

# Preparation, characterization, and antiproliferative activities of biotin-decorated docetaxel-loaded bovine serum albumin nanoparticles

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The aim of the present study was to characterize biotin-decorated docetaxel-loaded bovine serum albumin nanoparticles (DTX-BIO-BSA-NPs) and evaluate their antiproliferative activity *in vitro*. The particle size of prepared DTX-BIO-BSA-NPs was found to be always lower than 200 nm, with sizes of 166.9, 160.3, 159.0, 176.1 and 184.8 nm and the zeta potential was -29.51, -28.54, -36.54, -36.08 and -27.56 mV after redissolution with water for 0, 1, 2, 4 and 8 hours, respectively. The polydispersity index (PDI) was stable in the range of 0.170 - 0.178. In the *in vitro* drug-release study, the DTX-BIO-BSA-NPs targeted a human breast cancer cell line MCF-7 effectively. The x-ray diffraction spectrum and DSC curve of DTX-BIO-BSA-NPs suggested that docetaxel was in an amorphous or disordered crystalline phase in DTX-BIO-BSA-NPs. *In vitro* cytotoxicity results showed that DTX-BIO-BSA-NPs inhibits proliferation of MCF-7, SGC7901, LS-174T and A549 cells in a concentration-dependent manner after exposure to DTX-BIO-BSA-NPs for 48 hours. Taken together, these results indicate that DTX-BIO-BSA-NPs may have potential as an alternative delivery system for parenteral administration of docetaxel.

**Keywords:** Albumin/nanoparticles/characterization. Docetaxel. Antiproliferative activities. Biotin.

## INTRODUCTION

Docetaxel (DTX) is a semi-synthetic, taxane-derived, highly potent anticancer drug. It represents the second generation of taxane anticancer drugs and has shown broad spectrum antitumor activity against prostate, breast, pancreatic, lung, gastric and hepatic carcinomas (Xu *et al.*, 2009; Hwang, 2012; Zhao, Astruc, 2012; Chen *et al.*, 2016). Docetaxel binds irreversibly with  $\beta$ -actin and stabilizes the microtubule assembly which is responsible for inhibition of cell division and finally cell death (Musumeci *et al.*, 2006). Therefore this drug is currently used as a first-line treatment in clinical chemotherapy. However, the current preparations of docetaxel have some drawbacks such as water-insolubility, sensitization and toxic side effects.

Nanoparticles are made of a variety of polymers, such as polysaccharides (Fernandez-Urrusuno *et al.*, 1999; Liu *et al.*, 2008), proteins (Elzoghby, 2013; Harsha 2013)

and synthetic polymers (Fattal *et al.*, 1998; Breunig, Bauer, Goepferich, 2008). Among all the available materials, biological molecules have attracted considerable interest for application to nanomedicine because they are fully biocompatible and water-soluble (Kratz, 2008). Biological molecules have several advantages over small molecules and polymers for biomedical applications. First, the water-solubility of surface-modified nanoparticles generally increases with increasing mass of the ligands (Ackerson, Jadzinsky, Kornberg, 2005; Sperling, Parak, 2010) and thus, biological molecules will provide enhanced water-solubility for nanoparticles. Second, conjugating biological molecules to nanoparticles controls the release of drug in a sustained manner over a longer period of time (Roney *et al.*, 2005).

As a water-soluble protein, serum albumin is the most abundant and basic protein of blood plasma. It is primarily involved in the transport of substances in the blood that are poorly soluble in water, including drugs, and also alters the disposition of endogenous and exogenous compounds present in blood (Moreno, Cortijo, Gonzalez-Jimenez, 1999). Albumin is also reported to associate with nanoscale particles to promote

