Superparamagnetic Iron Oxide Nanoparticles As Drug Delivery Vehicles

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ABSTRACT

In recent years, nanotechnology has emerged as one of the most promising fields for biomedical applications and healthcare. The use of nanoparticles has earned much attention due to their small size, high surface area to volume ratio along with surface charge, and their optical and semiconducting properties. Superparamagnetic iron oxide nanoparticles (SPIONs), which show superpara magnetism, have been reported to be reliable and safe for many biological applications. This property can be exploited for various purposes including drug delivery to targeted tissues. In the present study, 10nm SPION were loaded with anticancer drug, Docetaxel. Docetaxel conjugation to SPION was confirmed by FTIR, UV-vis spectroscopy and gel electrophoresis. SPIONs uptake by cells was investigated using Prussian blue dye staining. *In vitro* drug release was studied which showed 55% drug release in 24 h. SPIONs and drug toxicity to cell lines were tested using MTT assay. SPIONs showed 80% cell viability to HEp-2 cells at 100 µg/ml in 48 hours whereas drug alone was very toxic to HEp-2 cells reducing cell viability to 20%. Drug loaded SPIONs were then tested on Mammary tumor cells (4T1) and showed 55% cell viability with 0.1M Docetaxel conjugated to SPIONs. The results indicate that reduced concentration of Docetaxel is needed to treat 4T1 cancer cells when it is conjugated to SPIONs.

Keywords: SPIONs, Docetaxel, 4T1 cell, Cell viability

1. INTRODUCTION

Nanomaterials are being employed for treatment and diagnostics in recent years. One of such nanomaterial is SPIONs. SPIONs show superparamagnetism, which is the capacity of these particles to orient their magnetic domain according to the magnetic field being applied. This property can be exploited for various purposes including drug delivery to targeted tissues (1). Applications of SPIONs include usage in Magnetic fluid hyperthermia as well as MRI contrast agents (2-3). In the present study, Docetaxel, a semi synthetic analogue of paclitaxel was conjugated with carboxyl functionalized SPIONs. Docetaxel interferes with function of microtubule growth and destroys the cell's flexibility and is used for treatment for various cancers such as breast, gastric, prostate, etc. We hypothesized that Docetaxel-SPIONs can target specific cancerous tissues efficiently when guided by magnetic field with low amount of drug required, hence reducing the side-effects of the drug.

2. MATERIALS & METHODOLOGY

2.1 Materials

Carboxyl terminated SPIONs (10 nm) and Magnetic Nanocrystal coupling kit were purchased from Ocean NanoTech (Springdale, Arizona). The CellTiter96 Cell Proliferation Assay kit was purchased from Promega™ (Madison, Wisconsin). A Beckman Coulter Z1 Particle Counter (Fullerton, California) was used to perform cell counts. Docetaxel was obtained from Sigma-Aldrich (St. Louis, Missouri). Prussian blue Staining kit was obtained from ENG Scientific (Clifton, New Jersey).

2.2 Preparation of Docetaxel-SPIONs

Docetaxel was conjugated to the 10 nm carboxyl terminated SPIONs using The Ocean NanoTech Magnetic Nanocrystal coupling kit. Docetaxel 10 mM stock solution was prepared in DMSO. Further dilution of Docetaxel was achieved with coupling buffer to give a final concentration 2 mg/mL. For conjugation, 0.2 mL of the functionalized SPIONs was mixed with 0.1 mL of activation buffer and 100 µL of the EDAC/NHS solution. With continuous mixing at room temperature 2mg/ml of Docetaxel was added to this mixture and stirred continuously at 2h. At the end of incubation, 10 µL of the quenching solution was added and mixed for 10 min. This was followed by an addition of 3 mL of wash/storage buffer to the samples. The samples were centrifuged at 30,000 rpm and the wash/storage buffer was removed. The nanoparticles were then placed in 1 mL of washer/storage buffer and stored at 4°C.

2.3 Docetaxel-SPION Characterization

2.3.1 Agarose Gel Electrophoresis

The Docetaxel-SPIONs size was compared to the carboxyl terminated SPIONs using 1% agarose gel

electrophoresis. The carboxyl-SPIONs served as a control for the drug loaded SPIONs.

