

Synthesis and Cytotoxicity Assessment of Gold-coated Magnetic Iron Oxide Nanoparticles

Keshtkar M.¹, Shahbazi-Gahrouei D.^{1*}, Mehrgardi M. A.², Aghaei M.³, khoshfetrat S. M.²

ABSTRACT

Introduction: One class of magnetic nanoparticles is magnetic iron oxide nanoparticles (MIONs) which has been widely offered due to of their many advantages. Owing to the extensive application of MIONs in biomedicine, before they can be used in vivo, their cytotoxicity have to be investigated. Therefore, there is an urgent need for understanding the potential risks associated with MIONs.

Materials and Methods: Firstly, gold-coated Fe₃O₄ nanoparticles (GMNP) were synthesized. The size, structure and spectroscopic properties of the nanoparticles were characterized by transmission electron microscopy (TEM), X-ray diffractometry (XRD) and UV-Visible spectrophotometer, respectively. Cytotoxicity of nanoparticles was studied with different concentrations ranging from 10 µg/mL up to 400 µg/mL and for different incubation times (12 hours and 24 hours) on MCF-7 and HFFF-PI6. Cytotoxicity study was performed by MTT assay.

Results: XRD pattern confirmed the structure of GMNPs and TEM image shows that GMNPs are under 50 nm. For MCF-7 and HFFF-PI6 cells, at concentration of 300 and 400 µg/mL, Fe₃O₄ nanoparticles are toxic, respectively. Moreover, for both cells, cell viability for GMNPs is higher than %80, therefore, up to 400 µg/mL they are not toxic. Results show that for both cells, Fe₃O₄ nanoparticles have higher cytotoxicity than GMNPs.

Conclusion: This finding suggests that gold coating reduces the toxic effects of uncoated Fe₃O₄ nanoparticles. Less toxicity of GMNP may be attributed to controlled release from Fe²⁺ ions in intracellular space. Moreover, cell toxicity increased with raise in dose (concentration) and incubation time.

Keywords

Iron Oxide Nanoparticles, Gold-coated, Cytotoxicity, MCF-7

Introduction

Nowadays, nanotechnology and molecular biology are widely developing nanoparticles with useful properties for overcoming the shortcomings of traditional disease diagnostic and therapeutic agents [1]. Among many nanoparticles used, magnetic nanoparticles have attracted great attention due to their specific magnetic properties [2]. One class of magnetic nanoparticles is magnetic iron oxide nanoparticles (MIONs) which has been widely offered because of its reactive surface that can be readily modified by biocompatible coatings as well as targeting, imaging and therapeutic molecules. MIONs are being extensively used in many biomedical applications such as magnetic

¹Department of Medical Physics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

²Department of Chemistry, University of Isfahan, Isfahan, Iran

³Department of Clinical Biochemistry, School of Pharmacy and Isfahan Pharmaceutical Sciences, Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

*Corresponding author:
D. Shahbazi-Gahrouei,
Professor of Medical
Physics, Department of
Medical Physics, Isfahan
University of Medical Sci-
ences, Isfahan, Iran
E-mail: shahbazi@med.
mui.ac.ir

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resonance imaging [3-8], drug delivery [2, 9, 10] and magnetic hyperthermia [11, 12].

There are several methods for fabricating magnetic iron oxide nanoparticles. The most common method, known as co-precipitation, includes co-precipitation of ferrous and ferric salts in an alkaline medium. In this method, the size and shape of iron oxide nanoparticles depend on several factors such as a type of salt used, temperature, pH value and so on. Another method is thermal decomposition, which usually requires relatively higher temperatures. In the microemulsion method, magnetic nanoparticles are fabricated in oil-in-water microemulsions by suspending a ferrous salt-surfactant precipitate from an aqueous solution; the next, a base is added. Hydrothermal method includes various wet chemical technologies of crystallizing substance in a sealed container from the high temperature aqueous solution at the high vapor pressure. Another method that has been extensively used is a sonochemical method in which the acoustic cavitation is formed by the ultrasound waves, generating a localized hotspot through adiabatic compression [13].