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their transportation through cells (Mehta *et al.*, 2004; Zhang *et al.*, 2008). Additionally, albumin nanoparticles exhibited high binding capacity of various drugs (Jithan *et al.*, 2011; Kratz, 2014) and were well tolerated without any serious side effects (Ibrahim *et al.*, 2002). Bovine serum albumin (BSA) is an important carrier protein in blood plasma for several ions and molecules. It is one of the most extensively-studied proteins and has structural homology to human serum albumin as a substitute for research (Yu *et al.*, 2012). Accordingly, albumin nanoparticles have received considerable attention (Elzoghby, Samy, Elgindy, 2012). Encouragingly, a paclitaxel-loaded human serum albumin nanoparticles (Abraxane) was approved by FDA for clinical use in 2005. It improved water solubility of paclitaxel with enhanced efficacy and tolerability compared with a Cremophor-based paclitaxel formulation (Cortes, Saura, 2010). Targeted therapy is among the most attractive strategies for cancer, the major cause of death in most advanced countries in the world. Conventional cancer chemotherapy, which usually utilizes small molecule drugs, is far from successful, mostly due to the lack of tumor selectivity, which leads to dose-limiting toxicity, resulting severe adverse effects that limits usage. To overcome these drawbacks, targeted anticancer therapy is aims to have tumor-selective anticancer effects with less systemic side effects. To increase the delivery of a given drug to a specific target site, albumin can be surface conjugated using various targeting moieties or ligands, such as folate, saccharide, biotin and peptides. Among them, biotin is used widely as a tumor targeting ligand for various anti-cancer drugs.

Biotin (vitamin H) participates as a coenzyme in reactions involving CO<sub>2</sub> such as carboxylations, transcarboxylations, and decarboxylations. Biotin is widely applied in two-step pretargeting strategies (Balthasar *et al.*, 2005; Mårtensson *et al.*, 2007; Lesch *et al.*, 2010; Lindegren, Frost, 2011; Uppal *et al.*, 2011; Marysael *et al.*, 2012; Urbanska *et al.*, 2012). Recent studies revealed that biotin receptors were over-expressed on numerous tumors characterized by rapid dividing and aggressive growth (Russell-Jones *et al.*, 2004; Patel *et al.*, 2012; Li *et al.*, 2013).

In this paper, we introduced biotin ligand on the surface of docetaxel-loaded bovine serum albumin nanoparticles (DTX-BIO-BSA-NPs) in an attempt to specifically target the corresponding receptors of carcinoma. This study aimed to formulate DTX-BIO-BSA-NPs and evaluate their physicochemical properties, measure *in vitro* release and determine their effectiveness in tumor cells.

## MATERIAL AND METHODS

### Material

Bovine serum albumin was provided by Instituto Grifols, S.A. (Barcelona, Spain). The docetaxel was obtained from Shanghai Kairen Bio-Technology Co. Ltd (Shanghai, China). Fetal calf serum was obtained from Shanghai Solarbio Bioscience and technology Co. Ltd (Shanghai, China). Phosphate-buffered saline (pH7.4) and trypsin were purchased from Sigma Chemicals Co. (St. Louis, USA). Fluorescein isothiocyanate (FITC) and Lyso-Tracker Red were purchased from Beyotime Institute of Biotechnology (Haimen, China). RPMI 1640 medium was obtained from HyClone Inc. (Logan, USA). Acetonitrile and methanol (HPLC-grade) were obtained from J&K Chemical Ltd (Beijing, China). Water was collected from a Milli-Q organic free water system (Millipore, Bedford, USA). All the other chemical agents and chemicals were used as analytic reagents.

### Preparation of DTX-BIO-BSA-NPs

The DTX-BIO-BSA-NPs were prepared as previously described using a desolvation technique (Jun *et al.*, 2011). Briefly, 75 mg bovine serum albumin was first dissolved in 7.5 mL deionized water, then 33.75 mg docetaxel dissolved in 22.5 mL ethanol was added using a peristaltic pump BT01-100 (Kejian, Changzhou, China) at room temperature (1 mL/min). Afterwards, 220  $\mu$ L of 0.25% (v/v) glutaraldehyde was added to the resultant solution for amino groups crosslink in the nanoparticles. The cross-linking process was performed during stirring of the suspension for 15 h. The organic reagents were removed by evaporation using a rotary evaporator RE-52AA (Yarong, Shanghai, China) at 45 °C, and the nanoparticles were then redispersed in an ultrasonication bath FRQ-1008T (Front, Hangzhou, China). The nanoparticles were collected by centrifugation at 10000 g for 10 min (Sigma, Harz, Germany), followed by washing twice with ethanol and deionized water. N-hydroxysuccinimide ester of biotin (NHS-biotin) was prepared according with the method described by Xu *et al.* (2007). NHS-biotin 5 mg was dissolved in 0.1 M carbonate/bicarbonate buffer (pH9.0, 1.0 mL) and the stirred DTX-BSA-NPs suspension was added. It took 16 h for reaction. The carboxylic groups of NHS-biotin were conjugated with the amino groups of the BSA-NPs under alkaline conditions. With centrifuged at 8000 g for 10 min twice, the unassociated NHS-biotin in the mixture was then removed. Followed by freeze-drying (Scientz-

10N, NingBo, China) under a condition of controlled temperature (-40 °C for 24 h followed by -70 °C for 72 h) and pressure (1 pa) using 5% mannitol as a freeze-drying agent, the DTX-BIO-BSA-NPs were obtained.

