Rev. Adv. Mater. Sci. 53 (2018) 74-78

APPLICATION OF IRON OXIDE NANOPARTICLES IN CONTEMPORARY EXPERIMENTAL PHYSIOLOGY AND CELL BIOLOGY RESEARCH

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Received: July 21, 2017

Abstract. Recent findings have suggested that iron oxide nanoparticles (IONPs) have some exceptional chemical characteristics which make them useful in both experimental physiology and cell biology research. These nanoparticles might be applied as drug delivery systems for anti-cancer and other medications. Also, IONPs might be a valuable part of many novel bioassays in various fundamental medical fields. In recent years, several studies have indicated that IONPs may have certain cytotoxic and genotoxic potential in living systems. During *in vitro* conditions, IONPs might induce generation of reactive oxygen species and cause oxidative stress in some cell populations. The toxicity of IONPs is not yet fully understood, and additional research is needed to confirm if IONPs have detrimental effects on human health. This short article focuses on the recent developments and trends in the fields of nanomedicine and nanobiology regarding iron oxide nanomaterials and their application in fundamental medical disciplines such as experimental physiology. We discuss our previously published works on structural effects of IONPs and other metallic nanoparticles on cell nucleus in *in vitro* conditions. We also describe our recent findings regarding the impact of IONPs on certain biophysical properties of subcellular components.

1. INTRODUCTION

Iron-based nanomaterials have in recent years been a focus of extensive research in the field of cell biology and fundamental medical sciences. Similarly to other nanomaterials, iron oxide nanoparticles (IONPs) have some important physical and chemical features which may enable them to be applicable as drug-delivery systems, part of various bioassays and potentially even a part of diagnostic procedures. Because of all these possible medical uses, IONPs are considered to be excellent candidates for introduction to some forms of clinical practice, providing that they do not exhibit significant toxicity on human tissues and organs [1-3].

Iron oxide nanoparticles may display some unique paramagnetic properties because of which they may be used during magnetic hyperthermia, an experimental treatment for some types of cancers. Generally, ferrite particles, when they are sufficiently small, become superparamagnetic. This essentially means that there is no self-agglomera-

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tion, while magnetic behavior exists after exposure to an external magnetic field. This induction and control by an external source, may be used to specifically target and damage tumor tissue while surrounding structure remains relatively intact [2,4,5].

This short article focuses on the recent developments and trends in the fields of nanomedicine and nanobiology regarding iron oxide nanomaterials and their application in fundamental medical disciplines such as experimental physiology. We discuss our previously published works on structural effects of IONPs and other metallic nanoparticles on cell nucleus in *in vitro* conditions. We also describe our recent findings regarding the impact of IONPs on certain biophysical properties of subcellular components.

2. CHEMISTRY OF IRON OXIDE NANOPARTICLES

As with other nanoparticles, the size of IONPs ranges between 1 and 100 nanometers. There are two major chemical types of IONPs with different characteristics: magnetite (Fe_3O_4), with both Fe^{3+} and Fe^{2+} in its structure, and maghemite (γ-Fe_2O_3), where iron is in in the trivalent state [2,4-6]. Magnetite can easily be transformed to maghemite because it is prone to oxidation:

$$2Fe_3O_4 + O_2 \rightarrow 2\gamma Fe_2O_3 .$$

There are several ways to synthesizing IONPs. Coprecipitation is relatively commonly used in many laboratories. Ferric and ferrous hydroxides can be combined in aqueous solutions to produce relatively stable magnetite nanoparticles:

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O .$$

One of the main potential problems during synthesis of IONPs is the control of the particle shape and size. They depend on numerous factors, some of which can be controlled in laboratory setting. They include acidity of the solution, temperature, the overall concentration of ions, as well as the addition of other compounds.