After synthesis, iron oxide nanoparticles are usually coated in order to improve their stability, facilitate the bonding of various biological ligands to nanoparticle surfaces and reduce their toxicity. Usual coating materials are classified to inorganic and organic materials such as gold, silica, polyethylene glycol (PEG), polyvinyl alcohol (PVA), dextran, chitosan and other polymers [14].

Gold is one of the noteworthy coating materials because of its chemical stability, biocompatibility and various applicability [15]. Gold-coated MIONs (GMNPs) can be heated by an external magnetic field for hyperthermia application and they are useful in photothermal therapy because of the excellent near-infrared (NIR) light sensitivity and strong adsorptive ability of the Au layer [16].

Owing to extensive applications of MIONs in biomedicine, before they can be used in

vivo, their cytotoxicity must be investigated. Therefore, there is a distinct need of understanding the potential risks associated with MIONs. The aim of this work is to investigate the cytotoxicity of GMNPs and bare uncoated MIONs (Fe_3O_4).

In this study, cytotoxicity effects of GMNPs and Fe_3O_4 on two cell lines were investigated. These two cell lines were human breast adenocarcinoma (MCF-7) and human foreskin fibroblast (HFFF-PI6). MCF-7 was used as a cancerous cell line and HFFF-PI6 was chosen as a normal cell line.

Material and Methods

Synthesis of Gold -coated Fe_3O_4 Nanoparticles (GMNPs)

The Chemical co-precipitation method was used for the nanoparticle synthesis in four steps.

Step 1

1.28 M of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.64 M of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were dissolved in deoxygenated HCl (0.4 M). Then, 200 mL NH_3 was quickly added to the mixture. The black product is Fe_3O_4 nanoparticles and after collecting, it was washed several times with deionized water.

Step 2

Then, Fe_3O_4 nanoparticles were coated with silica ($\text{Fe}_3\text{O}_4@\text{SiO}_2$). Briefly, Fe_3O_4 nanoparticles (30 mL) were first diluted with water (30 mL), 2-propanol (300 mL), and then ammonia (15 ml) was added. The solution was well-dispersed by ultrasonic vibration for 15 minutes. Finally, 1.9 mL TEOS was added into resultant solution under vigorous stirring for 16 hours. $\text{Fe}_3\text{O}_4@\text{SiO}_2$ was collected by a magnet and washed with water and ethanol.

Step 3

In the next step, the previous product was amino-functionalized ($\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{NH}_2$). The surface of silica-coated nanoparticles can be easily modified by Aminopropyltriethoxysilane (APTES). Briefly, with vigorously stirring, 12 gr $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and 32 mL

APTES were added into 120 mL anhydrous toluene. After that, the product was magnetically collected and washed with acetone.

Step 4

Finally, the gold nanoparticles were deposited in the previous product surface (15 mg Fe₃O₄@SiO₂@NH₂ was dispersed in 30 mL water at pH = 4) by the reduction of uric acid (8 mL, 1.71 mM) using NaBH₄ solution (8 mL, 0.1 M). Consequently, the product was separated and dispersed in PBS (pH = 7).

Characterization

Transmission electron microscopy (TEM) imaging (Philips CM 30) was used for the evaluation of morphology and size of nanoparticles. The UV/visible spectra were obtained using a JASCO UV-vis spectrophotometer. X-ray diffraction (XRD) pattern was recorded using a Bruker D8/Advance X-ray diffractometer with Cu-K α radiation.

Cell Culture

Roswell Park Memorial Institute (RPMI-1640) medium, fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA) and penicillin–streptomycin were obtained from Invitrogen (Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich.