### Morphology, particle size, and zeta potential of the DTX-BIO-BSA-NPs

A suitable amount of the lyophilized sample was dispersed in water. A drop of the solution was dropped on a round coverslip then blow-dried with sterile wind using a sterile bench, which also prevented dust from affecting the drop. The coverslip coated with the sample was used to investigate the morphology by SEM (FEI Co., Eindhoven, The Netherlands).

DTX-BIO-BSA-NPs were dispersed in water to attain a concentration of 5 mg/mL. Then 3 mL of the solution was placed in a sample cell to detect the particle size by laser light scattering and the zeta potential using a ZetaPlus Zeta Potential Analyzer (Brookhaven Instruments, Holtsville, USA). The data were obtained from the average of three measurements.

### Physical status of docetaxel and DTX-BIO-BSA-NPs

An x-ray diffractometer (Philips, The Netherlands) was used to determine the physical status of docetaxel in the nanoparticles. The diffraction angle ( $2\theta$ ) was recorded from 3° to 80° with a scanning speed of 5°/minute. CuK $\alpha$  radiation was used as the x-ray source at 40 kV and 30 mA.

### Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. In this paper, Five milligrams of samples were sealed in the aluminum pan and investigated by differential scanning calorimeter (TA instruments, Wilmington, USA) at a temperature heating rate of 10 °C/min that from 35 to 350 °C with nitrogen at a flow rate of 100 mL/min and pressure of 40 bar. The DSC was calibrated with indium, with an empty pan used as reference.

### Docetaxel determination by high-performance liquid chromatography

The docetaxel in ethanol was examined by high-performance liquid chromatography, i.e. a Waters liquid

chromatograph (Waters Corporation, Milford, USA), consisting of a Waters 600 Controller equipped with a Waters 717 Plus autosampler and a Waters 2487 UV detector. The samples were chromatographed at 25 °C by injection of a 20  $\mu$ L sample into a Hypersi C18 column (250 mm  $\times$  4.6 mm  $\times$  5  $\mu$ m, DEAC Company, Dalian, China). The mobile phase was a mixture of deionized water and acetonitrile in a volume ratio of 53:47 (v/v). The elution rate was 1.0 mL/min and the wavelength was set at 231 nm.

### Drug release

Drug release from DTX-BIO-BSA-NPs was studied and compared with drug release/diffusion from DTX suspension using a dialysis bag method (Liu *et al.*, 2011). The phosphate-buffered saline (0.1 M, pH 7.4) at 37 °C was used to determine the docetaxel release from the nanoparticles. After adding DTX-BIO-BSA-NPs suspension (0.5 mg/mL, 2 mL, without trehalose) or docetaxel solution (0.05 mg/mL, 2 mL) into Slide-A-Lyzer dialysis cassettes (Sigma-Aldrich, St. Louis, USA), the dialysis cassettes were then immersed in a 250 mL beaker containing 200 mL release buffer, which was placed in an incubator shaker (Jiangsu Jintan Medical Instruments Co., Ltd., Jintan, China) at 37 °C and 120 rpm. Later, with drawn from the beaker, 50 mL release buffer was replaced with 50 mL fresh release buffer by regular intervals. Each collected release buffer sample was concentrated to 1 mL by rotary evaporator, docetaxel was resolved by high-performance liquid chromatography and extracted from the removed medium after dividing into the organic phase. After being added to the medium, 5 mL chloroform was fully vortexed for 5 min and allowed to stand for 15 min for phase separation. Upon phase separation, the denser organic chloroform layer was carefully partitioned from the aqueous buffer phase and permitted to evaporate at the indoor temperature overnight. The dried sample containing docetaxel was then dissolved in 4 mL of methanol and analyzed by high-performance liquid chromatography. It was plotted to a profile showing cumulative drug release on the basis of time. And each diffusion experiment was operated repeatedly in triplicate, the average values and normal deviations were calculated in detail.