The variations of the structure and diameter of IONPs are the important issue in physiology and cell biology with potentially significant implications on the research results and validity of experiments. In biomedical research, cells and tissues are exposed to IONPs after which adequate measurements are recorded (i.e. parameters of oxidative stress, apoptosis, changes in gene expression, changes in signaling pathways etc.). However, intracellular environment may significantly differ in comparison to extracellular space in terms of concentrations of different ions, acidity and other chemical and physical factors. All these factors may influence the chemistry of iron-based nanomaterials, changing their size, surface area, shape and other parameters [2]. In some cases, iron oxide nanoparticles could become ordinary iron oxide compounds which are larger than 100 nanometers in diameter, causing them to lose the status of nanoparticles.

Therefore, one of the major questions posed in nanobiology is the following: Is the effect (morphological or physiological) of the nanoparticle on a living cell the result of the nanoparticle itself, or is the nanoparticle transformed into a different compound/ion/larger molecule which then exhibits its effects on the cell? Unfortunately, in many experiments and published studies, this question remains unanswered which may hamper future efforts to understand true biological effects of metallic nanomaterials.

3. IRON-BASED NANOMATERIALS AND PARAMETERS OF OXIDATIVE STRESS

Oxidative stress is usually the result of the increased production of substances belonging to reactive oxygen species, and/or the decreased ability of organism/cell to eliminate them or repair the damage induced by them. Peroxides, superoxide, hydroxyl radical, and singlet oxygen are all being produced in physiological conditions either as a byproduct of various chemical and metabolic reactions or with a specific role in a physiological function (i.e. in immune system). Products of oxidative stress may damage genetic material of the cell in many ways, including the braking of strands in DNA molecule. This can in turn cause disruptions in normal protein production and in some cases the malignant transformation of the cell [7-12].

Metal ions may in some circumstances increase the production of reactive oxygen species. This may be done by redox cycling since metal ions can act as a donor or receiver of electrons. Iron ions are also capable of these interactions. With the principles of “Fenton chemistry” ferrous iron and hydrogen peroxide can efficiently oxidize organic compounds. Both Fe^{2+} and Fe^{3+} may react with hydrogen peroxide to produce additional free radicals:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ ,$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^- ,$$

Fenton reaction may play a significant role in accumulation of DNA damage, protein inactivation, disruption of signaling pathways and the process of aging.

Despite several important studies and publications, it remains unclear if and at what rate IONPs may induce the generation of reactive oxygen species. In a recent article, Yarjanli et al. (2017) indicate that iron oxide nanoparticles may cause damage to the neural tissue by affecting the glutathione levels and substantially increasing the redox cycling due to their large surface area [13]. Glutathione is an important antioxidant which, after being oxidized, is reduced back by glutathione reductase with the help of nicotinamide adenine dinucleotide phosphate (NADPH). In the future it would be interesting to clarify the relationship between iron-based nanomaterials, other non-nanoparticle iron compounds and glutathione levels, not only in neural, but also in other tissues.

Gaharwar and Paulraj (2015) studied potential oxidative damage in rat peripheral blood cells [14]. The study was done on Wistar rats Fe_2O_3 nanoparticles induced oxidative stress perhaps by starting an inflammatory response. Ahamed et al. (2013) found that Fe_3O_4 nanoparticle also induce oxidative stress and even genotoxicity in in vitro conditions [15]. Also, in the human breast cancer cell line Fe_2O_3 nanoparticles were found to generate oxidative stress, DNA damage, and caspase activation [7]. The study of Alarifi et al. (2014) is particularly interesting since it provides a comprehensive and detailed insight not only on Intracellular ROS but also on catalase activity, superoxide dismutase, extent of membrane lipid peroxidation and glutathione estimation [7].

4. IRON OXIDE NANOPARTICLES AS DELIVERY SYSTEM FOR ACTIVE SUBSTANCES

Iron oxide nanoparticles can be used as vehicles for targeted drug delivery to damaged tissues and cells. This concept is not new, and there have been numerous studies trying to connect the unique physical and chemical features of IONPs with the ability to deliver a medication or some other active substance to a specific part of the organism [4,16-27].