MCF-7, human breast adenocarcinoma, and HFFF-PI6, human foreskin fibroblast, cell lines were purchased from the National Cell Bank of Iran (Pasteur Institute, Iran). The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μ g/mL). The cells were grown and maintained at 37 °C in a humidified incubator with 5% CO₂.

In-vitro Cytotoxicity

A simple, a non-radioactive and colorimetric assay named MTT was used for a quantitative

cytotoxicity assessment of nanoparticles [17]. In this assay, metabolically active cells convert a yellow and water soluble tetrazolium salt to a water insoluble and dark blue Formazan [18]. For the MTT assay viability studies, MCF-7 and HFFF-PI6 cells were seeded into 96-well cell culture plates at the density of 20,000 cells per well and incubated for 24 hours in a humidified incubator with a CO₂ concentration of 5% to allow adherence of the cells. Then, the cells were incubated with different concentrations of both Fe₃O₄ and GMNPs ranging from 10 to 400 μ g/mL for 12 and 24 hours. The control wells were a culture medium with no particles.

After 24 hours, the culture medium was removed, 100 μ L fresh medium and 20 μ L MTT (5 mg/mL) were added into each well and the plates were incubated for 4 hours. Then, the culture medium was carefully removed, and 200 μ L dimethyl sulfoxide (DMSO) was added to each well to dissolve the Formazan crystals for 10 minutes. The absorbance was measured at 570 nm using an ELISA reader (Synergy H1, Bio Tek). Experiments were performed in triplicate and cell survival was determined as a percentage of viable cells in comparison with control wells.

Results and Discussion

Figure 1 shows XRD pattern related to GMNPs and demonstrates the crystalline nature of GMNPs. 6 peaks at about 30.3° (220), 35.6° (311), 43.2° (400), 53.4° (422), 57.2° (511), 62.7° (440) could be assigned to Fe₃O₄ [19]. Also, four peaks positioned at 2θ values of 38.2° (111), 44.4° (200), 64.7° (220) and 77.7° (311) are corresponding to the planes of the cubic-phase Au [20]. XRD analysis shows that the recorded pattern matches the reference pattern for magnetite (Fe₃O₄) and gold.

Figure 2 shows TEM image of synthesized GNMPs. The core size and the shell thickness were less than 25 nm and 5 nm, respectively. In addition, nanoparticles are spherical from a morphological point of view.

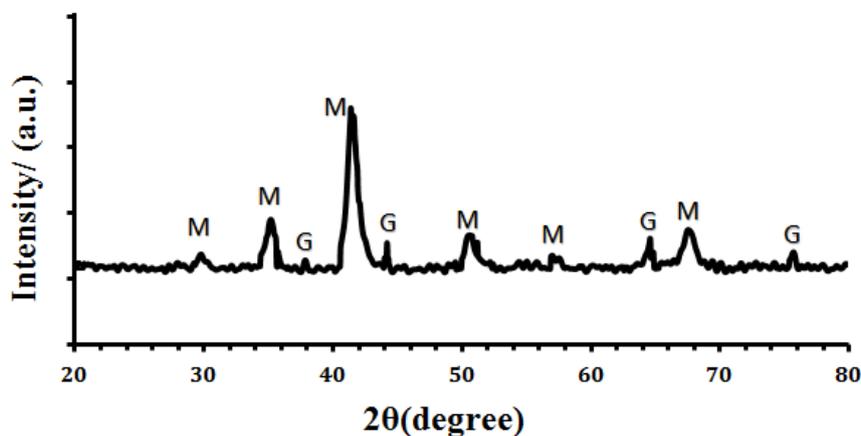


Figure 1: XRD pattern of GMNP ('M' and 'G' are diffraction peaks for Fe_3O_4 and GMNP, respectively).

