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Photoemission study of metallic iron nanoparticles surface aging in biological fluids. Influence on biomolecules adsorption

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ABSTRACT

Iron nanoparticles (nFe) prepared by vaporization and cryogenic condensation process (10–100 nm) has been exposed to Hank's balanced salt solution (HBSS) and the B-Ali cell growth fluids. These media can be used for cellular growth to study nFe penetration through cell membrane and its induced cytotoxicity. Surface chemistry of nFe exposed to such complex fluids has been characterized as the nanoparticles surface can be strongly changed by adsorption or corrosion processes before reaching intracellular medium. Particle size and surface chemistry have been characterized by scanning electron microscopy (SEM) and high-resolution X-ray photoelectron spectroscopy (HR-XPS). Exposition of nFe particles to growth and differentiation media leads to the formation of an oxy-hydroxide layer containing chlorinated species. We found that the passivated Fe_2O_3 layer of the bare nFe particles is rapidly transformed into a thicker oxy-hydroxide layer that has a greater ability to adsorb molecular ions or ionic biomolecules like proteins or DNA.

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1. Introduction

Nowadays particulate matter is strongly present in the ambient air and it has been associated with health effects such as respiratory diseases, even suspected to induced cancer and cardiovascular diseases. Some studies have shown that mortality can be correlated with the fine particles concentration in ambient air (diameter < 2.5 μm) [1,2]. Particles present in the air can be different by their size or their chemical composition (metal/oxide/organic). Ultrafine particles are potentially more toxic as they can diffuse more easily in biological systems as suggested by some studies [3].

Concerning iron nanoparticles, they have received considerable attention for their potential applications in both environment and biomedical fields. For example, zero-valent iron nanoparticles have shown potentiality for the transformation of organic contaminants and heavy metals. Extensive studies have demonstrated that nanoscale iron particles are effective for the transformation of a wide array of environmental contaminants such as chlorinated organic solvents, organo-chlorine pesticides, PCBs [4–9]. On the biomedical side, fully oxidized iron nanoparticles (called SPION) are being used for biomedical applications as for example in magnetic resonance imaging or for local drugs delivery.

These nano-sized or micro-sized particles can also appear in the environment in an unwanted way as they can be product in industrial processes (smokes in iron and steel industry) or can occur naturally in the environment. For example, small sized iron oxides as magnetite (Fe_3O_4) or maghemite (γFe_2O_3) can be produced in volcanoes smokes.

As these particles can be found in the environment, one has to wonder about their interaction with a biological system. More precisely, we have to wonder if any physiological or cellular damage could not be associated with the presence of these molecular sized particles in a living body.

In a cell, nanosized particles–cells interaction can induced biological damages resulting from DNA alterations or some protein denaturation or some oxidative stress in some cellular organites. In such mechanisms, interactions between the nanoparticle surface and the biological system are of prime importance. Different particle surface chemistry may cause alteration in gene expression and alteration in cellular responses such as activation of signaling pathways cell mechanism regulation. This peculiar cytotoxicity can be evidenced by strong perturbations of the major components of the cell as for example mitochondria, nucleus and DNA. Some studies demonstrated that exposure to SPION has been associated with inflammation, formation of apoptotic bodies, membrane leakage

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and generation of reactive oxygen species (ROS). In some studies, iron has been associated with carcinogenesis following the generation of ROS that can induce damage to DNA or proteins [10].

During SPION interaction with a biological system, particles are supposed to be corroded and release iron ions within cellular organites. This iron ions can cross the mitochondrial membrane can react with hydrogen peroxide and oxygen produced by the mitochondria leading to reactive hydroxyl radicals and Fe^{3+} ions. Transport processes involve the formation of iron complexes (for example divalent metal transporter DMT1) that allow iron to be transported across the enterocyte cell to enter in the labile iron pool [11]. Different type of iron complexes can be produced in biological fluids. In the case of chelators containing "hard" coordinating groups such as hydroxamate oxygens, the coordination sphere is full and thus prevents access of oxygen or peroxide to the complexed iron ion. For complexes with lower coordination such as bidentate or tridentate, dissociation and access to the iron center is easier and facilitate the production of damaging radical species by induction of oxidative stress [11].

Moreover, SPION can accumulate in organs such as liver or lungs subsequently to inhalation and Karlsson et al. [12] have shown that magnetite particles can cause genotoxicity via the induction of oxidative stress.