For example, in 2008, the study by Beata Chertok and associates [28], investigated the possibility of IONPs being used as a magnetic-targeted delivery system for orthotopic 9L-gliosarcoma in a rat experimental model. The authors used magnetic resonance imaging for estimation of the extent and selectivity of nanoparticle accumulation in cancer tissue. The exposure of the tumor to nanoparticles was proved to be significantly increased after magnetic targeting [28].

In breast cancer cells, magnetic IONPs can also be applied as drug delivery medium. In 2013, the research of Marcu et al. showed that MCF-7 cell line of breast adenocarcinoma, IONPs associated with the antracyclinic antibiotic Violamycine B1 exhibit certain anti-tumor effects [29]. However, it should be noted that nanoparticles do not make the drug more efficient, but rather just increase the cellular uptake and availability/delivery.

Also in 2013, Xing et al. managed to apply chemical changes to hollow iron oxide nanoparticles and to encapsuated anti-cancer drug doxorubicin (DOX) in the hollow porous of the particle system [30]. This was done using dopamine-plus-human serum albumin (HSA) method. This carrier proved efficient in increasing drug uptake to multidrug resistant OVCAR8-ADR cells [30].

Hollow magnetic iron oxide nanoparticles were successfully used in yet another study, for delivery of an anti-inflammatory drug, sodium meclofenamate. Alan et al. (2016) manufactured the IONPs using solvothermal decomposition of an urea/$FeCl_3 \cdot 6H_2O$ complex, and solvent ethylene glycol [17]. This was an *in vitro* chemical study, testing the adsorption and release capacity of sodium meclofenamate [17].

In the future, before IONPs can be successfully applied as drug carriers in clinical practice, several important issues need to be resolved. First, it is important to do extensive tests on animal experimental models to better understand pharmacokinetic properties of IONPs of different shapes and sizes. This includes the studies on bioavailability, distribution, accumulation and elimination of the particles in a living organism. Interactions between IONPs and kidney and liver tissues also need to be clarified. Second, the efficiency of IONPs as drug carriers in tumor tissues need to be determined. This includes not only the effectiveness of delivery but also the rate of cellular uptake and accumulation, as well as the deposition of IONPs in healthy tissues. Third, toxicity of IONPs need to be further investigated. Iron oxide as a compound is generally viewed as relatively non-toxic, however in nanoparticle form, important chemical features may change leading to cell and tissue damage. Oxidative stress is only one possible consequence. Genotoxicity, nephrotoxicity, and hepatotoxicity also need to be examined. Finally, little is known about interactions between IONPs and the immune sys-

tem. It is unclear if iron-based nanomaterials can have immunostimulative, immunosuppressive or any other immunomodulatory effects in living organisms. Only after all these questions are answered, IONPs may become an integral part of conventional therapeutic approaches in oncology, internal medicine and other clinical disciplines.

5. EFFECTS OF IRON OXIDE NANOPARTICLES ON STRUCTURE OF CELL NUCLEUS

Recently, there have been many studies trying to investigate possible interactions between IONPs and genetic material in the nucleus. The findings that IONPs can generate compounds belonging to reactive oxygen species, indicate that these nanoparticles may in some circumstances have detrimental effects on the cell nucleus and DNA. Indeed, some authors have suggested that IONPs may have certain genotoxic potential, however, these assumptions need to be confirmed in future research.

Particularly interesting is the possibility that IONPs may in some way change the structural features of the entire nucleus. Our recent study has indicated that IONPs induce time-dependent changes in nuclear fractal characteristics in buccal epithelial cells [31]. We treated these cells with IONPs, after which we measured the values of fractal dimension (parameter of complexity) and lacunarity (indirect parameter of fractal heterogeneity) in different time points. We observed statistically significant reduction of fractal dimension and increase of nuclear lacunarity after the treatment. This study indicated that changes in genetic material of the cells after exposure to IONPs may be present on a higher level of structural organization (nucleoplasm level) compared to previous studies that only tested genotoxicity on the molecular level. The detected changes in nuclei were partially similar to the ones seen in the previous study on silver nanoparticles [32].