### *In vitro* cellular uptake

Human breast cancer cell line MCF-7 were used for studying the *in vitro* cellular uptake of the DTX-BSA-NPs and DTX-BIO-BSA-NPs labeled with fluorescein

isothiocyanate (FITC) and Lyso-Tracker Red. MCF-7 cells were plated in 6-well transparent plates of 300,000 cells per well, and incubated for 24 hours before labeling with FITC and Lyso-Tracker Red. After labeling with FITC and Lyso-Tracker Red for 2 hours at 37 °C, the cells were observed by confocal laser scanning microscopy (Nikon, Tokyo, Japan) with fluorescence view imaging software.

### Cytotoxic activities

To evaluate the cytotoxicity of DTX-BIO-BSA-NPs and free docetaxel, MTT assay was used to test five different concentrations (1.25 nM, 12.5 nM, 125 nM, 1.25 μM and 12.5 μM) of DTX-BIO-BSA-NPs and docetaxel against MCF-7 cells, SGC7901 cells, LS-174T cells and A549 cells at 48 h, and compared the cytotoxicity between the DTX-BIO-BSA-NPs and free docetaxel. MCF-7 (Human breast cancer cell line), SGC7901 (human gastric carcinoma cell line), LS-174T (human colon adenocarcinoma cell line) and A549 (human lung adenocarcinoma cell) were obtained from the Harbin Tumor Institute (Harbin, Heilongjiang, China). All cells were cultured in RPMI 1640 containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO<sub>2</sub> incubator. Cells (50,000 per well) were seeded in 96-well tissue culture plates for 12 h and were then treated with the DTX-BIO-BSA-NPs of different concentration or docetaxel control solution for 48 h. The media were then removed and cells were washed with PBS. An MTT assay was performed by adding 10 μL MTT reagent (5 mg/mL) to each well and cells were then incubated for 4 h, after which culture media were replaced with 150 μL of DMSO. Light absorbance of the solution was measured at 492 nm using a multi-plate reader (Awareness Technologies, Palm City, USA). The cell inhibitory rate was calculated as follows: inhibition rate =  $[(Abs_{492\text{control cells}} - Abs_{492\text{treated cells}}) / Abs_{492\text{control cells}}] \times 100\%$ . All assays were done with six parallel replicates. Cell viability at different concentrations of compounds was plotted and 50% inhibition of growth was calculated as IC<sub>50</sub>.

### Statistical analysis of the data

Data are presented as the mean ± standard deviation for all treatments. Student's t-test was used to evaluate the significance of differences between the treated and control groups. A value of  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

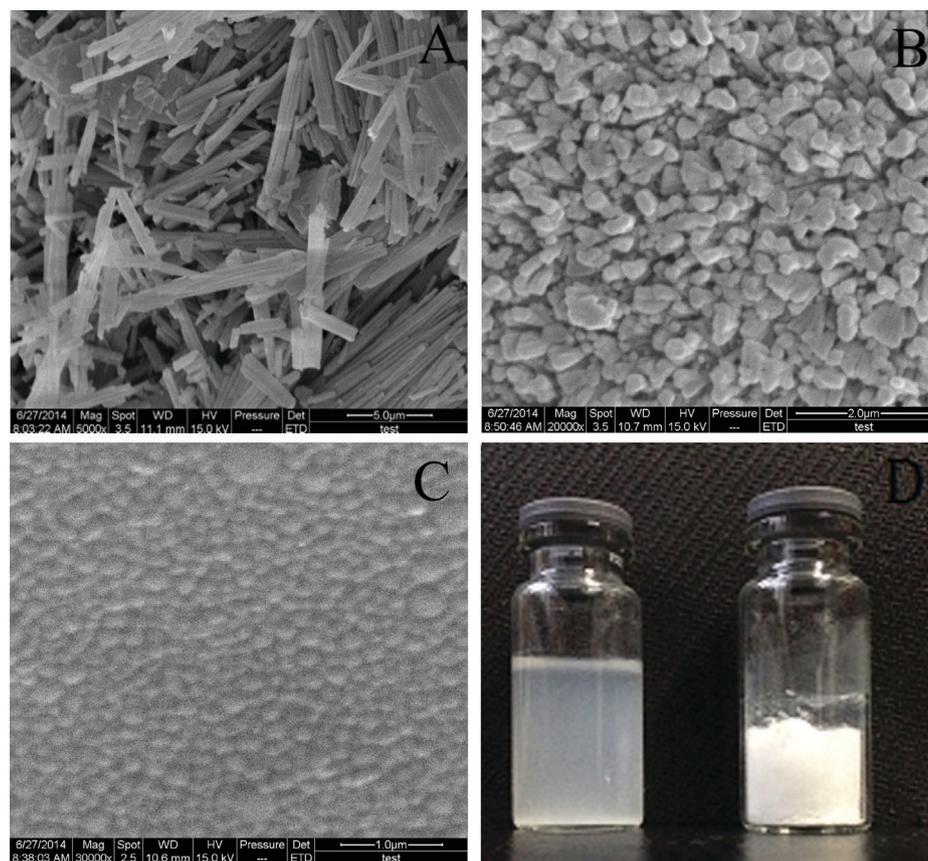
### Morphology, particle size and zeta-potential of DTX-BIO-BSA-NPs

