parkinson's and Alzheimer's disease. Regardless of the

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route used to deliver drugs into the brain, the diffusion of the drug delivery devices into the brain parenchyma must be controllable and must avoid activation of microglial cells, since the brain possesses its own macrophage population, the microglia, which is involved in the development of neurodegenerative disorders.

Due to IONPs diameter variability, coating material used and surface charge, all result of toxic experiments have presented a greater variability reflexed over literature, for this reason, it is necessary assess one by one, every single IONPs bare or coated used or in development process.

At the present, IONPs are involved in biomedical and biotechnological applications, this is due to their good tolerance evidenced by *in vivo* studies (Pisanic *et al.*, 2007; Wang *et al.*, 2011). Furthermore, the surface engineered IONPs (e.g. with targeting ligand/ molecules attached to their surfaces) used together with the aid of an external magnetic field is recognized as a modern technology to introduce particles to the desired site, where the drug is released locally, and it represents a great advantage to IONPs *per se*. Engineered IONPs, such a system, have the potential to minimize the side effects and to release the required dosage of the drugs at the target site/tissue (Kim *et al.*, 2012). These affirmations and the fact that some IONPs (with different coating materials) are approved by FDA (Carenza *et al.*, 2014; Araki *et al.*, 2013; Kong *et al.*, 2012; Gupta *et al.*, 2007), implies IONPs are safe for human uses, biomedical applications and nanobiotechnological developments (Kim *et al.*, 2012), on the contrary consumer safety are still unknown, and it would be needed to test the "real risk" exposure, since, once the engineered IONPs are inside the cells, the coating is likely digested leaving bare particles exposed to cellular components and organelles thereby potentially influencing the overall integrity of the cells.

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