A central problem is thus the information on the nanoparticules surface chemistry evolution under given medium constraints (vapor or liquid phase) as some modifications can yield to drastic changes in their reactive properties. While macroscopic iron sample strongly reacts with oxygen or water (corrosion), leading to bulk oxidation and sample properties degradation, nano-sized iron particles look like passivated solids, that's to say it exists a thin oxide layer (some nanometers) protective toward an full oxidation of the metal. Surface oxides can be of different type (Fe_2O_3, Fe_3O_4, FeOOH) depending on material oxidation or ageing that are correlated to environment conditions and leading to different surface reactivity. The surface oxide type of the nano-sized particles should be driving the reactivity in the living medium. Magnetite (Fe_3O_4) and maghemite (Fe_2O_3) can effectively show different cellular responses. The different crystallographic structures must induce different surface sites that have different ability to undergo oxidation/reduction reactions. Magnetite has been shown to cause higher levels of oxidative DNA lesions as compared to maghemite [13]. In the living medium, nano-sized particles are in strongly ionic aqueous liquid and SPION exist as magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) [14] and corrosion processes can lead to the formation of products with surfaces that are in the range between magnetite and maghemite and maghemite or a non-stoichiometric intermediate [15] can be found in fluids as a common by-product of magnetite oxidation.

For other oxidized nano-scaled metallic particles, it has been shown that nanoparticles of CuO were more cytoxic than micrometer particles. In contrast, the micrometer particles of TiO_2 caused more DNA damage compared to the nanoparticles [16]. So no general trends can be deduced from the particle sizes and a careful attention must be paid to particle chemistry and surface reactive sites, especially after chemical modifications when exposed to biological fluids.

In this work, we present a surface characterization of iron nanoparticles (nFe) prepared by vaporization and cryogenic condensation process. These particles can be representative of industrial smokes emitted by the metallurgical industry. Previous studies on these nFe particles by transmission electron microscopy show spherical nanoparticle shape with a diameter in the range 10–100 nm [17,19]. BET method has been used to measure the specific surface of nFe particles and to confirm electron microscope images. X-ray diffraction experiments reveals that these nFe nanoparticles are crystalline (BCC) [19]. nFe nanoparticles are agglomerated in bunches due to magnetic interaction as shown in Fig. 1. Surface compositions have been characterized by high-resolution X-ray photoelectron spectroscopy (HR-XPS). Iron nanoparticles have been exposed to different biological fluids that can be used for cellular growth to study nFe penetration through cell membrane and its induced cytotoxicity. Surfaces exposed to such complex fluids must be carefully characterized as the nanoparticles surface chemistry can be strongly changed by adsorption or corrosion processes before reaching intracellular organites or systems.

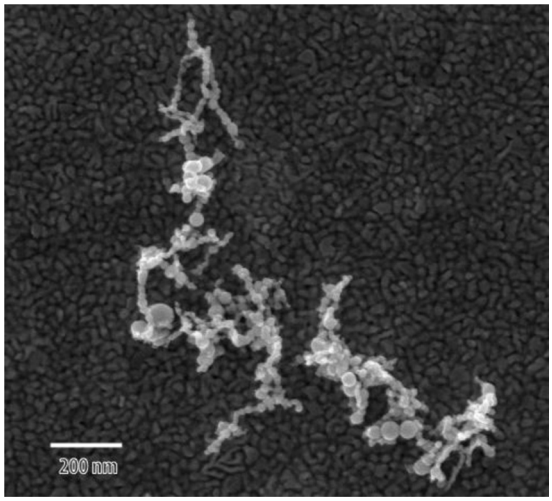

Fig. 1. MEB image of nFe nanoparticles deposited onto a gold cover silicon wafer realized with 5.0 keV electron beam energy.

Immersion in two growth and differentiation fluids have been studied: Hank's balanced salt solution (HBSS) that is a highly ionic solution the essential function of which is to maintain pH and osmotic balance as well as provide your cells with water and essential inorganic ions. The other media is the B-Ali medium that is also highly ionic nutritive medium solution but that also contains complex biological molecules for the cell culture.

We compare the influence of these two media on nFe surface chemistry by also exposing nanoparticles to a more neutral aqueous medium (Volvic mineral water). Modifications of surface oxide thin film chemistry and thickness have been investigated by photoemission to characterize the influence of growth media on the nanoparticles surface chemistry. In a final step, we investigate the influence of the surface ageing in these fluids on DNA adsorption.

2. Materials and methods

2.1. Chemicals

Metallic iron nanocrystals (nFe) have been produced using a gas condensation technique (cryogenic melting) that is described in Ref. [20]. Biologically active ionic solutions used in this work are HBSS and B-Ali. The HBSS medium is a buffered solution that mainly contains carbonate, sulfate, phosphate, calcium, sodium and chloride ions. HBSS was purchased from Life Technology. The B-Ali medium (Clonetics) that is also highly ionic solution but that also contains complex biological molecules such as insulin, hydrocortisone, transferrin or epinephrine, essential molecules in this nutritive medium for the cell multiplication. B-Ali medium was purchased from Lonza. In this study, we used a reference ageing aqueous media (mineral Volvic water) to compare the oxidation power of HBSS and B-Ali solutions. DNA solution was prepared from human lung cells. DNA extractions from lung cells were performed with QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's recommendations. The DNA was eluted in high purity water.

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