The effect of iron- gold core shell magnetic nanoparticles on the sensitization of breast cancer cells to irradiation

Hamidreza Kheiri Manjili[1], Hossein Naderi-Manesh[2,*], Maedeh Mashhadikhan[3], Leila Ma'mani[4], Safoora Nikzad[5], Sharafaldin al mussawi[1]

[1]Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[2]Department of Biophysics and Biochemistry Faculty of Science, Tarbiat Modares University, Tehran, Iran
[3] Departments of Biology, Faculty of Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[4] Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[5] Department of medical physics, Faculty of Medical sciences, Tarbiat Modares University, Tehran, Iran

*Corresponding Author: email address: Naderman@modares.ac.ir (N. Naderi-Manesh)

ABSTRACT

Herein, iron-gold core shell magnetic nanoparticles Fe@Au NPs was investigated as contrasting agent in radiation therapy in the breast cancer. Assessment of cytotoxic and radio sensitizing potential was done by MTT method and Flow cytometry. Radiation was done using Co 60 source. The response of cells to treatment with radiation alone and radiation with nanoparticles was assessed. The study demonstrates that Fe@Au nanoparticles do not have considerable cytotoxic effects, but they increase the effectiveness of radiation that means the survival of the group without nanoparticles exposed to 5 Gy radiations is 75%while the group with nanoparticles is 33%. With 2 Gy radiations the survival of the two groups are 87% and 80% respectively.

Keywords: Exposure; Iron-gold core shell nanoparticles; Radio sensitizing.

INTRODUCTION

Radiation therapy applying the ionizing radiation as a cancer treatment is used in over 50% of cases [1-3], but these radiations are injurious for the normal tissue around the tumor. Therefore, several techniques have been proposed to reduce this effect and make the distinction between normal and malignant tissue. Due to the importance of this fact, the introduction of efficient and robust manner to improve and fix the drawback has emerged [4, 5]. The radiation damages caused by the interaction of radiation with matter, which is based on the incident beam energy and target atomic number and severity of interactions changes. In this regard, the nanotechnology could be useful tool to overcome this problem and reduce the side effects of the radiation therapy in the normal cells around the cancerous cell. The nanoparticles with high atomic numbers can concentrated in the cancer cells as sensitizing agents and increased their absorption property [6-9]. The atomic number of soft tissue is around 7.5. Also, applying the heavy metals, their adsorption and uptake in cancerous cells could increase the atomic number depending on the material which lead to increase the photoelectric interaction in cancerous cells versus the normal ones [10-13].

Additionally, gold nanoparticles (Au NPs) have been well studied because of their large optical adsorption coefficients in near-infrared region, where the biological tissues are transparent [14]. Gold (with atomic number 79) nanoparticles are widely used as an agent for enhancing the radiation sensitivity.

Recently, the magnetic nanoparticles have opened new horizon to introduce new systems and improve the efficiency of cancer diagnosis and treatment. They have attracted a great deal of attention due to their potential biomedical utilizes [15]. On the other hands, magnetic nanoparticles have exciting different biomedical usages such as magnetic separation, magnetic resonance imaging (MRI) contrast agents, and targeted drug delivery

for cancer therapy[16]. Due to the advantageous characteristics including the super paramagnetic property, non-toxicity, inertness, ease of detection in the human body, and high biocompatibility, the gold-coated magnetic nanoparticles have been used for a variety of biological applications[17]. Therefore the productions of the biocompatible core-shell iron-gold nanomaterials have emerged as a promising strategy over the recent years for their wide usages in the biotechnological and biomedical areas, including bio-targeting for cancer treatment, drug carrier, bio-detection and bio-separation[18-21]. Regarding to these efforts and reports, the introduction of novel low-cost and efficient MRI contrast agent integrating magnetic iron-gold core shell nanoparticles with various surface characteristics would be of great significance. Therefore, the aim of this study was to construct and evaluate the combined effect of gold nanoparticles (iron–gold nanoparticles) due to the presence high atomic number gold and imaging of tissue using the magnetic fields, and so the possibility of nanoparticles' accumulation in tumor tissue than in normal tissue increased.

MATERIALS AND METHODS

All chemicals were purchased from Aldrich and used without further purification. Distilled water was used throughout. All liquid starting materials were degassed for 2 h prior to the experiment. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), streptomycin, penicillin G, all were obtained from Sigma Aldrich chemical company. Fetal bovine serum (FBS) and culture medium (DMEM= Dulbecco's Modified Eagle's Medium), and supplements were obtained from Gibco (Germany). Phosphate buffer (20 mM, pH 7.8) and corresponding salts were used throughout this research were purchased from Merck. All cell lines were grown in DMEM medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2% l-glutamine, 2.7 % sodium bicarbonate, 1% Hepes buffer, and 1% penicillin-streptomycin solution (GPS, Sigma) at 37 °C in humidified atmosphere with 5 % CO_2. All cells were trypsinized in the solution of 0.05% trypsin and seeded into 96-well micro-plates at the density of 1×10^5 cells/well.

