The Effects of Iron Oxide Nanoparticle on Differentiation of Human Mesenchymal Stem Cells to Osteoblast

Tahereh Foroutan [1*], Mohamad Zaman Kasaie [2]

1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.
2. Department of Chemistry, Faculty of Basic Science, Tarbiat Modares University, Tehran, Iran.

Tahereh Foroutan received her BS in biology from Kharazmi University. Then, she studied histology in Tarbiat Moadares University. She received her PhD in developmental biology from Kharazmi University in 2010. She is a member of animal sciences department of biological sciences faculty of Kharazmi University. She is interested in developmental biology, Stem cell application for regenerative medicine respectively.

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ABSTRACT

Introduction: Iron oxide nanoparticles (IO NP) have an increasing number of biomedical applications. To date, the potential cytotoxicity of these particles remains an issue of debate. Little is known about the cellular interaction or toxic effects of IO NP on differentiation of stem cells. The aim of the present study was to investigate the possible toxic role of different doses of IO NP in differentiation of human mesenchymal stem cells (hMSCs) derived bone marrow to osteoblast.

Methods: hMSCs were seeded on normal stem cell medium with added 20 and 70µg/ml of IO NPs. Post confluence (2 weeks), cells growing cell viability was measured by 3-(4, 5-Dimethylthiazol-2-yl) -2, 5 -diphenyltetrazolium bromide (MTT) assays. hMSCs at passage 2 were cultured in osteogenic medium added with 20 and 70 µg/ml of IO NPs. The expression of osteogenesis markers of osteopontin, osteocalcine in different groups was compared by RT-PCR assay.

Results: Our findings showed that cell viability of hMSCs cultured in normal stem cell media containing 20µg/ml IO NPs was significantly ($P>0.05$) lower than control and 70µg/ml dose of IO NPs groups, while there was no significant difference between control and 70µg/ml dose of IO NP. The expression level of osteoblast markers osteopontin and osteocalcin in hMSCs differentiated to osteoblast in dose with 20µg/ml IO NPs was significantly lower than the other groups, while the expression level of osteopontin and osteocalcin in 70µg/ml IO NPs dose was insignificantly higher than control group.

Conclusion: To summarize, the presence of IO NPs with low dose influenced the cell viability cells in normal stem cell media, demonstrating toxicity of this material with 20µg/ml. It could be probably due to penetrating particles throughout cell membrane. This represents a critical aspect to its successful use for stem cell-based regenerative medicine strategies.

Key Words:
Iron oxide nanoparticle, Mesenchymal stem cells, Cell differentiation, Osteogenesis

*** Corresponding Author:**
Tahereh Foroutan, PhD
Address: Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.
E-mail: foroutan@khu.ac.ir

1. Introduction

As such in vitro characterization, it is essential to ensure the bio-safety of these particles.

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical fields. The use of IO NPs in biomedical research is steadily increasing, leading to the rapid development of novel IONP types and an increased exposure of cultured cells to a wide variety of IO NPs. IOs are iron oxide particles with diameters between about 1 and 100 nanometers. They have attracted extensive interest due to their super paramagnetic properties and their potential applications in many fields.

It is still unclear whether IO NPs are generally safe or should be used with caution. During the past years, several contradictory observations have been reported, which highlight the great need for a more thorough understanding of cell–IONP interactions. To improve our knowledge in this field, there is a great need for standardized protocols and toxicity assays that would allow to directly compare the cytotoxic potential of any IO NP type with previously screened particles. During the past years, several contradictory observations have been reported, which highlight the citotoxicity effect of IO. To date, the potential differentiation of these particles remain an issue of debate. Interestingly, nano graphen has recently been shown to accelerate the osteogenic differentiation of human mesenchymal stem cells without harming the cell shape and attachment [1]. This represents a critical aspect to nanomaterials successful use for stem cell-based regenerative medicine strategies.

Mesenchymal stem cells (MSCs) are critical for numerous ground breaking therapies in the field of regenerative medicine. A myriad of environmental factors including their interaction with soluble growth factors, extracellular matrices and neighboring cells are crucial for their survival, proliferation and differentiation into specific lineages [2-4]. One of the main goals of tissue engineering is to control these factors by creating physical and chemical microenvironments designed to guide the ultimate fate of stem cells. Materials with different elasticity, rigidity and texture have been extensively investigated for this purpose. Stem cell scaffolds, which can be both 2D and 3D in nature, have been fabricated to mimic the intrinsic characteristics of natural substrates such as muscle, bone and cartilage [5-7].

In particular in the field of bone tissue engineering, almost all artificial materials require the multiple administrations of growth factors to promote hMSC differentiation.

