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Synergistic effect of the combination of triethylene-glycol modified Fe_3O_4 nanoparticles and ultrasound wave on MCF-7 cells

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ABSTRACT

Cancer is a group of disease characterized by uncontrolled growth and spread of abnormal cells in the body. The clinical treatments for cancer include surgery, chemotherapy and radiotherapy. Currently, employing new approaches for treatment has attracted more attentions. One of these approaches is sonodynamic therapy, which is an analogous approach based on the synergistic effect of ultrasound and a chemical component referred to as sonosensitizer. Recent years applications of nanotechnology have witnessed a tremendous expansion of research in medicine especially in treatment of cancers. The combination of sonodynamic therapy and nanotechnology can introduce a new way for cancer therapy. In this study, we used therapeutic ultrasonic waves with intensity of 1 MHz and different concentrations of Fe_3O_4 nanoparticles, as sonosensitizer, to investigate their combination effect on MCF-7 cell line. Briefly, we divided cells into four different groups; control, cells which got in touch with nanoparticles, cells that with exposure to ultrasound waves and cells which were influenced with combination of nanoparticles and ultrasonic waves. Finally, cell viability assay was used for detection of cytotoxicity effects. Experimental results revealed a significant decrease in viability of cells, which were affected by the combined action of ultrasound field and Fe_3O_4 nanoparticles, compared to the separate exposure of Fe_3O_4 nanoparticles or ultrasonic field. The synergic effect of ultrasound waves and Fe ions might be due to the production of toxic free radicals.

1. Introduction

Cancer is a complex, multifactorial and devastating disease with a leading cause of morbidity and mortality worldwide that has baffled researchers over the years [1,2]. Use of new approaches for cancer treatment has attracted a lot attention these years.

Combination therapy for the treatment of cancer is also becoming more popular because it generates synergistic anticancer effects. It seems that sonodynamic therapy (SDT) could be considered as a potentially crucial alternative way in comparison to the traditional treatment manner. In this technique, a chemotherapeutic agent known as sonosensitizers are used to increase the influences of ultrasound's preferred effects on cells [3].

Ultrasound is a mechanical wave with properties like: ability to periodic vibrations of particles, short wavelengths and frequencies above 20 kHz [4]. Its irradiation appears to be an appropriate method for damaging malignant cells [5] due to the intrinsic cellular responses. Indeed, it could generate microbubbles, micron sized (1–10 μm) bubbles that fluctuate in response to the incident ultrasonic waves [6], that will collapse under enough intensity to produce the inertial (or stable) cavitation [7,8].

When ultrasound is used in its therapeutic levels, microbubbles oscillations can lead to an increase in the permeability of microvessels and hence enhance the cellular uptake of different molecules, nanoparticles and therapeutic agents. This event is normally due to sonoporation which is generated by microbubbles oscillating in a stable motion (inertial cavitation) and temporarily open pores in the plasma membrane [6].

Inertial cavities are gas bubbles that expand by mechanical resonance and produce energy through collapsing. Enormous energy release by the expulsion of cavities produce temperature and pressure above 5000 K and 800 atm [7].

This energy could produce reactive oxygen species (ROS) which lead to a severe cytotoxic effect. Indeed, in the present of enough ROS in the cell, a cascade of events will activate inside the cell that

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eventually result in apoptosis [9–11]. There are two main apoptotic pathways; the extrinsic (receptor mediated) and the intrinsic (mitochondria mediated). While the extrinsic pathway requires sonosensitizers for interaction with their favorable receptors, the intrinsic pathway of apoptosis could be stimulated by both internal and external triggers, such as ultrasound [12]. Briefly, it could disrupt normal function of mitochondria. Moreover, the hydroxyl radicals, produced by ROS, could cause lipid peroxidation in plasma membrane through misappropriating distribution of electrons [13]. The accumulation of oxidants eventually leads to destruction of cellular proteins, enzymes, lipids, and nucleic acids, consequently the normal processes of the cell disrupt that lead to the improvement of diseases and cell death [14–17].

To enhance the effect of ultrasonic irradiation, there are enormous pure varieties of sonosensitizers, which could be terminated by cell death through different mechanisms ranging from increasing ROS content to disrupting the vascular networks of malignant cells [13,18].

The purpose of the present study is to obtain a way to promote the preferential damage of ultrasonic irradiation by using nanoparticles as sonosensitizers.

Nanotechnology is a new emerging frontier of this century which has potential to revolutionize different fields of science. It has a wide range of applications from informational technologies to medicinal applications [19,20]. In spite of the recent developments in conventional treatment strategies, the effective use of nanotechnology in medicine and pharmaceuticals is a fast growing field that generate a new research field called nanomedicine, which could introduce new suitable approach for cancer diagnosis and treatments because of the properties of nanoscale structures [16,21–23].