Synthesis of Gold Coated Iron Nanoparticles (Fe@Au)

The synthesis reaction was carried out under inert atmosphere. The procedure for the synthesis of nanoparticles is schematically shown in Figure 1. In a typical experiment, in a beaker (A), 0.665 g $FeSO_4$ was dissolved in 4.8 ml deionised water and 12 g CTAB, 10 g 1-butanol and 30 g octane were added. In another beaker (B), sodium borohydride ($NaBH_4$) was dissolved in 4.8 ml water and mixed with 12 g CTAB, 10 g 1-butanol and 30 g octane. Micellar solutions containing $FeSO_4$ (beaker A) and $NaBH_4$ (beaker B) were mixed together under stirring, color change from pale green to black was observed. Once the iron nanoparticles formed, a micellar solution containing 0.0490 (M) $HAuCl_4$ (aq), 6 g CTAB, 5.0 g 1-butanol and 30 g octane was added followed by the addition of a micellar solution of $NaBH_4$ (aq). Au (III) was reduced to Au (0) by $NaBH_4$ and gold forms a coating on the outer surface of the iron particles. The iron containing nanoparticles were separated by an external magnetic field. The remaining surfactants and byproducts were removed by repeated wash in with 1:1 methanol/water mixture. The particles were dried under vacuum and stored, the resulting powder was black.

MTT assay

To perform MTT assay the 96 well culture plates were used and the cells were cultured at concentration of 1×10^4 cell per well. Then, Plates were incubated for 12h at 37˚C and CO_2 5% for allowing the cells to attach the plate. Following that, the 96 well plates were irradiated using Co 60 source. MTT solution stock was prepared prior to use and filtered through a 0.22 µm filter. MTT solution was added to each well (10 µl in each 100 µl of culture medium) and cells were incubated for 4 hours at 37□C. Afterward, the medium was removed and the Formazan product was dissolved by adding 100 µl dimethylsulfoxide (DMSO) to wells. To ensure that crystals solved completely, the plate was put on a shaker for 5 min. The absorbance of each well measured at 540 nm using Multiscan plate reader (England). DMSO absorbance measured as control. Each experiment was performed at least in triplicate.

MTT assay is related to number of cells in exponential phase.

Flowcytometry analysis

The cells were trypsinized and counted, then were cultured in 6 well plates with the density of 1×10^4 cells per well and exposed to ionzing radiation. After 3 days of irradiation, cellular apoptosis was assessed with Annexin V apoptosis detection kit according to manufacturer's instructions (eBioscience, USA). In brief, cells were harvested and centrifuged at 300g for 5 min. Then cells were washed once with Phosphate buffer saline (PBS) and once with 1X binding buffer. After that, cells were suspended in 1X binding buffer and 5 µl of flurochrome-conjugated Annexin V was added to 100 µl of cell suspension and incubated for 10 min in dark at room temperature. Cells then washed with 2 ml of binding buffer and suspended in 200 µl of 1X binding buffer. Finally, 5 µl of Propidum iodide staining solution was added to 200 µl cell suspensions and assessed by Flow Cytometry (BD FACSCantoII, USA).

RESULTS

Preparation of Nanoparticles

The magnetic property of iron nanoparticles can be used to increase the contrast in imaging and also in targeted navigation to the tumor area by usage of magnetic fields. The goal of this research is evaluation of the effect of gold coated magnetic nanoparticles in increasing the sensitivity of malignant cells to radiation. Therefore, the magnetic Fe@Au core shell nanoparticles were prepared as shown in Figure **1**. Gold coated magnetic nanoparticles (Fe@Au) with mean diameter of 70 nm were synthesized. The nanoparticles were applied as contrast agent was investigated to enhance radiation absorption and sensitize the cancerous tissues to radiation.

Figure 1. Synthesis of iron gold core shell magnetic nanoparticles.

Cytotoxicity of Nanoparticles

To evaluate the nanoparticle cytotoxicity, cell lines of breast cancer (MCF-7) and normal mammary epithalia cells (MCF10A) were treated by various concentrations of nanoparticles. Serial 2-fold dilutions of nanoparticles were prepared (2 µg/ml to 48 μg/ml) and applied to both cell lines, then cells were incubated for 24h at 37 and MTT assay was performed. MTT results for both cell lines and all concentration are shown at Figure **2**.
The results illustrate that the cells viability of MCF-7 and MCF10A cell line is more than 90% for concentrations less than 20 µg/ml. However, in all concentrations the effect of nanoparticle on MCF-10A cells was less than MCF-7. Thus the concentration of 18 µg/ml was selected to use in combination with irradiation.

