Supermagnatic Iron Oxide Nanoparticles Toxicity to Mammalian Cells

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ABSTRACT

The present study investigates developing superparamagnetic iron oxide nanoparticles (SPION) as controlled release anticancer agents. SPIONs were synthesized (NP1, NP2, NP3) and large crystal growth particles (NP3, 50 nm) were loaded with anti-cancer drug, doxorubicin (NP4). Drug release assay from NP4 revealed 44% drug release in 24 h with no further release up to 96 h. The nanoparticles were tested for their toxicity to human cells, HEp-2 using MTT assay. HEp-2 cells, showed 90% viability upto 250 µg/ml nanoparticles concentration. Both bare and drug loaded nanoparticles were then tested for their efficacy to kill mouse mammary tumor 4T1 cells. NP4 were able to kill 50% 4T1 cells at 75 µg /ml whereas NP3 caused 50% cell death at 150 µg/ml concentration. Our results indicate SPIONs to be a good drug delivery agent against cancer cells with low toxicity towards normal healthy cells.

Keywords: superparamagnetic iron oxide nanoparticles, toxicity, HEp-2 cells, 4T1 cells

1. INTRODUCTION

The use of nanoparticles, particularly magnetic nanoparticles have earned much attention recently due to their small size, high surface area to volume ratio, surface charge, and magnetic properties. Such properties have enabled these particles to be used in the biomedical applications including magnetic resonance imaging as contrast agents, hyperthermia agents to kill tumor cells and, as magnetic vehicles for targeted delivery of genes, drugs, peptides etc. to specific cells [1-4]. Recently their application for systemic delivery of anti-cancer drugs is being investigated [5-6]. There are many limitations when using conventional chemotherapy for cancer treatment. However, using magnetic nanoparticles as potential drug delivery vehicles offers many advantages such as low dosage requirement of the drug, reduction of therapeutic side effects on healthy sites, and the ability to achieve site specific drug delivery. When using nanomaterials as drug delivery agents, few rationales have to be considered such as the loaded amount of drug, its physical state, its release kinetics and the influence of environmental factors. The present study investigates the possibility of developing superparamagnetic iron oxide nanoparticles (SPION) as controlled release drug formulation and use as anticancer therapeutic agents.

2. MATERIALS & METHODS

2.1. Synthesis of Nanoparticles

2.1.1. Np-1 Synthesis- Synthesis of Fe_3O_4 nanoparticles (from Iron Pentacarbonyl, size 5-10nm)

Fe_3O_4 nanoparticles were synthesized using ultrasonic irradiation of iron pentacarbonyl (2mL) in decaline (40 mL) in presence of PVA (250 mg) and distilled water (20 mL). The final solution was irradiated with a high-intensity ultrasonic probe (Sonics and Materials Model VCX 750, SS-Horn, 20 kHz, 750 W/cm^2) for 3 hours at 50% power. The reaction was carried out at room temperature in open air using a stainless steel vessel. The reaction temperature was maintained at approximately 30 °C by circulating the thermostatic water through a stainless steel vessel. The solution was rinsed with distilled water followed by ethanol and centrifuged (Allegra 64R centrifuge) at 12,000 rpm at 6°C. The precipitate was washed several times with ethanol and dried in vacuum chamber for 24 hours. These nanoparticles were further used for crystal growth as a nucleating agent.

2.1.2. Np-2 Synthesis (size 50-100 nm, crystal growth particles using EDA+HMT)

Crystal growth of Fe_3O_4 nanoparticles was carried out by using nanoparticles as a seed. For this method, Poly Vinyl Alcohol (PVA) (15 mg), distilled water (100 mL), as prepared nanoparticles (5mg), iron (II) acetate (500 mg) and ethylenediamine (EDA) (5 ml) and HMT (210mg) were used. PVA was first homogeneously dissolved in hot water. This solution was mixed with iron oxide nanoparticles, iron (II) acetate and EDA. The final reaction mixture was heated to 90°C for 2 hours. The mixture was kept

overnight. The solution was then diluted in ethanol and centrifuged as described in earlier section. Finally solution was separated and the precipitate was collected, dried and powdered.

2.1. 3. Np-3: Synthesis (size 50-100 nm, crystal growth particles using EDA)

Crystal growth of Fe_3O_4 nanoparticles was carried out by using nanoparticles as a seed. For this method, Poly Vinyl Alcohol (PVA) (15 mg), distilled water (100 mL), as prepared nanoparticles (5mg), iron (II) acetate (500 mg) and ethylenediamine (EDA) (5 ml) were used. PVA was first homogeneously dissolved in hot water. This solution was mixed with iron oxide nanoparticles, iron (II) acetate and EDA. The final reaction mixture was heated to 90 °C for 2 hours. The mixture was kept overnight. The solution was then diluted in ethanol and centrifuged as described in earlier section. Finally solution was separated and the precipitate was collected, dried and powdered.