By reduction in the size of particles until nanoscale range, the ratio of surface to volume increases and therefore different properties of particles such as magnetic, optical and mechanical are changed [24–27] leading to their applications in different fields of science. For example in medicine, they could be used as a carrier in targeted drug delivery systems to convey therapeutic agents exactly to a certain biological entity [28] or act as therapeutics and interact on a cellular (10–100 nm), subcellular (20–250 nm), protein (3–50 nm) or genetic scale (10–100 nm) [29,30].

Among the various types of nanoparticles, magnetic nanoparticles (MNPs) especially Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) have attracted lots of attention particularly those that exhibit superparamagnetic properties (SPIONs), which are desired nanoparticles for use in biomedical applications [31,32]. For instance they could be used for a specific biological purpose such as cell isolation, drug delivery, diagnostics through magnetic resonance imaging (MRI), cellular imaging and hyperthermia [29,33–35].

The toxicity of MNPs in biological environment is dependent on a range of factors related to the properties of the MNP such as size [36], concentration [37], surface properties [38–40] and structural properties [41,42].

According to the cellular study, the main cause of toxicity by MNPs is oxidative stress that is primarily formed by the incomplete reduction of oxygen and can impair cell metabolism and increase apoptosis. Therefore, MNPs could be used in cancer therapies to destroy cancer cells [14,15].

In this study, the combination effect of Fe_3O_4 nanoparticles and therapeutic ultrasound waves in the viability of cancer cells was investigated. To this end, therapeutic ultrasonic waves with intensity of 1 MHz and different concentrations of Fe_3O_4 nanoparticles, as sonosensitizers, were used. Briefly, the cells were assigned to four different groups; control cells; cells which got in touch with nanoparticles; cells with exposure to ultrasound and cells which were influenced with the combination of nanoparticles and ultrasonic waves. Finally, MTT assay has been carried out for detection of cytotoxicity effects.

2. Materials and methods

2.1. Materials

Iron (III) acetylacetonate ($Fe(acac)_3$) and ethyl acetate were purchased from Merck. Triethylene glycol (TREG) was obtained from Novachem. For cell culture we purchased Dulbecco's Modified Eagle's Medium (DMEM) and penicillin–streptomycin from BioIdia and Fetal Bovine Serum (FBS) from Gibco. Moreover we obtained 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma. MCF-7 cell line was obtained from Pasteur Institute of Iran.

2.2. Synthesis of SPION

Thermal decomposition method was chosen for synthesis of SPION, briefly 0.53 g of $Fe(acac)_3$ and 30 ml TREG were mixed and magnetically stirred under a flow of nitrogen. They were heated until reflux temperature during 3 h. TREG in this reaction played a triple role as high-boiling solvent, reducing agent, and stabilizer to efficiently controlled the particle growth and prevented interparticle aggregation [43,44].

After cooling the blackish suspension to room temperature, 20 ml ethyl acetate was added to it and mixed through magnetic stirred. After the precipitation of nanoparticles, they were washed 3 times with ethyl acetate and dried under vacuum [45].

2.3. Size, structure and morphology of SPION

Transmission electron microscopy (TEM) (JEM-2100F, Japan operating at an accelerating voltage of 200 kV) was used to determine the size and morphology of SPION. The crystal structure was identified by X-ray diffraction (XRD) on a Philips B-6797 diffractometer with Cu Kα radiation and the diffraction pattern was accumulated in the 2θ range of 5–90° at a scan speed of 2° 2θ/min [46].

2.4. Fourier transform infrared (FT-IR) spectrum

JACSO 6300 FT-IR spectrometer used for Fourier transform infrared (FT-IR) with a resolution of 4 cm^{-1}. To characterize SPION, small amount of nanoparticle powder was milled with KBr and a mixture of them was pressed into a disc for analysis.

2.5. Biocompatibility testing

2.5.1. Cell culture

Human breast adenocarcinoma cell line, MCF7, was cultured in DMEM medium with 10% fetal bovine serum and 100 mg/ml streptomycin/ penicillin. The cell line was grown in cell culture flasks in an atmosphere of 5% CO_2 at 37 °C. The cells were separated from flask surface by trypsin addition. The solution of iron oxide nanoparticles – SPIONs in medium culture in concentration of 200, 100 and 50 μg/ml – was prepared through mechanical agitation due to ultrasonic field.

2.5.2. Experimental design

The cells were incubated for up to 72 h after the following modes of treatment:

- Addition of SPIONs only (Fe_3O_4).
- 1 min exposure to ultrasound only (us).
- Addition of SPIONs and subsequent 1 min exposure to ultrasound (Fe_3O_4 + us).