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$ZnFe_2O_4$ nanoparticles as radiosensitizers in radiotherapy of human prostate cancer cells

Alireza Meidanchi [a,b], Omid Akhavan [b,c,*], Samideh Khoei [d], Ali A. Shokri [a,e], Zahra Hajikarimi [d], Nakisa Khansari [f]

[a] Department of Physics, Payame Noor University (PNU), P.O. Box 19395-3697, Tehran, Iran
[b] Department of Physics, Sharif University of Technology, P.O. Box 11155-9161, Tehran, Iran
[c] Institute for Nanoscience and Nanotechnology, Sharif University of Technology, P.O. Box 14588-89694, Tehran, Iran
[d] Department of Medical Physics, Iran University of Medical Sciences, Tehran, Iran
[e] Computational Physical Sciences Research Laboratory, School of Nano-Science, Institute for Studies in Theoretical Physics and Mathematics (IPM), PO Box 19395-5531, Tehran, Iran
[f] Department of Cardiology, Hamadan University of Medical Sciences, Hamadan, Iran

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ABSTRACT

Nanoparticles of high-Z elements exhibit stronger photoelectric effects than soft tissues under gamma irradiation. Hence, they can be used as effective radiosensitizers for increasing the efficiency of current radiotherapy. In this work, superparamagnetic zinc ferrite spinel ($ZnFe_2O_4$) nanoparticles were synthesized by a hydrothermal reaction method and used as radiosensitizers in cancer therapy. The magnetic nanoparticles showed fast separation from solutions (e.g., ~1 min for 2 mg mL^{-1} of the nanoparticles in ethanol) by applying an external magnetic field (~1 T). The $ZnFe_2O_4$ nanoparticles were applied in an in vitro radiotherapy of lymph node carcinoma of prostate cells (as high radioresistant cells) under gamma irradiation of ^{60}Co source. The nanoparticles exhibited no significant effects on the cancer cells up to the high concentration of 100 μg mL^{-1}, in the absence of gamma irradiation. The gamma irradiation alone (2 Gy dose) also showed no significant effects on the cells. However, gamma irradiation in the presence of 100 μg mL^{-1} $ZnFe_2O_4$ nanoparticles resulted in ~53% inactivation of the cells (~17 times higher than the inactivation that occurred under gamma irradiation alone) after 24 h. The higher cell inactivation was assigned to interaction of gamma radiation with nanoparticles (photoelectric effect), resulting in a high level electron release in the media of the radioresistant cells. Our results indicated that $ZnFe_2O_4$ nanoparticles not only can be applied in increasing the efficiency of radiotherapy, but also can be easily separated from the cell environment by using an external magnetic field after the radiotherapy.

1. Introduction

In all over the world, cancer is known as one of the major causes of death. For example, the first cause of death in Iran is due to cancer, after coronary heart disease and accidents [1]. Among the cancers, prostate cancer is one of the main causes of death in men [2]. Surgery (open, laparoscopic or robotic-assisted), external beam radiation, or radioactive seed implants (brachytherapy) may be used to treat early stages of disease. Combination of hormonal therapy, chemotherapy, radiation, or one of them alone can be used to treat more advanced disease. Radiotherapy was often used as an effective treatment for clinically localized cancer cells. However, radioresistance of cancer cells is one of the major causes of ineffective treatments [3].

In recent years, nanostructures with unique physico-chemical properties, such as small particle size, high effective surface area, and capability of functionalization, are promisingly used in cancer therapeutic researches and applications (see, for example, [4–9]). Meantime, the use of magnetic nanoparticles not only provides imaging of the cancer cells, but also can lead to more effective localized treatments of tumors by targeted drug delivery [10,11].

On the other hand, radiotherapy is one of the most current and important cancer therapeutic methods. Using radiotherapy cancer cells can be destructed through direct and indirect mechanisms. In direct destruction, the gamma radiation directly affects the DNA through ionization and/or excitation of target atoms. Based on the indirect mechanism, gamma radiation interacts with other atoms or molecules around DNA (i.e., water and/or nanoparticles) to produce free radicals or Auger electrons which can destruct the cells. The interaction of gamma radiation with nanoparticles having high Z-elements (as radiosensitizers) can further excite the photoelectric effects and/or Auger electron ejections. Hence, some nanoparticles with high Z-elements (such as gold and gadolinium) have been used as effective radiosensitizers in cancer therapy [12–14]. Moreover, Choi et al. [15] investigated the potential of sensitizing effect of iron oxide nanoparticles for photon activated therapy. They observed a more significant reduction in viability of the FeO-treated irradiated cells, compared to the radiation alone group.