Interestingly, some nano particles such as geraphene have recently been shown to accelerate the osteogenic differentiation of human mesenchymal stem cells without harming the cell shape and attachment [1]. Given the promise of adult stem cells in regenerative medicine and IO materials as biomaterials, the overriding objectives of this study were to evaluate the differentiation of culturing MSCs in osteogenic medium in the presence of IO with 50 nm in diameter and dose of 20 and 70µg/ml, and to assess how the expression of the osteoblast markers is compared to control group.

2. Materials & Methods

Cell lines and markers

Human MSCs were purchased from Royan institute and cultured in low-glucose Dulbecco's modified eagle medium (Sigma) supplemented with 10% FBS (Invitrogen), 1% penicillin/streptomycin (Gibco), 1% Non-essential amino acids (Sigma) and 1% sodium pyruvate (Sigma). hMSCs at passage 2 were used in this study. Osteogenic medium consisting of DMEM basal medium

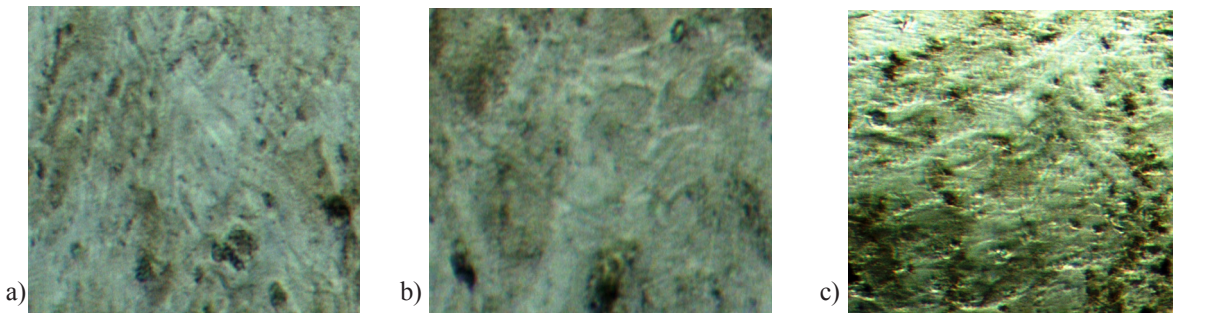

Figure 1. The hMSCs cultured in the osteogenic medium contained 20 (a) and 70qg/ml (b) NP incubated for 21 days. (c) control group showing hMSCs cultured in the osteogenic medium without any IO NP.

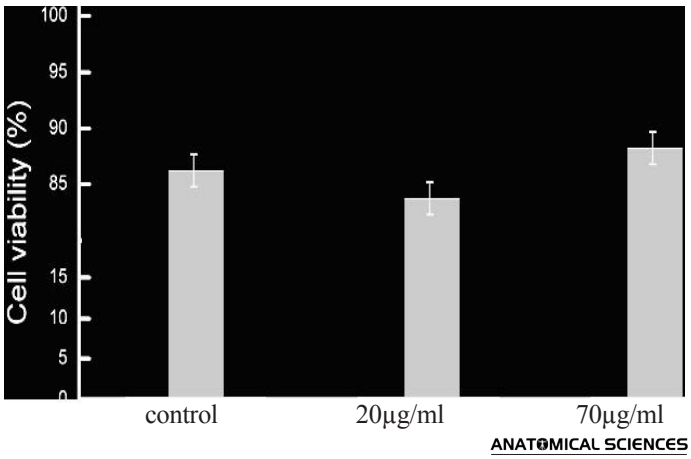

Figure 2. Graph showing the percentage of cell viability of hMSCs after 24 hours exposure to 20 (b) and 70 mg/ml ©of IONP in comparison to hMSCs grown on medium without particle, control group (a), as determined by MTT assay.

(Sigma) added with dexamethasone, L glutamine, ascorbic acida and Beta-glycerophosphate was prepared according to a known procedure [8].

Cell viability

hMSCs (20,000 cells/well (24 well plate)) were seeded on uncoated (control) and graphene coated (test) chips and cultured in normal stem cell medium. Post confluence (2 weeks), cells growing on each chip were transferred to new well plate and washed 3 times with 2 ml of PBS. 1 ml of PBS was added to each well followed by 5µl of 1mM Calcein acetoxymethyl ester (Calcein AM) and incubated at room temperature for 15 minutes. After removing the unbound stains, the chips were inverted onto glass slides mounted with vectashield with DAPI (H 1200, Vector labs) and visualized under fluorescence microscope (Nikon AZ-100 multipurpose microscope). Figures were taken at 40 different positions of the chips and processed by image J software to count the number of viable cells to the number of nucleus as determined by staining with DAPI. Cell viability was measured by comparing the cell numbers for each substrate with the cells counted on cover slips. In addition, (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays were carried out, in which cytotoxicity evaluation was based on the activity of enzymes to reduce MTT to formazan dyes, giving a purple color.