2.3.2 Fourier transformed infrared spectroscopy (FT-IR)
The characteristic bands of the conjugated samples and carboxyl-SPIONs were studied using Thermo Fisher Nicolet 380. The spectra were collected at 400 - 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.3.3 Visible Spectroscopy
The absorbance spectra of carboxyl-SPIONs and Docetaxel-SPIONs were characterized using Beckman Coulter DU 800 spectrophotometer.

2.3. *In Vitro* Drug Release
Docetaxel-SPIONs were dispersed in buffer and placed in a dialysis membrane cassette (molecular weight cut-off of 3.5 kDa) and placed into 1L of PBS (pH 7.4). The entire system was kept at room temperature in a beaker (220 $\pm$2 r/min). After a predetermined period, 100 µl of solution was removed from the dialysis bag and the amount of docetaxel was analyzed by UV-Vis spectrophotometer at 400nm. The released docetaxel was determined by a calibration curve.

2.4. Cellular Uptake of Nanoparticles
2.4.1 Cell Culture
HEp-2 cells (CCL-23) and HeLa cells (CCL-2) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Mouse mammary tumor cells (4T1) were provided by Dr. S. Soni from Alabama State University. HeLa and HEp-2 cells were cultured in minimal essential medium (MEM/H) (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Life technologies, Carlsbad, CA), 2 mM L-glutamine (Life Technologies) and 1 μg/mL antibiotic and antimycotic solution (Life Technologies). 4T1 cells (mouse mammary tumor cells) were cultured in Dulbecco Modified Eagle Medium (DMEM) (ATCC) supplemented with 10% heat-inactivated FBS, 1 μg/mL antibiotic and antimycotic (Life Technologies). All cells were maintained at 37°C in a humidified incubator containing 5% CO_2.

2.4.2 Cellular Uptake of Nanoparticles by Cell Lines
The cells were plated in 35 mm culture dishes. After 24 hr the cells were incubated with SPIONs. The following day, the cells were fixed with 0.5 M of 4% paraformaldehyde solution for 10 min followed with a wash with PBS. The cellular uptake of the nanoparticles was viewed using the Prussian blue stain kit via microscope. Images were taken using the bright field light mode of the immunofluorescence microscope Nikon Ti Eclipse at a total magnification of 200X magnification.

To investigate effect of magnetic field on SPION uptake by cells a magnet was placed on the bottom of the dish for 24 hr and the cells were incubated at 37 °C in 5% CO_2. After incubation, the cells were fixed and stained using the Prussian blue stain as mentioned above.

2.5. Cell Viability with Nanoparticles
The cytotoxicty 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).

3. RESULTS & DISCUSSION

3.1. Characterization of SPIONs
The carboxylic acid functionalized SPIONs used for this study were 10nm in size and spherical shape with coating of an oleic acid and monolayer amphiphillic polymer. The zeta potential of the particles was -79.5 mV, which is a strong zeta potential indicating the particles to be very stable.

3.2. Drug Conjugation to SPIONs
The mobility of the Docetaxel-SPIONs, Docetaxel, and SPIONs were observed on a 1% Agarose gel (Fig.1). The Docetaxel-SPIONs migrated slower than the SPIONs which reveal that the conjugation was successful.

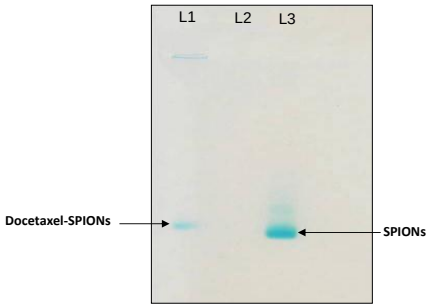
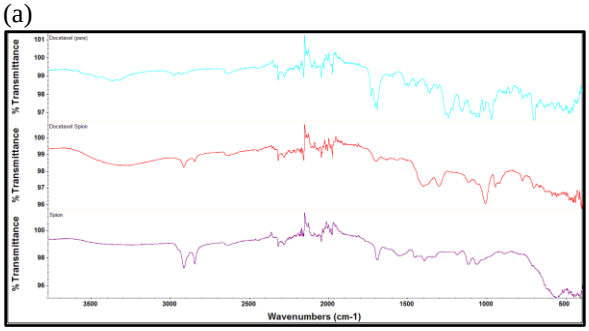


Fig 1. Gel Electrophoresis analysis of Docetaxel-SPIONs (L1), Docetaxel (L2), and bare SPIONs (L3).