Radio sensitization of nanoparticles

To determine the radio sensitization of nanoparticles, the cells were incubated with nanoparticles 24h before irradiation. After irradiation the culture medium was changed and the cells were incubated at 37˚C and CO_2 5%. MTT assay was done after 48 and 72 h of incubation. The results are shown at Figure **3** (A & B).
MTT assay was used to determine the effects nanopartcles on radiosensitization of the cells using radiotherapy. Results indicate that after 48h of radiation there are no significant differences between groups of treatments. Considering the results after 72h of treatments there is no significant difference between groups with radiation alone in comparison to radiation together with nanoparticles at 2 Gy. The cells viability of group treated without and with nanoparticles were 87% and 80%, respectively. But at the 5 Gy irradiations after 72h, the cells viability was 75% for group without nanoparticle which significantly decreased to 33% in group treated with nanoparticle and radiation.

Flow cytometry analysis

In order to assess the rate of radio sensitization of nanoparticles by flow cytometry, MCF7 cells

were cultured in 6 well plates. Cells were incubated with nanoparticles, 24 hours prior to irradiation. On the next day, cells were irradiated and after 72 hours, MCF-7 cells were harvested and assessed for apoptosis by flow cytometry. Results have been shown in Figure **4**. Flow Cytometry results are compatible with MTT results, 72 h after irradiation.

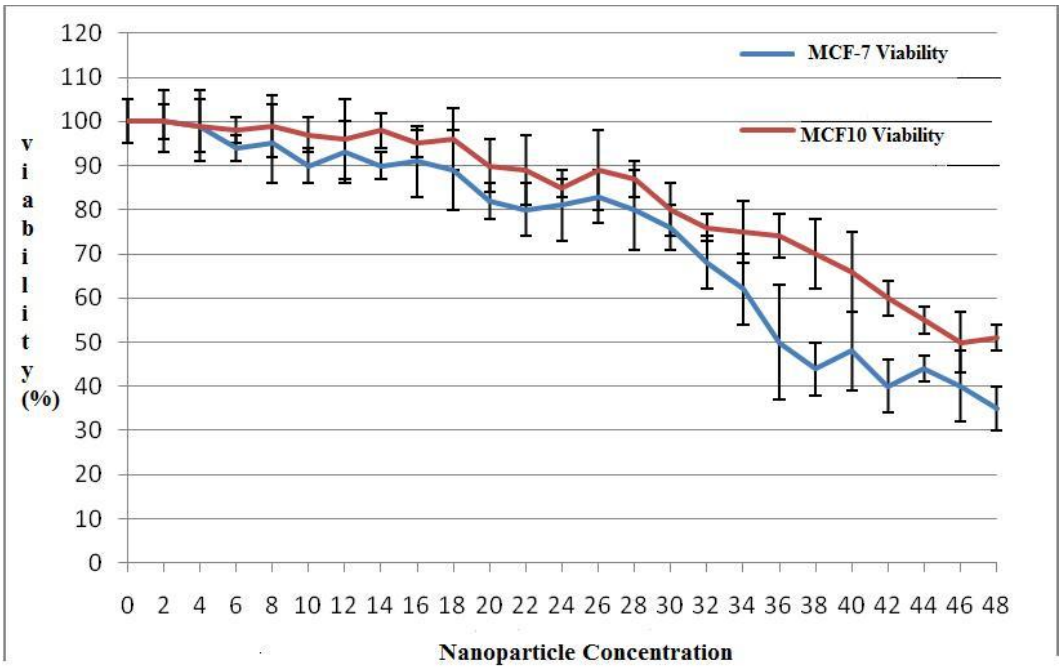

Figure 2. Graph of MTT results shows the cell's viability in base of various doses of nanoparticles