2.1.4. Np-4 Synthesis (Drug loaded Nps)

A solution of doxorubicin (DOX) in water (1 mg/mL) was added to the SPIONs in water (1 mg/mL) at a ratio of 1 mg to 3 mg of iron oxide (Fe_3O_4). The mixture of SPIONs in DOX was shaken in a rotary shaker at room temperature for 24 hours to facilitate DOX uptake. To reduce degradation of DOX, the solution was covered with aluminum foil since doxorubicin is highly sensitive to light. After 24 hours, the drug loaded NPs were removed from the liquid by using a permanent magnet and the free drug in the supernatant was analyzed at 502 nm by UV-vis spectrometer (NANODROP 2000 Spectrophotometer). The drug loaded magnetic nanoparticles were then magnetically separated and dried.

The synthesized nanoparticles were analyzed using XRD, FTIR, TEM and magnetic measurements.

2.2. Drug Release Assay

The drug loaded nanoparticles (2 ml) were placed inside a dialysis membrane (molecular weight cut-off: 3.5 kDa, Novagen). The membrane was immersed into a beaker containing 1 L PBS buffer(10mM, pH 7.4) under mechanical shaking (40 rpm) at room temperature. The dialysate was sampled at predetermined time points and the amount of DOX was determined by absorbance measurements (490 nm) using UV-vis spectrophotometer.

2. 3. Cell Line and Cell Culture

Mouse mammary tumor cells (4T1) were provided by Dr S. Soni from Alabama State University. 4T1 cells were propagated in DMEM with 10% FBS and 1% antibiotics and antifungal mixture.

HEp-2 cells were purchased from American Type Culture collection (ATCC; CCL-23) and were propagated by standard methods using Minimum Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-Glutamine, 75 U/ ml Penicillin, 100 μg/ml Kanamycin and 75 μg/ml Streptomycin.

2.4. Cell Viability Studies

The cytotoxicity of nanoparticles was determined by MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) dye reduction assay using CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) kit (Promega). HEp-2 cells were seeded in a 96-well plate at a density of 20,000 cells per well in MEM containing 10% FBS and grown overnight. Subsequently nanoparticles at different concentrations (0 to 250μg/ml) were added to the cells. Cell toxicity was determined at 24 and 48 h after post-incubation with nanoparticles. 15 μl of MTT was added to each well and incubated at 37 °C for 4 h to allow formation of formazan crystals. After 4 h, 100 μl of stop solution was added and formazan crystals were allowed to dissolve for 1 h at 37°C. The absorbance of formazan products was measured at 570 nm using a microplate reader (Tecan™ Instruments). The results were expressed as percentage viability compared to the untreated control. Concentrations of nanoparticles showing 50% reduction in cell viability (IC_{50} values) were then calculated.

Similar studies were conducted on 4T1 breast cancer cells which were seeded at a density of 1000 cells per well in DMEM supplemented with 10%FBS and 1% antimycotic-antibiotic.

3. RESULTS & DISCUSSION

3.1. Characterization of Nanoparticles

All synthesized nanoparticles were superpara-magnetic and highly porous. Among the synthesized nanoparticles, the Np3 (Fig 1a) were chosen for drug loading. Synthesized Np-3 nanoparticles were uniformly dispersed with minimum agglomeration

Fig. 1a). These particles were soft agglomerates loosely connected which is advantageous for holding more drug and to reduce the formation of a clot in the blood. These particles were further chosen to load with drug doxorubicin (Np-4).

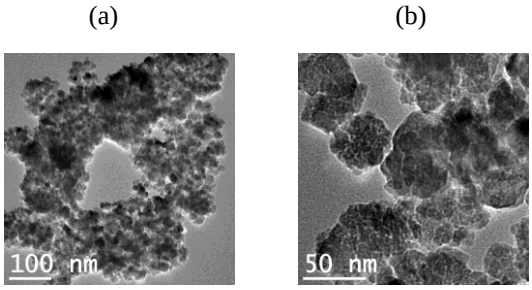

Fig 1: (a) Np-3 (50-100nm); (b) Np-4 (drug loaded nanoparticles)

3.2. Drug Release from Nanoparticles

Release of doxorubicin loaded on nanoparticles was estimated in PBS by measuring the absorbance (490nm) of the solution. In 24 h, 44% of doxorubicin was released from the nanoparticles with no further release up to 96 h (Fig. 2) indicating the nanoparticles to be good drug delivery agents for slow release to the targeted tissues.