FT-IR spectroscopy was conducted to confirm the presence of Docetaxel on SPION surface (Fig. 2a-b). The FTIR spectroscopy was done for Docetaxel-coated SPIONs, Docetaxel-free SPIONs and Docetaxel alone (Fig. 2a-b). SPION showed strong characteristic peak at 584 cm^{-1}which is characteristic of the Fe-O vibration, in agreement with other studies (4-5). Between SPIONs and Docetaxel electrostatic

bonds may form between carbonyl oxygen and hydroxyl groups of Docetaxel with Fe in iron oxide crystal. This interaction lead to disappearance of hydroxyl bands centered at 3487 and 3377 cm^{-1} in FTIR spectrum of Docetaxel-coated SPIONs.

(a)



(b)

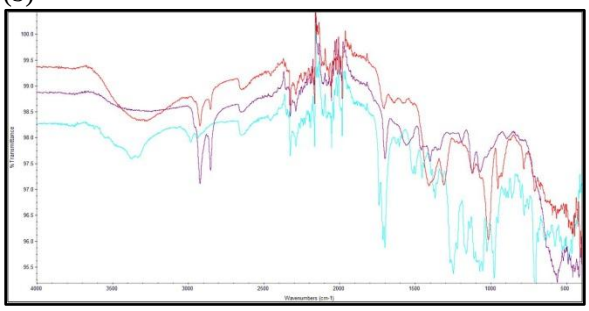


Fig 2. (a) FTIR analysis of SPIONs, Docetaxel and Docetaxel-SPIONs (b) Overlapped FTIR spectra of SPION, Docetaxel and Docetaxel-SPIONs.

Docetaxel conjugation to the SPIONs was also evaluated using UV-Vis spectroscopy which showed a shift in the absorbance (Fig. 3).

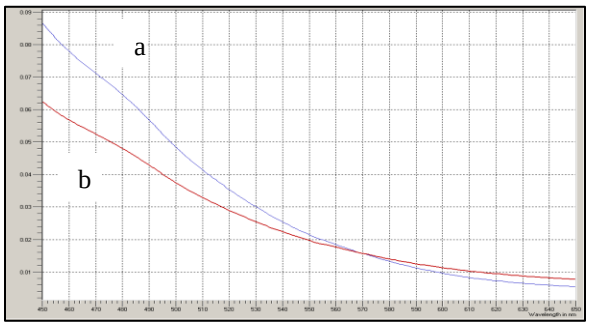


Fig 3. Visible Spectroscopy Spectra of (a) SPIONs (b) and Docetaxel-SPIONs.

These results indicate that the conjugation was successful.

3.3. In Vitro Drug Release Assay

The drug release behavior of the Docetaxel conjugated with SPION was investigated by dialysis. We observed 55% of Docetaxel release at 24 h (Fig. 4). By 72 h, 85% of the drug was released indicating there is a gradual release of the drug from the SPION.

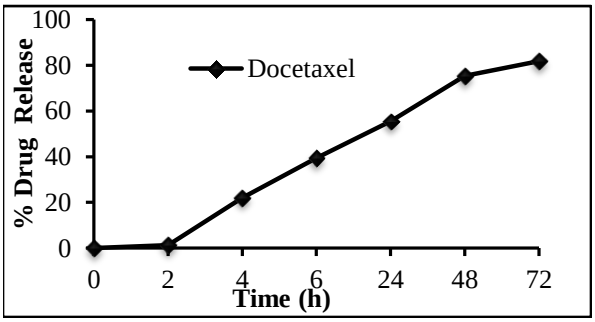


Fig 4. *In Vitro* Drug release from Docetaxel-SPIONs.

3.4. Cellular Uptake of Nanoparticles

The cellular uptake of SPIONs by HEp-2, HeLa, and 4T1 cells were studied using Prussian blue staining. The Prussian blue dye stains SPIONs a blue color. The control cells were not incubated with SPIONs and did not show any blue color staining (Fig. 5a). The 4T1 cells that were incubated with SPIONs stained blue with Prussian blue staining (Fig. 5b).

(a) (b)

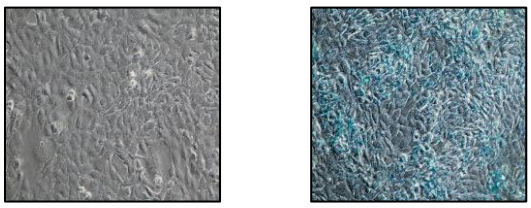


Fig 5. Uptake of SPIONs by 4T1 cells (a) Control (b) Cells incubated with SPIONs.