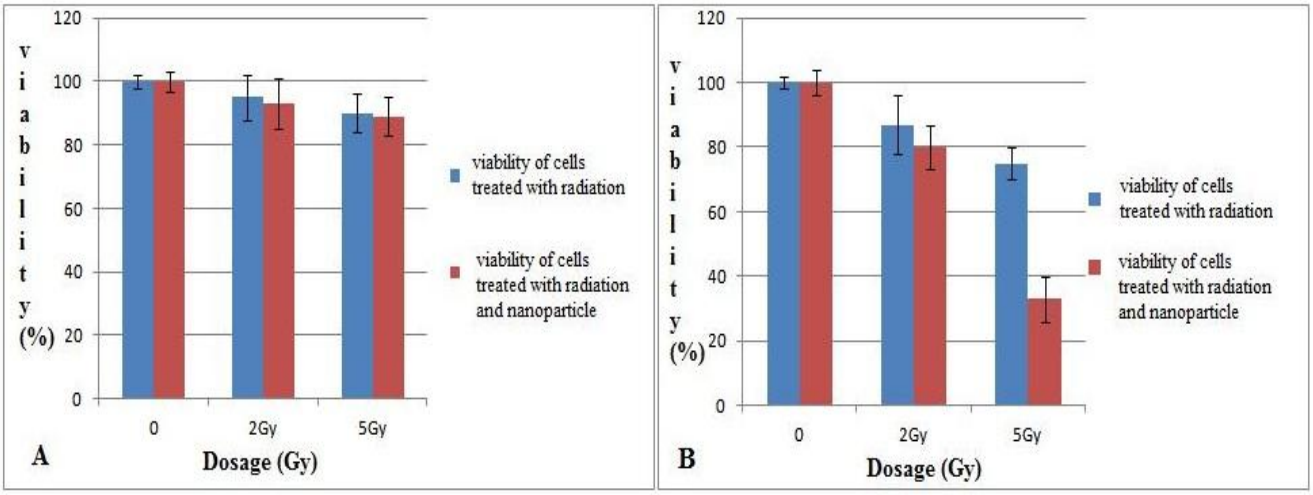

Figure 3. Graph of MTT results shows the levels of cell sensitivity after nanoparticle treatments in control group (irradiation without nanoparticles) and radiated groups exposed to 2 and 5 Gy after 48h (A) and 72h (B)

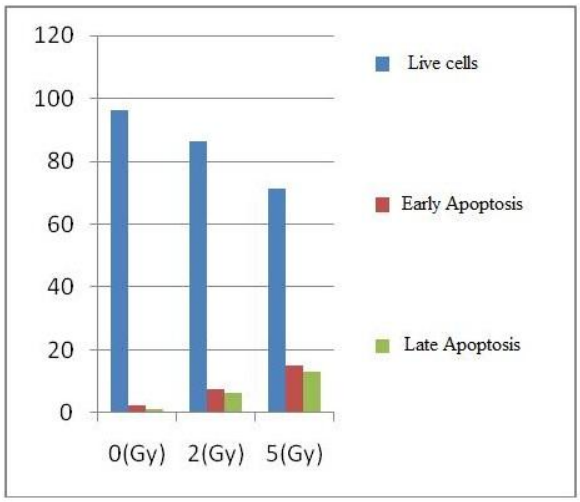

Figure 4. Percentage of live cells and apoptotic cells in early and late phase in control group (without irradiation) and groups with 2Gy and 5 Gy irradiation after 3 days following irradiation

DICSUSSION

Nanotechnology can be used to concentrate nanomaterial with high atomic number in malignant cells and their property of increasing radiation absorption can be used to sensitize the cancerous tissues to radiation. On the other hand, the magnetic property of iron nanoparticles can be used to increase the contrast in imaging and also in targeted navigation to the tumor area by the use of magnetic fields.

Gold coated magnetic nanoparticles (Fe@Au) with mean diameter of 70 nm were synthesized. Assessment of cytotoxic and radio sensitizing potential was done by MTT method and Flow cytometry. Radiation was done using Co 60 source. The response of cells to treatment with radiation alone and radiation with nanoparticles was assessed.

Radiation was done using Co 60 source. The response of cells to treatment with radiation alone and radiation with nanoparticles was assessed. The study demonstrates that Fe@Au nanoparticles do not have considerable cytotoxic effects, but they increase the effectiveness of radiation that means the survival of the group without nanoparticles exposed to 5 Gy radiations is 75%while the group with nanoparticles is 33%. With 2 Gy radiations the survival of the two groups are 87% and 80% respectively.

The results of MTT assay and Flow Cytometry analysis demonstrate that the cytotoxicity of nanoparticle is not considerable at concentrations less than 20 µg/ml. Moreover, there are no significant differences between group treated with or without nanoparticles at radiation of 2 Gy. While, nanoparticle treatment with 5 Gy irradiations significantly decrease cell viability of MCF-7 cells and have considerable therapeutic results. Therefore, we found Fe@Au magnetic nanoparticles could be used to specify irradiation in breast cancer cell line (MCF-7).

