# Nanotransporter for the delivery of anticancer drugs : A potential therapeutic tool against drug resistant cancers

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## INTRODUCTION AND OBJECTIVE

Tumor is a tissue constitution of heterogeneous populations of drug sensitive and drug resistant cells. Chemotherapy kills drug-sensitive cells, leaving behind a higher proportion of drug-resistant cells and hence limiting the effectiveness of therapeutic agents (Fan, 2013).

Our objective is to increase the cytotoxic efficacy of anticancer drugs in drug resistant cells by exploring the role of nanotransporters for anticancer drug delivery against drug resistant cervical cancer cells).

We also report a novel biopolyelectrolyte pair protamine and carboxymethyl cellulose for fabrication of multilayered nanotransporter and further incorporation of ferrite nanoparticles for magnetic field based targeted drug delivery studies.

## MATERIALS AND METHODS

### *Materials*

Protamine, carboxymethyl cellulose, doxorubicin hydrochloride, apoptosis detection kit (Annexin V conjugated with FITC) and MTT reagent were purchased from Sigma Aldrich, India. Ferrite nanoparticles ($Fe_3O_4$) having size of about 15±5nm was a kind gift from Prof. Chandan Srivastava (Dept. of Materials Engineering, IISc, Bangalore).

### *Preparation of nanotransporters*

Nanotransporter used in this study is a multilayered polyelectrolyte nanocapsule formulated by layer by layer deposition of alternatively charged biopolymers, protamine and carboxymethyl cellulose, on silica nanoparticles. The core was leached out to produce hollow nanocapsules and utilized for delivery of an anticancer drug, doxorubicin (dox) into drug-resistant cells at physiological conditions.

### *Microscopic characterization of nanotransporters*

The morphology of the nanotransporters such as size, shape and polymer coating thickness were studied by field emission scanning electron (FESEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The magnetic strength at the surface of the ferrite nanoparticles embedded magnetic nanocapsules was calculated by magnetic force microscopy (MFM).

### *Cell culture*

Drug-resistant human adenocarcinoma cell line (HeLa) was used for drug internalization studies. About 1x103 number of cells per well were grown in 24-well plates with DMEM supplemented with 10% FBS at 37°C incubator and 5% $CO_2$ supply. All experiments were carried out with 70% confluence and supplemented with 10% FBS in DMEM to mimic the human physiological condition.

### *Nanotransporter internalization against dox-resistant cells and its trafficking*

Cells were treated with nanotransporter and magnetic nanotransporter encapsulated with dox (5µg/ml) for 1h, 4h and 12h. Cells were also treated with free dox as a control. For magnetic field based drug delivery, the magnetic field was created externally to the cells by keeping bar magnets under 24-well plates. The experiment was terminated at different time intervals and cells were washed with PBS to remove unattached nanotransporters from the cell surface. The cells were then subjected to confocal microscopy (CLSM) imaging and flow cytometry analysis (FACS).

### *Cytotoxicity assay*

Cytotoxicity assay was done to study the effect of various concentrations of dox on viability of dox-sensitive and dox-resistant HeLa cells. To determine the IC50 value of dox in sensitive cells, cells were treated with 0.1 to 50µg/ml of dox for 48 hrs. Similarly, to determine IC50 value in dox resistant cells, cells were treated with 40 - 130µg/ml dox for 24, 48 and 96 hrs and cytotoxicity was measured by using MTT assay. After comparing the difference between IC50 values of dox for sensitive and resistant HeLa cells, we analyzed cytotoxicity of encapsulated drug by MTT assay.

### *Apoptosis assay*

Dox resistant HeLa cells were treated with free dox and encapsulated dox. Cells were then processed for determining the percentage of apoptotic cells by using apoptosis detection kit as per the manufacturer's protocol. The samples were later analyzed using FACS.

## RESULTS AND DISCUSSION

### *TEM characterization of nanotransporter*

TEM analysis showed that the nanotransporters possessed a spherical shape with diameter of about 150nm (Fig 1). The removal of silica core from the

coated particles was verified using EDX software attached with FESEM (data is not shown here).