Our current research is focused on the potential effects of IONPs on textural features of cell nucleus. For quantification of nuclear texture, we apply Grey level co-occurrence matrix algorithm which is in essence a mathematical method that utilizes second order statistics to calculate textural characteristics such as entropy, angular second moment and inverse difference moment. Our preliminary results indicate that there is a time-dependent increase of nuclear textural entropy after the treatment with IONPs. These findings suggest that IONPs may increase the level of structural disorder in cell nuclei. For example, on a sample of 30 buccal epithelial cells, we measured the increase of nuclear entropy from the value of 5.32 to the average value of 5.81 (more than 9% increase), which is considerable, having in mind the exactness and relative accuracy of the method. To our knowledge, this is the first study to investigate the effects of IONPs in this experimental setting applying this kind of mathematical method.

6. CONCLUDING REMARKS

Iron oxide nanoparticles are potentially important drug delivery systems, applicable in various medical disciplines. Recent findings have suggested that IONPs have some exceptional chemical characteristics which make them useful in both experimental physiology and cell biology research. In recent years, there have been indications that IONPs may induce the generation of compounds belonging to reactive oxygen species. Oxidative stress induced by IONPs is an important issue in current pharmacology research since it may prevent further IONP application in clinical medicine. Before IONPs are introduced in clinical practice as part conventional diagnostic/therapeutic procedures, questions on their toxicity need to be answered. This is particularly the case with genotoxicity, nephrotoxicity and hepatotoxicity. In the future, it is expected that IONPs will become the focus of many new research efforts in the fields of molecular biology, physiology and oncology.

ACKNOWLEDGEMENTS

The authors are grateful to the project 92018 of the Mediterranean Society for Metabolic Syndrome, Diabetes and Hypertension in Pregnancy DEGU (Dr Igor Pantic, principal author of this manuscript, is the Head of the project), as well as to the projects of The Ministry of Education and Science, Republic of Serbia (projects 175059, 41027 and 175015). Prof. Igor Pantic is also grateful to NSF Center for Advanced Knowledge Enablement, Miami, FL, USA (I. Pantic is an external research associate).

REFERENCES

[1] A.C. Sintov, C. Velasco-Aguirre, E. Gallardo-Toledo, E. Araya and M.J. Kogan // *Int Rev Neurobio*l **130** (2016) 199.

[2] J. Paunovic, D. Vucevic, T. Radosavljevic, S. Pantic, D. Nikolovski and I. Pantic // *Rev Adv Mater Sci* **49** (2017) 123.