CONCLUSION

Malignant cells treated with magnetic nanoparticles coated with gold (Fe@Au) have decreased viability during radiation than the cells without nanoparticles.

REFERENCES

1.Alberts, B. The promise of cancer research. *Science* 2008, *320*, 19.

2.Varmus, H. The new era in cancer research. *Science* 2006, *312*, 1162-1165.

3.Sladowska A. Modern External Beam Radiotherapy Techniques - Intensity Modulated Radiotherapy2009;35- 115.

4.Stephens FO, Aigner KR. Targeting cancer. In: Stephens FO, Aigner KR, editors. Basics of Oncology. London-New York: Springer, 2009:87-120.

5.Porcel E, Liehn S, Remita H. Platinum nanoparticles: a promising material for future cancer therapy Nanotechnology 2010; 21: (085103) 1-7. doi:10.1088/0957-4484/21/8/085103.

6.(Zheng Y, Hunting DJ, Ayotte P, Sanche L. Radiosensitization of DNA by gold nanoparticles irradiated with high-energy electrons. Radiat Res 2008; 169: 19-27. doi:10.1667/RR1080.1.

7.Chen, W.; Zhang, J. Using nanoparticles to enable simultaneous radiation and photodynamic therapies for cancer treatment. *J. Nanosci. Nanotechnol.* 2006, *6*, 1159-1166.

8.Herold, D.M.; Das, I.J.; Stobbe, C.C.; Iyer, R.V.; Chapman, J.D. Gold microspheres: A selective technique for producing biologically effective dose enhancement. *Int. J. Rad. Biol.* 2000, *76*, 1357-1364.

9.Herold, D.M.; Das, I.J.; Stobbe, C.C.; Iyer, R.V.; Chapman, J.D. Gold microspheres: A selective technique for producing biologically effective dose enhancement. *Int. J. Rad. Biol.* 2000, *76*, 1357-1364.

10.Boudaïffa B, Cloutier P, Hunting D, Huels MA, Sanche L. Resonant formation of DNA strand breaks by low-energy (3 to 20 eV) electrons. Science 2000; 287(5458): 1658-60.

11.Carter JD, Cheng NN, Qu Y, Suarez GD, Guo T. Nanoscale energydeposition by Xrayabsorbing nanostructures. J Phys Chem B 2007; 111(40): 11622-5.

12.Chang MY, Shiau AL, Chen YH, Chang CJ, Chen HH, Wu CL. Increased apoptotic potential and dose-enhancing effect of gold nanoparticles in combination with single-dose clinical electron beams on tumor-bearing mice. Cancer Sci 2008; 99: 1479–84. doi:10.1111/j.1349-7006.2008.00827.x.

13.Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discov Today 2010; 15: 842- 50. doi:10.1016/j.drudis.2010.08.006

14.Huang XH, El-Sayed IH, Qian W, El-Sayed MA: Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. J Am ChemSoc 2006,128:2115-2120.

15. Xiaoting Meng, Xiaoting; Hugh C. Seton, Le T. Lu, Ian A. Prior, Nguyen T. K. Thanh and Bing Song (2011). "Magnetic CoPt nanoparticles as MRI contrast agent for transplanted neural stem cells detection". Nanoscale 3 (3): 977–984.

16. Wang J1, Huang Y, David AE, Chertok B, Zhang L, Yu F, Yang VC.Curr Pharm Biotechnol. 2012 Magnetic nanoparticles for MRI of brain tumors. Sep;13(12):2403-16.

17. Huff TB, Tong L, Zhao Y, Hansen MN, Cheng JX, Wei A: Hyperthermic effects of gold nanorods on tumor cells. Nanomedicine. 2007, 2: 125-132.

18.Liu HY, Chen D, Li LL, Liu TL, Tan LF, Wu XL, Tang F: Multifunctional gold nanoshells on silica nanorattles: a platform for the combination of photothermal therapy and chemotherapy with low systemic toxicity. AngewChemInt Ed 2011, 50:891-895.

19.Ma M, Chen H, Chen Y, Wang X, Chen F, Cui X, Shi J: Au capped magnetic core/mesoporous silica shell nanoparticles for combined photothermo-/chemo-therapy and multimodal imaging. Biomaterials 2012, 33: 989-998.

20.Hu M, Petrova H, Chen JY, McLellan JM, Siekkinen AR, Marquez M, Xingde Li, Younan Xia, and Gregory V. Hartland: Ultrafast laser studies of the photothermal properties of gold nanocages. J PhysChem B 2006, 110:1520-1524.

21.Chen M, Yamamuro S, Farrell D, Majetich SA: Gold coated iron nanoparticles for biomedical applications. Appl Phys 2003, 93:7551-7553.