* Corresponding author.
E-mail address: oakhavan@sharif.edu (O. Akhavan).

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Recently, spinel ferrite nanoparticles with superparamagnetic property are utilized in many areas including magnetic storage media [16], ferrofluids [17,18], biosensors [19], catalysts [20], environmental remediation [21,22], and water purification [23,24]. In addition, spinel ferrite nanoparticles with low toxicity have been used in some nanomedicine application such as hyperthermia treatments [25,26], magnetic resonance imaging [27,28], drug delivery [29] and DNA separation [30]. Among the various spinel ferrites, zinc ferrite (as a normal spinel structure with $(Zn)[Fe_2]O_4$ representation in which square brackets contain the octahedral or B sites and parenthesis shows the cations in tetrahedral or A sites) has attracted much interest, due to its low ordering temperature and antiferromagnetic ground state with a Néel temperature at ~10 K, paramagnetic behavior at room temperature and narrow band gap of 1.9 eV [31].

Concerning the application of $ZnFe_2O_4$-based composites in cancer therapy, Tomitaka et al. [32] found that $ZnFe_2O_4$ and $NiFe_2O_4$ nanoparticles exhibited cytotoxic effects on HeLa cells when exposed to 100 μg mL^{-1} of the nanoparticles. Then, Shah et al. [33] reported magnetic and bioactivity evaluation of ferromagnetic $ZnFe_2O_4$-containing glass ceramics for the hyperthermia treatment of cancer. Very recently, Liu et al. [34] suggested $ZnFe_2O_4$ nanoparticles with good magnetic property and neuro-cytocompatibility for potential applications in magnetic resonance imaging of cells. Since nanoparticles with high Z-elements (such as gold [12,13], iron oxide [15] and gadolinium-based [14] nanoparticles) have been used as radiosensitizing agents in radiotherapy of cancer, the $ZnFe_2O_4$ nanoparticles (including high Z-elements) can also be proposed as a suitable magnetic radiosensitizer in cancer treatments. However, no investigation concerning application of $ZnFe_2O_4$ nanoparticles as radiosensitizers in radiotherapy of cancer cells has been reported, yet.

In this work, $ZnFe_2O_4$ nanoparticles were synthesized by using a one-step hydrothermal process. The morphological, structural, optical, and magnetic properties of the nanoparticles were investigated. The concentration-dependent cytotoxicity of the nanoparticles on human prostate cancer cells was studied. Moreover, the nanoparticles were applied as radiosensitizers in an in vitro radiotherapy of human prostate cancer cells (as high radioresistant cells), under gamma irradiation.

2. Experimental

2.1. Materials

LNCaP human prostate cancer cells derived from a metastatic lymph node were prepared from the Pasteur Institute of Iran. Fetal bovine serum (FBS), Trypan blue dye, RPMI 1640 and penicillin/streptomycin (Invitrogen), 96-well microplates (JET BIOFIL) and ethanol (Merck, 99.9%) were prepared for the experiments. Other products such as MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), dimethyl sulfoxide (DMSO, ≥99%), trypsin-ethylenediaminetetraacetic acid, $Zn(NO_3)_2 \cdot 6H_2O$ (≥98%), and $Fe(NO_3)_3 \cdot 9H_2O$ (≥98%) were obtained from Sigma-Aldrich.

2.2. Hydrothermal synthesis of $ZnFe_2O_4$ nanoparticles

$ZnFe_2O_4$ nanoparticles were synthesized through the coprecipitation of $Zn(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$ in the presence of the ethanol [35], To do this, 298 mg $Zn(NO_3)_2 \cdot 6H_2O$ and 808 mg $Fe(NO_3)_3 \cdot 9H_2O$ were added to 80 mL ethanol and the mixture was stirred at 400 rpm for 30 min. After that, the mixture was sealed in a Teflon-lined stainless steel autoclave at 180 °C for 12 h. Then, the mixture was cooled down at room temperature. The obtained $ZnFe_2O_4$ powder was rinsed by distillated water (with purity of ≥98%) several times, and dried in an oven at 60 °C for 24 h. In the material characterization stage, $ZnFe_2O_4$ nanoparticles were dispersed in ethanol and distilled water, while for the biological tests (see the following) the desired amount of the nanoparticles was dispersed in culture media to obtain the desired concentrations.