[3] R. Xing, A. Bhirde, S. Wang, X. Sun, G. Liu, Y. Hou and X. Chen // *Nano Research* **6** (2013) 1.
[4] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst and R.N. Muller // *Chem Rev* **108** (2008) 2064.
[5] A.S. Teja and P.Y. Koh // *Progress in Crystal Growth and Characterization of Materials* **55** (2009) 22.
[6] K.H.G. Buschow, H*and Book of Magnetic Materials* (Elsevier, New York, 2006).
[7] S. Alarifi, D. Ali, S. Alkahtani and M.S. Alhader // *Biol Trace Elem Res* **159 (**2014) 416.
[8] H. Cichoz-Lach and A. Michalak // *World J Gastroenterol* **20** (2014) 8082.
[9] R. Ebabe Elle, S. Gaillet, J. Vide, C. Romain, C. Lauret, N. Rugani, J.P. Cristol and J.M. Rouanet // *Food Chem Toxicol* **60** (2013) 297.
[10] K.P. Kuruvilla, M.S. Nandhu, J. Paul and C.S. Paulose // *J Neurol Sci* **331** (2013) 31.
[11] P. Muriel // *Hepatol Int* **3** (2009) 526.
[12] H. Jaeschke // *J Gastroenterol Hepatol* **26** Suppl 1 (2011) 173.
[13] Z. Yarjanli, K. Ghaedi, A. Esmaeili, S. Rahgozar and A. Zarrabi // *BMC Neurosci* **18** (2017) 51.
[14] U.S. Gaharwar and R. Paulraj // *J. Biomedical Science and Engineering* **8** (2015) 274.
[15] M. Ahamed, H.A. Alhadlaq, J. Alam, M.A. Khan, D. Ali and S. Alarafi // *Curr Pharm Des* **19** (2013) 6681.
[16] A. Aires, S.M. Ocampo, B.M. Simoes, M. Josefa Rodriguez, J.F. Cadenas, P. Couleaud, K. Spence, A. Latorre, R. Miranda, A. Somoza, R.B. Clarke, J.L. Carrascosa and A.L. Cortajarena // *Nanotechnology* **27** (2016) 065103.
[17] V. Alan, C. Isabel, M. Eugenia, G. Roberto, J. Coffer and M. Mendez-Rojas // *J Nanomed Res* **3** (2016) 00071.
[18] J. Estelrich, E. Escribano, J. Queralt and M.A. Busquets // *Int J Mol Sci* **16** (2015) 8070.
[19] K. Hola, Z. Markova, G. Zoppellaro, J. Tucek and R. Zboril // *Biotechnol Adv* **33** (2015) 1162.
[20] M.H. Hsiao, Q. Mu, Z.R. Stephen, C. Fang and M. Zhang // *ACS Macro Lett* **4** (2015) 403.
[21] J. Hwang, E. Lee, J. Kim, Y. Seo, K.H. Lee, J.W. Hong, A.A. Gilad, H. Park and J. Choi // *Colloids Surf B Biointerfaces* **142** (2016) 290.
[22] S. Kossatz, J. Grandke, P. Couleaud, A. Latorre, A. Aires, K. Crosbie-Staunton, R. Ludwig, H. Dahring, V. Ettelt, A. Lazaro-Carrillo, M. Calero, M. Sader, J. Courty, Y. Volkov, A. Prina-Mello, A. Villanueva, A. Somoza, A.L. Cortajarena, R. Miranda and I. Hilger // *Breast Cancer Res* **17** (2015) 66.
[23] B. Rajan, S. Sathish, S. Balakumar and T. Devaki // *Environ Toxicol Pharmacol* **39** (2015) 727.
[24] K. Sathish Kumar and V. Jaikumar // *Iran J Pharm Res* **10** (2011) 415.
[25] H.I. Tong, W. Kang, Y. Shi, G. Zhou and Y. Lu // *Int J Pharm* **505** (2016) 271.
[26] H. Unterweger, D. Subatzus, R. Tietze, C. Janko, M. Poettler, A. Stiegelschmitt, M. Schuster, C. Maake, A.R. Boccaccini and C. Alexiou // *Int J Nanomedicine* **10** (2015) 6985.
[27] V. Valdiglesias, N. Fernandez-Bertolez, G. Kilic, C. Costa, S. Costa, S. Fraga, M.J. Bessa, E. Pasaro, J.P. Teixeira and B. Laffon // *J Trace Elem Med Biol* **38** (2016) 53.
[28] B. Chertok, B.A. Moffat, A.E. David, F. Yu, C. Bergemann, B.D. Ross and V.C. Yang // *Biomaterials* **29** (2008) 487.
[29] A. Marcu, S. Pop, F. Dumitrache, M. Mocanu, C.M. Niculite, M. Gherghiceanu, C. Lungu, C. Fleaca, R. Ianchis, A. Barbut, C. Grigoriu and I. Morjan // *Applied Surface Science* **281** (2013) 60.
[30] R. Xing, A.A. Bhirde, S. Wang, X. Sun, G. Liu, Y. Hou and X. Chen // *Nano Research* **6** (2013) 1.
[31] D. Nikolovski, S. Dugalic and I. Pantic // *J Microsc* (2017) **268** (2017) 45.
[32] I. Pantic, J. Paunovic, M. Perovic, C. Cattani, S. Pantic, S. Suzic, D. Nesic and G. Basta-Jovanovic // *J Microsc* **252** (2013) 286.