3. Results

Our results showed that each of the three groups revealed osteoblast morphology (Figure 1). Cell viability of hMSCs cultured in medium containing 20µg/ml IO NPs was significantly (P>0.05) lower than the other control groups, and 70µg/ml dose of IO NPs groups (Figure

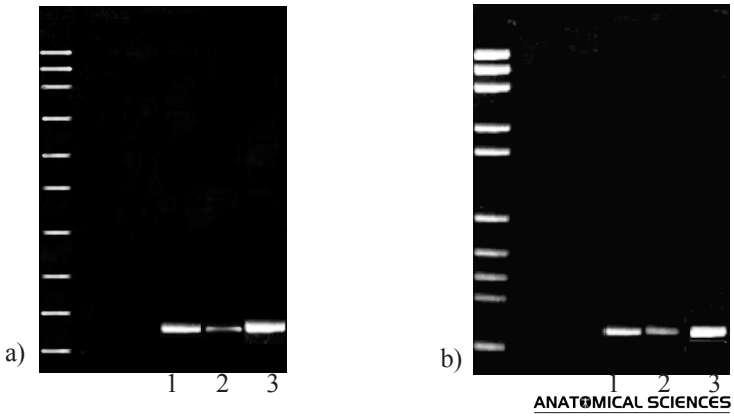

Figure 3. Comparison of the expression of osp (a) and osc (b) genes of hMSCs differentiated to osteoblast in control group (1) and either treated with 20qg/ml (2) or 70qg/ml (3) IO. We studied these samples at specific time points of 21 days. In 20qg/ml of IO, osteoblast showed a weak expression of osteopontin and osteocalsin compare to the other groups.

2). There was no significant difference between control and 70µg/ml dose of IO NP. Also the expression level of osteoblast markers of osteopontin and osteocalcin in hMSCs differentiated to osteoblast in 20µg/ml IO NPs was significantly lower than two other control groups and 70µg/ml IO NPs (Figure 3). The expression level of osteoblast markers of osteopontin and osteocalcin in 70µg/ml IO NPs dose was insignificantly higher than control group.

Cellular Growth and Survival was determined by MTT assay. There was no significant difference ($P>0.05$) in cell viability between IO NP and without substrates, demonstrating that cell growth was indeed not affected by the presence of nanoparticles. Specific markers were used to determine the conversion of hMSCs into specific cell types when cultured in osteogenic media. In none of substrates studied here, the osteogenic medium alone was sufficient to lead to osteogenic differentiation over the whole duration of the experiment (15 days). In the absence of IO, the expression of osteogenic markers were lower than experimental groups, this was demonstrated by RT-PCR assay of 3 typical protein markers, namely osteopontin, osteocalcin for osteoblasts. These three genes showed a thick expression in 70µg/ml groups compared to control and 20µg/ml groups. On the other hand, the expression of genes in control group was higher than 20µg/ml group. However, once these IO NPs with dose of 70µg/ml were added to different medium, hMSCs enhanced their differentiation into osteoblast.

4. Discussion

In this study we showed that there was no significant difference in cell viability and differentiation rate of hMSCs to osteoblast between experimental and control groups, indeed the expression of osteogenic markers at dose 20µg/ml of IO NPs did not change compared to control group.

The results of the present study suggest that IO is non-cytotoxic in mentioned dose. The in vitro labeling of cultured cells with iron oxide nano particles (NPs) is a frequent practice in biomedical research. One of current problems for nanomedicine involves understanding the issues related to toxicity of nanomaterials. Due to the large variation in incubation conditions, IO NP characteristics, and cell types studied, it is still unclear whether IO NPs are generally safe or should be used with caution [2]. Some reports have highlighted the importance of in-depth cytotoxic evaluation of cell labeling studies as at non-toxic concentrations, some particles appear to be less suitable for labeled cells. Some investigations indicate size- and surface coating-dependent toxicity to cells in terms of mass concentration. So in terms of the number of particles per well and the resultant total surface area per well, the larger diameter IO NPs are more toxic than those of smaller size [9]. In contrast to Ying, Zhu concluded that both of the two-sized 22 and 280 nm IO particle intra tracheal exposures could induce lung injury. He suggested that the nano-sized IO particle may increase microvascular permeability and cell lysis in lung epitheliums and disturb blood coagulation parameters significantly [10].