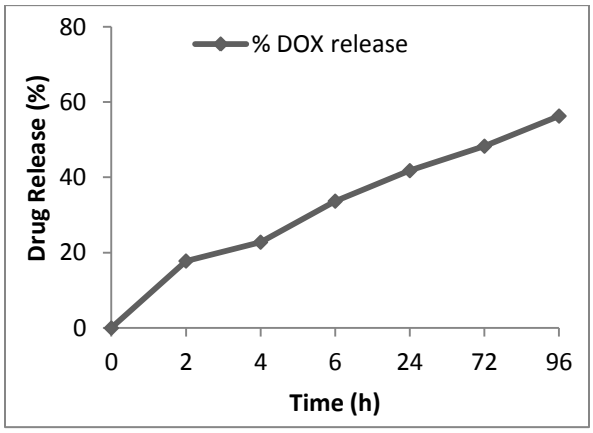

Fig 2. In vitro release of doxorubicin from Np-4 (drug loaded nanoparticles)

3.3. Cell Viability Analysis with Nano-particles

Viability assays are vital steps in toxicology that explain cellular response to a toxicant. Also, they give information on cell death, survival, and metabolic activities. Cell viability was estimated using MTT assay which evaluate mitochondrial function by measuring the degree of mitochondrial reduction of tetrazolium salt to formazan by succinic dehydrogenase. Concentrations ranging from 0 to 250 µg /ml of each nanoparticle sample were tested for toxicity to HEp-2 cells and 4T1 cells. HEp-2 cells showed very low toxicity to NP1, NP2 and NP3 with 90% viability even at 250 µg/ml concentrations. Toxicity of nanoparticles increased with time from 24 to 48 h. Nanoparticles loaded with drug were able to kill 50% 4T1 cells at 75 µg /ml whereas the same nanoparticles without drug showed 50% cell viability up to 150 µg/ml concentration (Fig. 3a-b).

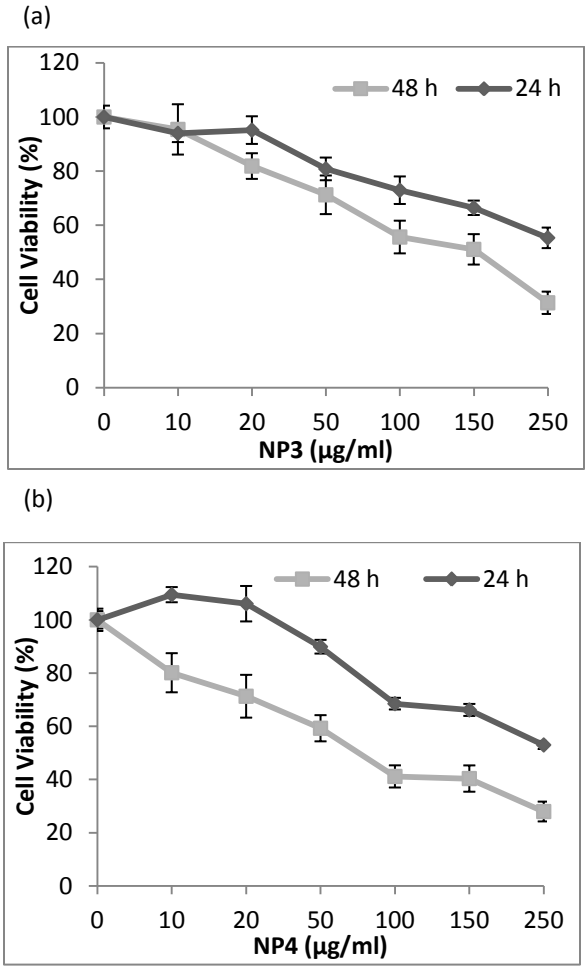

Fig 3. Toxicity of nanoparticles to 4T1 cells (a) Np-3 (b) Np-4 (drug loaded nanoparticles)

CONCLUSIONS

Nanoparticles loaded with drug were able to kill 50% 4T1 cells at 75 µg /ml whereas the same nanoparticles without drug caused 50% cell death at 150 µg/ml concentrations. Our results indicate iron oxide nanoparticles to be a good drug delivery agent

against cancer cells with low toxicity towards normal healthy cells.

ACKNOWLEDGMENTS

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