**In vitro** Evaluation of Cobalt-Zinc Ferrite Nanoparticles Coated with DMSA on Human Prostate Cancer Cells

**Daryoush Shahbazi-Gahrouei**, Zeinab Ghasemian, Mohammad Abdolahi, Sohrab Manouchehri, Shaghayegh haghigho Javanmard and Nasim Dana

1Department of Medical Physics and Medical Engineering, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2Department of Medical Physics and Radiation Technology, School of Paramedical Sciences, Bushehr University of Medical Sciences, Bushehr, Iran
3Department of Physics, Malek-Ashtar University of Technology, Shahinshahr, Isfahan, Iran
4Physiology research center, Isfahan University of Medical Sciences, Hezar Jerib Street, Isfahan, Iran

**Abstract**

Toxicity of nanoparticles on the human health is one main feature for successful application of nanoparticles in medicine. In this paper, the cytotoxicity effects of CZF-MNPs and CZF-MNPs @ DMSA were evaluated. For this purpose, at first the characterization of Cobalt-Zinc Ferrite Magnetic Nanoparticles (CZF-MNPs) coated with biocompatible Dimercaptosuccinic Acid (DMSA) was investigated using of Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR) and Atomic Absorption Spectrophotometer (AAS). In following, the cytotoxicity effects of CZF-MNPs and CZF-MNPs @ DMSA were investigated on human prostate cancer cell lines, HPCs, (PC3 and DU145). The results showed that the average size of bare and coated nanoparticles was about 16 and 40 nm. The FTIR spectra results showed the presence of DMSA cover on the surface of nanoparticles. Furthermore, **in vitro** MTT assay CZF-MNPs @ DMSA at high concentrations (1.2 and 1.5 mM Fe) study results showed that they have some cytotoxicity on HPCs (PC3 and DU145).

**Keywords**: Cobalt-zinc ferrite; DMSA coated; Cytotoxicity; Human prostate cancer cells (HPCs); MTT assay

**Introduction**

The applicability of nanotechnology is strongly being attracted for various applications such as cytotoxicity, magnetic resonance imaging, biomedical applications, drug delivery and cancer therapy [1-6]. Recently, Spinel ferrite nanoparticles with different coatings have been widely investigated, due to their desirable magnetic properties in biomedicine and bioengineering fields [7-9]. These magnetic nanoparticles should have high magnetization values and size smaller than 100 nanometers. Also, these materials should have low toxicity and high biocompatible [10,11].

Various types of monomers such as DMSA were evaluated as anchors for simple attachment of polymer coatings on magnetic nanoparticles [12].

Toxicity of nanoparticles on the human health is one main feature for successful application of nanoparticles in medicine. Recently, the cytotoxicity effects of DMSA-Fe$_2$O$_3$ by tetrazolium dye assay on human aortic endothelial cells were reported and were shown that DMSA-Fe$_2$O$_3$ has some cytotoxicity [2]. On the other, choosing an appropriate surface coating for the desired application is important and complex [13]. There is controversy about cell viability in this regard. For instance, Pisanic et al. have demonstrated that DMSA coated nanoparticles were toxic to neurons [14], but in another study Wilhelm et al. have shown that DMSA coated nanoparticles were non-toxic to HeLa cells [15].

In this paper, the characterization of magnetic nanoparticles of Co$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ and Co$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ @ DMSA were investigated by Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Absorption Spectrophotometer (AAS). Also, the cytotoxicity effects of CZF-MNPs and CZF-MNPs @ DMSA were investigated for the first time on human prostate cancer cell lines, HPCs, (PC3 and DU145).

**Experimental**

**Preparation and characterization of MNPs**

Synthesis of cobalt-zinc ferrite nanoparticles and its coated with DMSA described by author and co-worker, previously [16]. Nanoparticles size and morphology were investigated by Transmission Electron Microscopy (EM 900 model, Co Zeiss.). The FTIR of nanoparticles were evidenced with FT Infrared Spectroscope (JASCO, FT/IR-6300, Japan) in the range of 400-4000 cm$^{-1}$. The content of Fe$^{2+}$ in Co$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ and Co$_{0.5}$Zn$_{0.5}$Fe$_2$O$_4$ @ DMSA MNPs was done by atomic absorption spectrophotometer (Shimadzu, AA-680).

**Cell culture**

HPCs (DU145 and PC3) were purchased from the National Cell Bank of Iran, Pasteur Institute of Iran. They were cultured in Roswell Park Memorial Institute medium (RPMI) media supplemented with 1% antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) and 10% fetal bovine serum until the third passage before experiments were performed. All the cell culture materials were from Gibco, USA. Cells were grown to confluence at 37°C in 5% CO$_2$/air.

**In vitro** cytotoxicity (MTT assay)

The cytotoxicity of coated and uncoated magnetic nanoparticles...
was evaluated using standard 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based colorimetric assay. Cancer cells were cultured in 96 well plates in 200 μL media per well at a density of 10^4 cells/well. After 24 h of incubation, the media was replaced with fresh media containing different concentration (0.3, 0.6, 0.9, 1.2 and 1.5 m mol) of coated and uncoated MNPs, and incubated for 24 h. After which time, the plate supernatant was discarded and 100 ml solution of MTT (25 mg MTT in 5 ml colorless RPMI solution) was added into each well. After 3 h of incubation with MTT, each well of the plate was added 100 ml Dimethyl Sulfoxide (DMSO) to dissolve the purple formazan. In order to being deposited the remaining nanoparticles in the solution of the wells, 96 well plate was centrifuged at 2500 rpm for 10 min. After centrifuge, supernatant was transferred to another 96-well plate. Then; absorbance of each well was read by using an Enzyme-Linked Immunosorbent Assay (ELISA) plate reader with a reference wavelength of 570 nm. The cell viability was determined by the following formula:

\[
\text{% Cell viability} = \left( \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \right) \times 100
\]

All experiments were repeated three times. All control groups included control samples containing PC3 and DU145 and RPMI 1640 culture medium in the absence of nanoparticles.