2.3. Material characterizations

The optical absorption spectra of the samples were recorded by using a UV–visible spectrophotometer (UV–vis. CARY 300 Conc) in the wavelength range of 200–800 nm. The band gap energy (E_{bg}) of the prepared sample was estimated by the following formula: E_{bg} (eV) ≤ 1240 / λ (nm) where λ is the wavelength (~550 nm) of the absorption peak of the $ZnFe_2O_4$ nanoparticles. Fourier transform infrared (FTIR) spectra were recorded by using a FTIR-4200, JASCO equipped with a pressed KBr pellets in wavenumber range of 400 to 4000 cm^{-1}. The morphology of the nanoparticles was characterized by field emission scanning electron microscopy (FE-SEM, Hitachi S4160) and transmission electron microscopy (TEM, Zeiss-EM10C) at accelerating voltages of 15 and 80 kV, respectively. The TEM samples were prepared by dispersing the desired powder in acetone, and then, dipping carbon coated copper grids into the suspension. Raman spectroscopy (BRUKER Model SENTERRA) was performed at room temperature using a high-energy diode excitation source operating at wavelength of 785 nm. The magnetic property of the $ZnFe_2O_4$ nanoparticle was studied by a vibration sample magnetometer (VSM/AGFM-Meghnatis Kavir Kashan Co.) with a maximum applied magnetic field of 10 kOe, at room temperature.

2.4. Cell culture, gamma ray irradiation and MTT assay

The human prostate cancer cell line LNCaP was selected, because LNCaP cells show higher radioresistance than parental cell lines [36]. The cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin, in T-25 tissue culture flasks in a 5% CO_2 atmosphere at 37 °C. The medium was changed every 2–3 days. After three passages, approximately 8×10^3 LNCaP cells/well were seeded in two similar 96 well plates (180 μL of cell suspension was added to the wells) and incubated to adhere overnight. After 24 h, 20 μL suspension containing medium and $ZnFe_2O_4$ nanoparticles (sonicated at frequency of 40 kHz and power of 100 W for 10 min) added to the plates until wells reach to various concentrations (0.01, 0.1, 1, 10, 100 μg mL^{-1}, 4 wells for each nanoparticle concentration) and incubated in a 5% CO_2 atmosphere at 37 °C for 24 h. Cell proliferation medium without the $ZnFe_2O_4$ nanoparticles was set as a control. Then, one of the plates was irradiated by a gamma ray of ^{60}Co source (2 Gy dose) of a cobalt therapy system (Shohada-e-Tajrish Hospital, Tehran, Iran). After irradiation, MTT dye was added to each well (two plates) and incubated at 37 °C for 4 h. Then, the MTT containing medium was then removed and replaced with DMSO (100 μL/well) to dissolve the formazin crystals. The absorbance at 590 nm was measured by ELISA micro plate reader (DYNEX MRX, USA) using a reference wavelength of 620 nm. The cell viability percentage was calculated by the following formula: cell viability (%) = [(optical density (OD) of the sample − OD of the medium) / (OD of the cell control − OD of the medium] × 100. All biological assays were repeated at least three times. To statistically analyze the data, Tukey's post-hoc test was utilized using SPSS software (version 16.0). The differences were considered significant for the *P*-values <0.05. It should be noted that, although investigation about the probable toxicity of the $ZnFe_2O_4$ nanoparticles on normal human cells is important, the ethical laws and institutional guidelines of the Iran University of Medical Sciences prevented us to test the effects of the nanoparticles on the human normal cells.

3. Results and discussion

3.1. Morphological study

The morphological structures of the $ZnFe_2O_4$ nanoparticles were investigated by SEM and TEM, as presented in Fig. 1. Using SEM (Fig. 1a