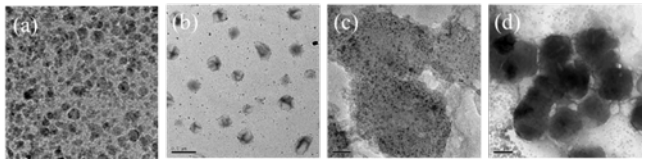


**Figure 1. TEM characterization of (a) Ferrite nanoparticles, (b) Hollow nanotransporters, (c) Magnetic nanotransporters and (d) Dox loaded nanotransporters.**

***Pharmacokinetic studies***

Dox loading studies revealed that 65% and 27% loading was achieved above the physiological pH (pH 8) and acidic pH (pH 4) respectively. At pH 8 and above, strong electrostatic repulsion force between the polymeric layers permits more drug loading. The dox release profile was studied after enzymatic triggering of dox loaded nanotransporters with trypsin enzyme. Results showed that about 90% dox was released over a period of 6hr (data is not shown here).

***Dox internalization studies in dox-sensitive and dox resistant HeLa cells***

Dox resistant as well as dox sensitive HeLa cells were treated with free dox (5µg/ml). Dox resistant cells did not show internalization of dox due to the pgp effect. Upon 12 hrs treatment with dox encapsulated with nanotransporters, a marked increase in the delivery of dox molecules into resistant cells was observed. CLSM images reveal that dox concentration in the cells increased with treatment time (Images are not shown here).

***FACS quantitation of dox in dox-resistant cells***

The mean fluorescent intensity (MFI) was calculated for different treatment times and highest MFI value was obtained after treating the cells with dox encapsulated magnetic nanotransporters along with external magnetic field (Fig 2).

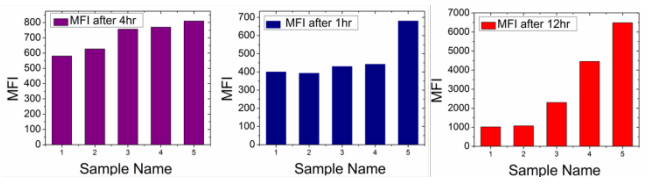


**Figure 2. Mean Fluorescence Intensity (MFI) of dox in dox resistant cells, (a) MFI after 1hr treatment, (b) after 4 hrs and (c) after 12 hrs. Sample information for the bar chart (1) Cells alone, (2) Dox alone, (3) Dox encapsulated nanotransporters, (4) Dox encapsulated magnetic nanotransporters without magnetic field, (5) With external magnetic field.**

***Cytotoxicity assay***

MTT assay showed 80% cell death in drug resistant cells treated with dox encapsulated nanotransporters (100µg/ml) while free dox showed 50% cell death after 96hr treatment. The empty nanotransporters did not show any cytotoxicity up to100µg/ml which confirms their biocompatibility (Fig 3).

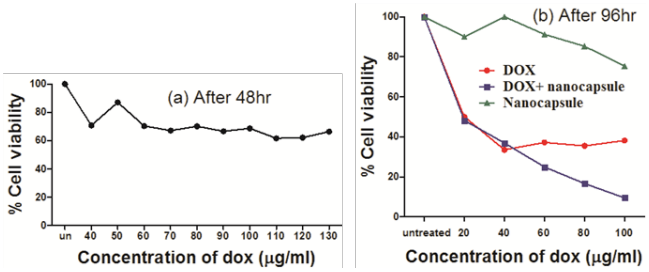


**Figure 3. Cytotoxicity assay. (a) Percentage cell viability of dox-resistant cells after 48hr of free dox treatment and (b) After 96hr treatment with free dox and encapsulated dox.**

## CONCLUSION

Herein we report that these nanotransporters are more efficient cargo systems for intracellular drug delivery in drug-resistant HeLa cells. Inhibition of the drug efflux through ABC transporter (p-glycoprotein efflux effect) by these nanotransporters might attribute to their increased cytotoxicity. Magnetic nanotransporters showed highest drug internalization in presence of an external magnetic field. This nanotechnology therapy can be useful in refining the treatment strategy of drug resistant tumors.

## REFERENCE


- Fan Bai et al. (2013) *Nanoparticle-mediated drug delivery to tumor neovasculature to combat Pgp expressing multidrug resistant cancer*. Biomaterials (In press) 1-12.


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