Chen and colleagues showed that IO NPs had a concentration-dependent cytotoxicity, and they could enhance H_2O_2-induced cell damage dramatically [11]. They showed that IO nanoparticles could catalyze H_2O_2 to produce hydroxyl radicals in acidic lysosome mimic conditions which was consistent with their peroxidase-like activities. They reported that IO NPs located in lysosomes increase acidic environment of lysosomes and therefore contribute to hydroxyl radical production [11]. However, in this study IO NP with 50 nm size did not induce toxicity in experimental groups significantly.

Our data showed that IO accelerates cell differentiation in osteogenic medium compared to control group. To date, osteogenic differentiation and signal transduction pathways of MSCs cultured on IO nano particles are poorly understood. In particular in the field of bone tissue engineering, almost all artificial materials require the multiple administrations of growth factors to promote hMSC differentiation. Both natural and synthetic materials have been fabricated for transplantation of stem cells and their specific differentiation into muscles, bones and cartilages [1]. One of the key objectives for bone regeneration therapy to be successful is to direct stem cells' proliferation and to accelerate their differentiation in a controlled manner through the use of growth factors and osteogenic inducers [1]. Here we show that IONP provides a promising biocompatible scaffold that does not hamper, but accelerates differentiation of human mesenchymal stem cells (hMSCs) into bone cells.

The differentiation rate is comparable to the one achieved with common growth factors, demonstrating IO NPs potential for stem cell research. Interestingly, some nano particles such as geraphene have recently been shown to accelerate the osteogenic differentiation of human mesenchymal stem cells without harming the cell shape and attachment [1]. In this research the difference in the expression of osteogenic markers in two experimental and control groups might affect the types of MSCs surface receptors that can bind to IO nano par-

ticles and differentially regulate the signal transduction pathways, thereby leading to the disparities in the differentiation propensity. In the field of bone tissue engineering, many approaches face challenges when it comes to scalability and compatibility with implants. For example, an alloplastic (non-biologic) material under mechanical stress may not respond in a similar way as the surrounding host bone, and thus resulting in structural failure of the implant or inflammatory changes in the original bone, as seen in stress shielding. Also, bioactive implants still face limitations in terms of potential pathogenic infections, low availability and high costs. IO NPs may provide an elegant material to some of these challenges. Our data indicate that the use of IO NPs in osteogenic differentiation would be an efficient way to diminish long-term toxic potential.

It seems IO NPs are attracted to each other to form larger cores that can not pass through the cell membrane, and therefore show less toxicity compare to the other groups. To summarize, the presence of IO NPs with low dose influenced the cell viability cells in normal stem cell media, demonstrating toxicity of this material with 20µg/ml. It could be probably due to penetrating particles throughout cell membrane. In the presence of an osteogenic medium, 70µg/ml IO NPs differentiation of hMSCs to osteoblast increased compare to control group. This represents a critical aspect to its successful use for stem cell-based regenerative medicine strategies. Apparently, in higher dose, IO NPs are attracted to each other to form larger cores that can not pass through the cell membrane, and therefore show less toxicity compared to the other groups. In addition, it is scalable and provides a cost effective way to enhance biological tissues.

References:

[1] Nayak TR, Andersen H, Makam VS, Khaw C, Bae S, Xu X, et al. Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells. ACS Nano. 2011; 5(6):4670e8.

[2] Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. Nature. 2001; 414(6859):98–104.

[3] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nature Biotechnology. 2005; 23(1):47–55.

[4] Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science. 2009; 32(5935):1673–1677.

[5] Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. Journal of Cellular Biochemistry. 1997; 64(2):295–312.

[6] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006; 126(4):677–689.

[7] Reilly GC, Engler AJ. Intrinsic extracellular matrix properties regulate stem cell differentiation. Journal of Biomechanics. 2010; 43(1):55–62.

[8] Fahmi H, Pelletier JP, Mineau F, Martel-Pelletier J. 15d-PGJ2 is acting as a 'dual agent' on the regulation of cox-2 expression in human osteoarthritic chondrocytes. Osteoarthritis and Cartilage. 2002; 10(11):845–848.

[9] Soenen SJ, De Cuyper M, De Smedt SC, Kevin B. Methods in Enzymology. 2012; 509:195–225.

[10] Zhu MT1, Feng WY, Wang B, Wang TC, Gu YQ, Wang M, et al. Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. Toxicology. 2008; 247(2-3):102-11.

[11] Chen Z, Yin JJ, Zhou YT, Zhang Y, Song L, Song M, Hu S, Gu N. Dual enzyme-like activities of iron oxide nanoparticles and their implication for diminishing cytotoxicity. ACS Nano. 2012; 6(5):4001-12.