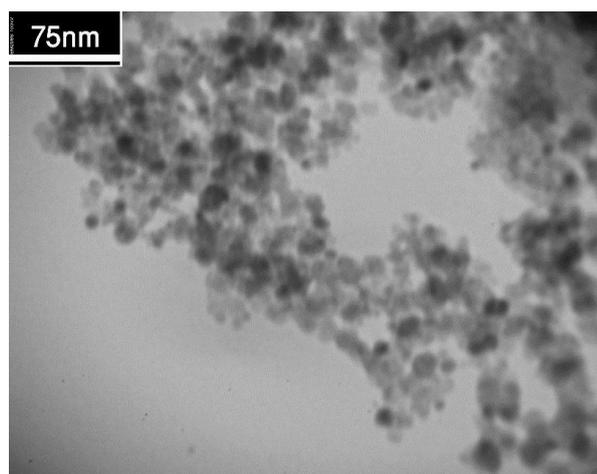


Figure 2: Transmission electron microscopy (TEM) image of GNMP.

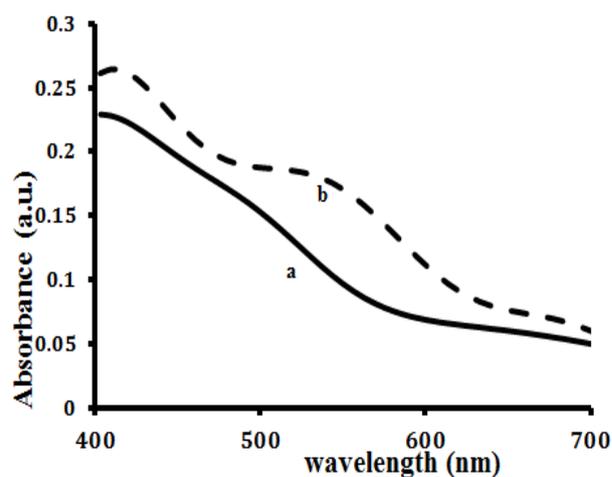


Figure 3: UV-visible spectra of nanoparticles (a) Fe_3O_4 nanoparticles, (b) GMNP.

Figure 3 demonstrates the UV-Vis absorption spectra for Fe_3O_4 and GNMP. Fe_3O_4 shows no measurable features in the visible region, while the aqueous dispersion of GNMP shows a characteristic absorption peak at 530 nm which is attributed to the surface Plasmon resonance bands of gold shell.

To study the viability of two cell lines, MTT assay was performed. In this assay, yellow tetrazolium is reduced into purple formazan crystals by mitochondrial succinate dehydrogenase of viable cell. Thereby, it can be con-

cluded that the rate of formazan crystal formation is directly proportional to the number of viable cells [21].

Cytotoxicity of nanoparticles was studied with different concentrations ranging from 10 $\mu\text{g}/\text{mL}$ up to 400 $\mu\text{g}/\text{mL}$ and for different incubation times (12 and 24 hours). All experiments were carried out in triplicate. After subtraction of the blank values, the mean values and standard deviation (SD) were calculated. The results are presented in terms of percent-

age cell viability. It should be noted that materials with cell viability more than 80% can be considered as being biocompatible [17].

Figures 4 and 5 illustrate the cytotoxicity data for MCF-7 and HFFF-PI6 cells, respectively. Results reveal that for both cells, Fe_3O_4 nanoparticles have higher cytotoxicity than GMNPs (Figures 4a and 5a). For MCF-7 and HFFF-PI6 cells, at concentrations of 300 and 400 $\mu\text{g}/\text{mL}$, Fe_3O_4 nanoparticles are toxic, respectively. Moreover, by increasing nanoparticle concentrations, cell viability de-

creases.

In 2009, Mahmoudi et al. synthesized superparamagnetic iron oxide nanoparticles with different polymer/iron mass ratios (r-ratio) by coating them with polyvinyl alcohol (PVA). MTT assay was used to investigate the cell toxicity of the samples. Their results showed that the biocompatibility of the nanoparticles, based on cell viabilities, could be enhanced by increasing the r-ratio due to increasing particle size causing lower cell toxicity effects [17].