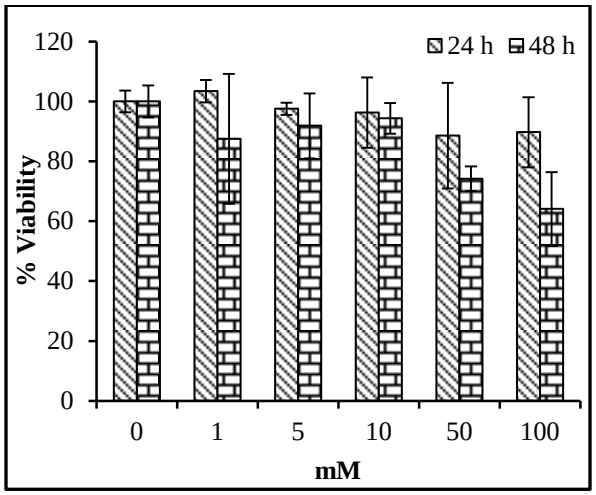
When a magnet was placed under the culture dish to investigate the effect of a magnetic field on cellular uptake of SPIONs, cells that were in the vicinity of the magnetic field displayed maximum blue staining with Prussia blue. The cells away from the magnetic field showed little to no staining. The results indicate an external magnetic field can increase the cellular uptake of SPIONs and guide the nanoparticle to a specific site.

3.5 Cell Viability with SPIONs and Docetaxel-SPIONs

SPION and drug toxicity to cell lines were tested using MTT assay. SPION showed 80% cell viability to HEp-2 cells at 100 µg/ml in 48 hours whereas drug alone was very toxic to HEp-2 cells reducing cell viability to 20% in 48 hours at 100 µg/ml.

When the cells were treated with Docetaxel, a drastic decrease in cell viability was observed with HEp-2 and HeLa cells. However, Docetaxel did not decrease 4T1 cell viability much. After 48h, there was a 35% reduction in 4T1 cell viability at 100 nM Docetaxel (Fig. 6a). Drug loaded SPION were then tested on 4T1 and showed 55% cell viability with 0.1M Docetaxel conjugated to SPION (Fig. 6b). The results indicate that the concentration of Docetaxel needed to treat 4T1 cancer cells is decreased by a 1000 fold when it is conjugated to SPIONs.

(a)



(b)

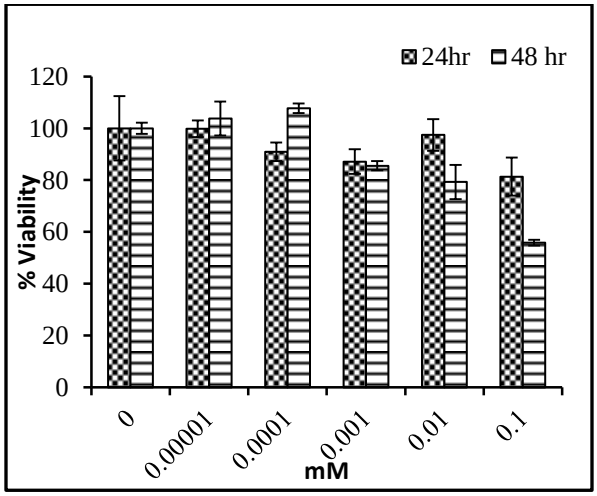


Fig. 6. Cell viability of 4T1 cells treated with (a) Docetaxel (b) Docetaxel-SPIONs for 24hr and 48hr.

CONCLUSIONS

In this study, SPIONs conjugated with Docetaxel were used as drug delivery vehicles to treat cancerous cells. The conjugation of docetaxel to SPIONs was confirmed using agarose gel electrophoresis, FT-IR, and Vis-Spectroscopy. In addition, Docetaxel was successfully released from SPIONs gradually over the period of time. It was observed that the cellular uptake of SPIONs can be increased by the addition of an external magnetic field. Furthermore, the efficacy of Docetaxel was increased when conjugated to SPIONs, thereby, decreasing the concentration of the drug required to treat 4T1 cells. SPIONs can be utilized as drug delivery vehicles in cancer therapy.

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ACKNOWLEDGEMENTS

This study is supported by NSF-CREST (HRD-1241701) and NSF-HBCU-UP (HRD-1135863).