Particle size is an important aspect for the passive targeting of nanoparticles (Stylianopoulos, 2013). Scanning electron microscopy (SEM) studies were carried out to determine the size and shape of nanoparticle, the images of which are shown in Figure 1. Raw docetaxel presented as irregular blocks, with a particle size ranging from 1 to 10 μm (Figure 1A). DTX-BSA-NPs and DTX-BIO-BSA-NPs exhibit a nearly spherical shape dispersed evenly (Figure 1B and 1C), consistent with the report of Zhu *et al.* (2016). The freeze-dried powder and aqueous dispersion of the powder are shown in Figure 1D. This image demonstrates that the freeze-dried powder reconstitutes well in solution, with a transparent opalescence and uniform state observable.

The dispersion of DTX-BIO-BSA-NPs in water was studied to measure the stability of the solution after 8 hours at a temperature of 25 °C. The particle size was always lower than 200 nm after redissolution with water during studied times as presented in Table I. DTX-BIO-BSA-NPs are small enough for the particles to accumulate in tumor tissue selectively via the enhanced permeation and retention effect (Nichols, Bae, 2014; Araki *et al.*, 2015). Restricting the size of nanoparticles to a range between 100-200 nm enhances extravasation from tumor blood microvessels as well as interstitial transport, leading to the accumulation and retention of nanoparticles in solid tumors (Vardhan *et al.*, 2017).

Zeta potential is defined as the difference of electrical potential between the surface of the cells and the bulk-surrounding medium. The surface charge has important effect on the stability of nanoparticles in solution (Ye *et al.*, 2016). In our experiments, the zeta potentials were -29.51, -28.54, -36.54, -36.08 and -27.56 mV for 0, 1, 2, 4 and 8 hours, respectively (Table I), and the polydispersity index (PDI) was stable in the range between 0.170 - 0.178. The phenomena of particle reunion, precipitation, and stratification did not occur when the solution was stored after 8 hours and the dispersion appeared equally distributed. Lower PDI indicated good dispersibility, a prerequisite of good stability. Higher zeta potential supported that the DTX-BIO-BSA-NPs did not aggregate much in the aqueous state in general and physiologically-relevant media in particular. Therefore, the DTX-BIO-BSA-NPs dispersion demonstrated good stability over the 8-hour observation period.

The x-ray diffraction spectrum of docetaxel and



**FIGURE 1** - Characterization of DTX, DTX-BSA-NPs and DTX-BIO-BSA-NPs. Scanning electron microscopic images of (A) raw docetaxel (magnification: 5000 $\times$ ). (B) DTX-BSA-NPs (magnification: 20000 $\times$ ). (C) DTX-BIO-BSA-NPs (magnification: 30000 $\times$ ). (D) Reconstitution appearance of DTX-BIO-BSA-NPs solution (left) and freeze-dried DTX-BIO-BSA-NPs powder (right).