Our results are in good agreement with Gai-

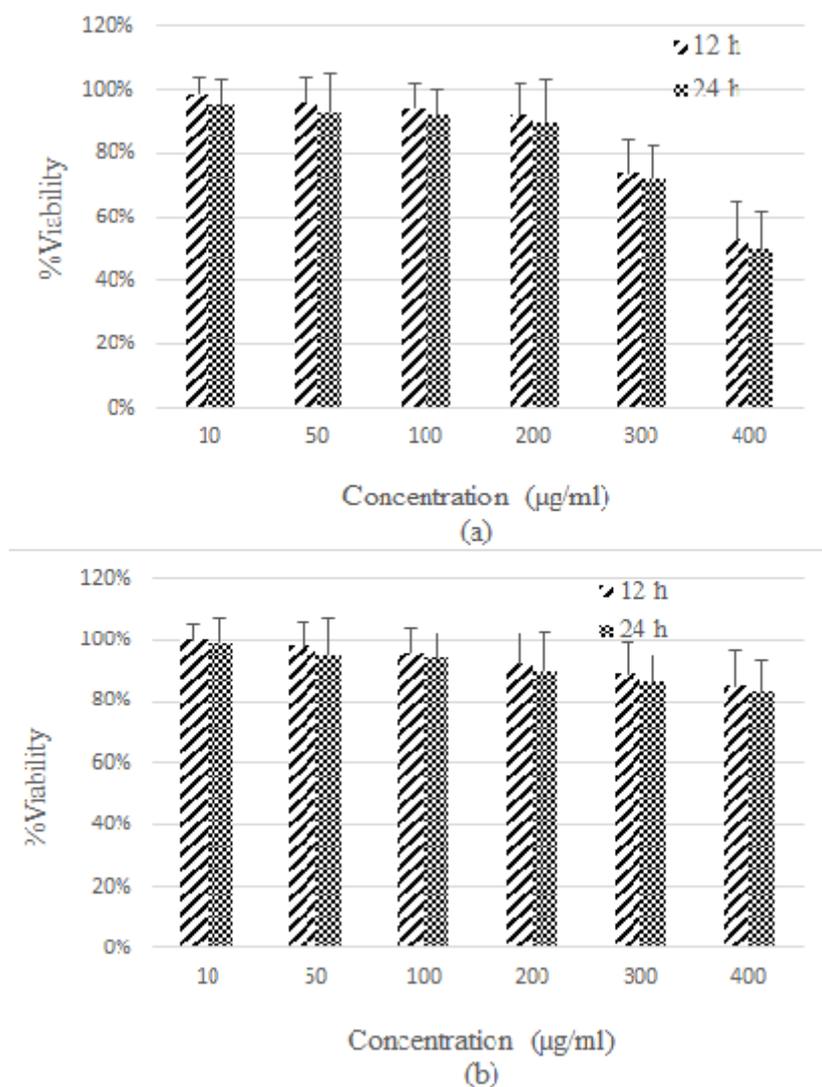


Figure 4: Representation of cytotoxicity of Fe_3O_4 nanoparticles (a) and GMNPs (b) in MCF-7 cells, with different concentrations at various time intervals.