**Incubation of cells with the bare and coated MNPs**

For the preparation of gelatin suspension 1 g of powder gelatin dissolved in 50 ml of PBS solution, and put it for 30 min in water bath to obtain uniform solution. Two prostate cell lines (PC3 and DU145) incubated with different concentrations (0.5, 1 and 1.5 m mol) of CZF-MNPs and CZF-MNPs@ DMSA for 2 h at room temperature in RPMI 1640 culture medium. In order to separation of particles sticking together, before adding bare and coated MNPs suspension with different concentrations into RPMI culture medium were sonicated for 20 min. Then, the cells were washed three times with PBS solution. In the next step, 1 mL of gelatin suspension is added to 1.5 mL Eppendorf tubes with different concentrations, and stirring to obtain uniform solution. Then, the Eppendorf tubes with different concentrations are placed on ice powder, until obtained solution is solidity. Control groups are containing only 1 ml of gelatin suspension.

**In vitro MR imaging**

These Eppendorf tubes with different concentrations were used to experiment the in vitro MRI characterizations. T2-weighted images were obtained using a 1.5 T MRI scanner (1.5 T GE Medical system).

**Results and Discussion**

**Characterizations**

The surface morphology was evaluated by Transmission Electron Microscopy (TEM) (EM 900 model, Co Zeiss, operating at 80 keV). TEM bright field images of CZF-MNPs and CZF-MNPs @ DMSA (co-precipitation) are shown in Figure 1. This figure shown that particles have almost spherical structures and also the average particles size of CZF-MNPs and CZF-MNPs @ DMSA (co-precipitation) are 16 and 40 nm, respectively.

In order to prove the presence of the surface coated, the MNPs pre- and post- DMSA coated were evaluated using the FTIR method (Figure 2). Peaks at 3425 and 1643 cm<sup>-1</sup> are related to OH bands which indicated the presence of water in the structure of matter. Peaks at 580 and 427 cm<sup>-1</sup> indicated that the spinel structure was formed. Peaks at 928, 1160, 1360 and 1701 cm<sup>-1</sup> which showed in Figure (2b) indicated that DMSA coated on the CZF-MNPs surface. The FTIR diagrams analysis confirmed the formation of Co-Zn ferrite phase and showed the presence of cover on the surface of nanoparticles and also the presence of water content in the samples. The concentration of Fe in CZF-MNPs and CZF-MNPs @ DMSA are 325 and 225 ppm. This loss concentration indicated the presence of DMSA coated on the surface of the bare MNPs.

**In vitro MR imaging**

The ability and suitability of synthesized nanoparticles as a MR contrast agents, was also confirmed with MR imaging techniques. T2-weighted images were obtained with a (1.5 T GE Medical system).
scanner. The results displayed in Figure 3 demonstrate that both uncoated and coated nanoparticles have been imported in PC3 and DU145 cell lines and induced grate signal intensity reduction.

**In vitro cytotoxicity (MTT assay)**

In vitro cell viability of PC3 and Du145 of HPCs after exposure to CZF-MNPs and CZF-MNPs @ DMSA was assessed by MTT assay at different concentrations of Fe. The viability of PC3 cells do not decreased versus control group by increasing Fe concentrations of CZF-MNPs, but only at a concentration of 0.3 mM has negligible toxicity (3%). The overall, the CZF-MNPs indicated that a high viability against PC3 cells. The viability of Du 145 cells at different concentrations of Fe of CZF-MNPs is more than 75% (Figure 4a).

As shown in Figure 4b, the same viability of PC3 and Du145 HPCs after exposure to CZF-MNPs @ DMSA was evaluated by MTT assay at different concentrations of Fe.

The viability of PC3 and Du145 cells is more than 75%, but only at concentrations of 1.2 and 1.5 mM it is less than 50%. The toxicity of coated nanoparticles at high concentrations (1.2 and 1.5 mM of Fe) is more than that of pure nanoparticles.

In this study CZF-MNPs @ DMSA at concentration of 0.9 mM Fe (after 24 h incubation), leading to proliferation of PC-3 and Du145 cancer cells, this result is similar to study which done by Ge et al. They have shown that after 24 h incubation of Fe₂O₃ @ DMSA with human aortic endothelial cells at concentration of 0.01 mg/ml, leading to proliferation of human aortic endothelial cells. In addition, at low concentration of 0.001 and 0.02 mg/ml, viability of human aortic endothelial cells is equal to control group (100%). The same result was obtained at 0.3 and 0.6 mM concentration of Fe (viability of PC3 and DU145 cancer cell lines is approximately 100%). Thus, certain doses of nanoparticles coated with DMSA can be used for biological researches, and in these special doses, no side effect was observed on healthy tissues.

**Conclusions**

The viability of the HPCs cells is dependent to concentration of MNPs. Also, the toxicity of these nanoparticles should be studied on the other cancer cell lines. The results showed that 14 nm CZF-MNPs did not induce toxicity in PC3 HPCs. Rather, increasing concentrations of CZF-MNPs is leading to proliferation of this cell line. It is also surprising, both in this study and another study which done by Ge et al. in 2013 [2], nanoparticles coated with DMSA, in a certain concentration, not only are not toxic but also are leading to proliferation of cancer cells. The mechanisms relative to proliferation of cancer cells are unknown. Therefore, further investigation is needed to demonstrate the cell viability effect. As a suggestion, the cytotoxicity effect of magnetic nanoparticles is needed, not only on cancer cell lines but also on normal cell lines.

**References**