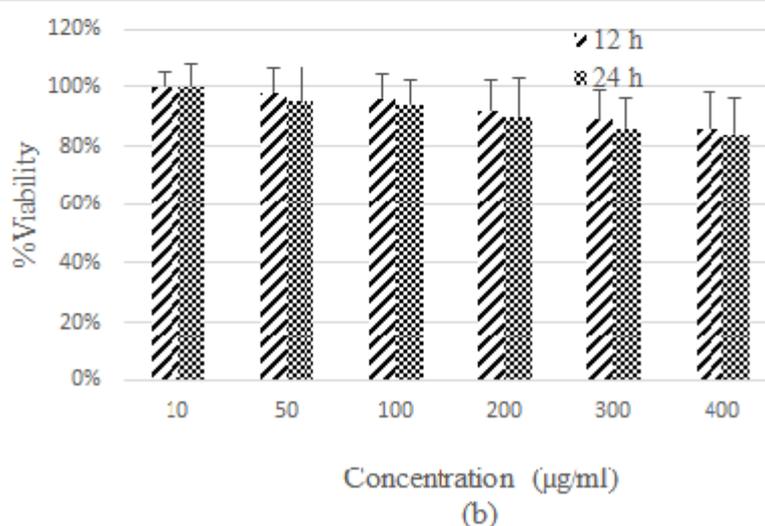
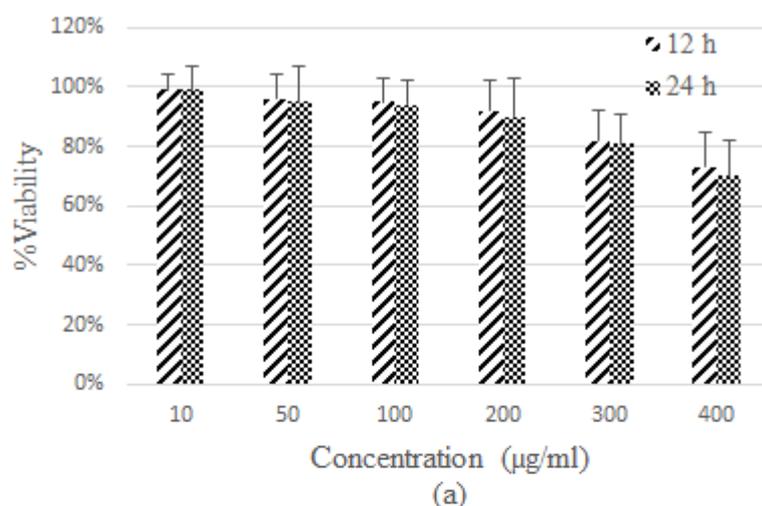


Figure 5: Representation of cytotoxicity of Fe₃O₄ nanoparticles (a) and GMNPs (b) in HFFF-PI6 cells, with different concentrations at various time intervals.

hre et al. too, where they modified the surfaces of magnetic iron oxide nanoparticles with gelatin in order to investigate their cytotoxicity and cellular uptake. MTT assay was used to assess the cytotoxicity of gelatin-coated nanoparticles and uncoated nanoparticles on human fibroblasts. Their results showed that gelatin coating would increase cell viability [22].

Figures 4b and 5b show the cytotoxicity results are related to GMNP for MCF-7 and HFFF-PI6 cells, respectively. The results

clearly indicate that the toxic effect of GMNP on MCF-7 and HFFF-PI6 cells were less as compared to Fe₃O₄ nanoparticles. This finding suggests that the gold coating reduces the toxic effects of uncoated Fe₃O₄ nanoparticles. Less toxicity of GMNP may be attributed to controlled release from Fe²⁺ ions in intracellular space. Higher toxicity of uncoated-MIONs (Fe₃O₄) could be attributed to high release from iron ions in intracellular space [23]. Moreover, the results show toxicity is time-dependent for all cells and nanoparticles. Notably, cell toxic-

ity increased with raise in dose (concentration) and incubation time.

Conclusion

In this study, the cytotoxicity effects of Fe₃O₄ nanoparticles and GMNPs were evaluated on MCF-7 and HFFF-PI6 cells by a simple and colorimetric method named MTT assay. Findings represent that for both cells, Fe₃O₄ nanoparticles have higher cytotoxicity than GMNPs, which could be attributed to high release from iron ions into intracellular space in case of Fe₃O₄ nanoparticles. Moreover, nanoparticle toxicity is dose- and time-dependent. It should be noted that at any concentrations, all kinds of nanoparticles are toxic depending on cell type.

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Conflict of Interest

None

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