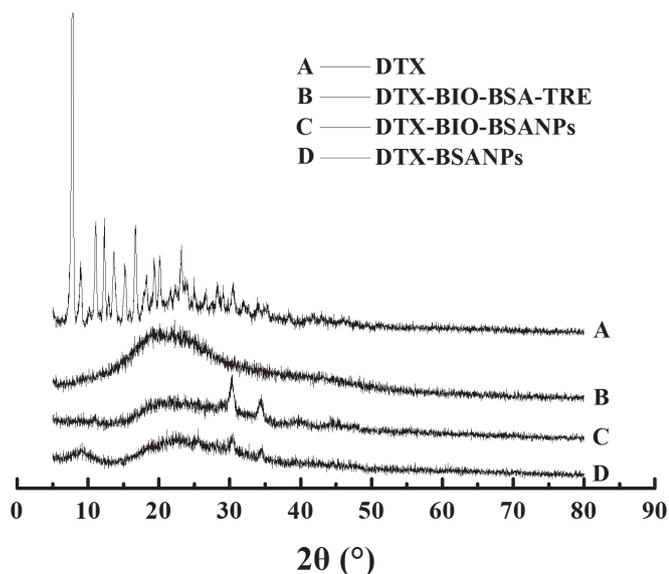
freeze-dried DTX-BIO-BSA-NPs is shown in Figure 2. The disappearance of the characteristic peak ( $2\theta=7.84^\circ$ ,  $12.36^\circ$ ,  $16.58^\circ$ ) of docetaxel in the DTX-BIO-BSA-NPs suggested that docetaxel was in an amorphous or disordered crystalline phase in the solid solution state. In addition, the DSC curves of samples (Figure 3) showed there is a peak at  $222^\circ\text{C}$  in the docetaxel curve which stands for docetaxel melting point peak in its crystalline form. This peak is not observed in DTX-BIO-BSA-NPs, which further demonstrated that docetaxel was in an amorphous or disordered crystalline state. (Musumeci *et al.*, 2006; Yallapu *et al.*, 2010).

### ***In vitro* drug release**

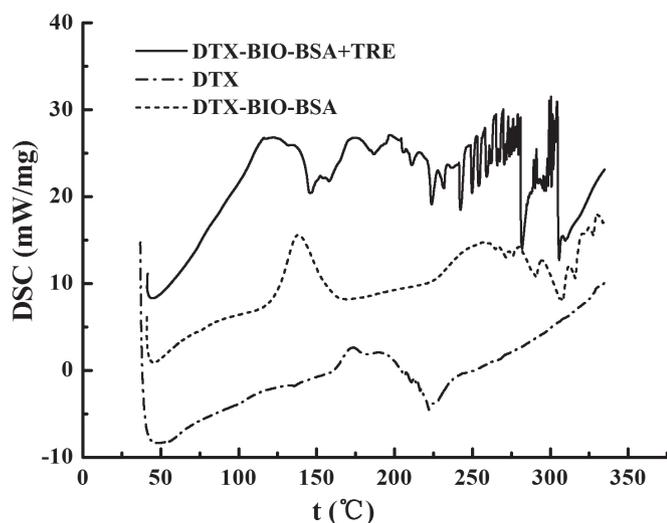
*In vitro* drug release studies are crucial to mimic the release of drug in a physiological environment. *In vitro* release of docetaxel from pure drug suspension and DTX-BIO-BSA-NPs were performed in PBS (pH 7.4). Among the experimental methods available for determining the *in vitro* release pattern of docetaxel from docetaxel suspension and DTX-BIO-BSA-NPs, the dialysis bag method is the most suitable. The dialysis bag retains the nanoparticles and allows the diffusion of free drug into release media, where samples were withdrawn from and analysed (Zuo *et al.*,

**TABLE I** - Particle size, zeta potential and polydispersity index changes of DTX-BIO-BSA-NPs over 8 hours

Redissolution time (h)	Mean diameter $\pm$ SD (nm)	Zeta potential (mV)	Polydispersity
0	$166.9 \pm 23.8$	$-29.51 \pm 1.66$	$0.170 \pm 0.016$
1	$160.3 \pm 25.6$	$-28.54 \pm 1.93$	$0.176 \pm 0.012$
2	$159.0 \pm 21.4$	$-36.54 \pm 1.77$	$0.178 \pm 0.010$
4	$176.1 \pm 23.0$	$-36.08 \pm 0.87$	$0.177 \pm 0.010$
8	$184.8 \pm 24.2$	$-27.56 \pm 1.79$	$0.171 \pm 0.010$



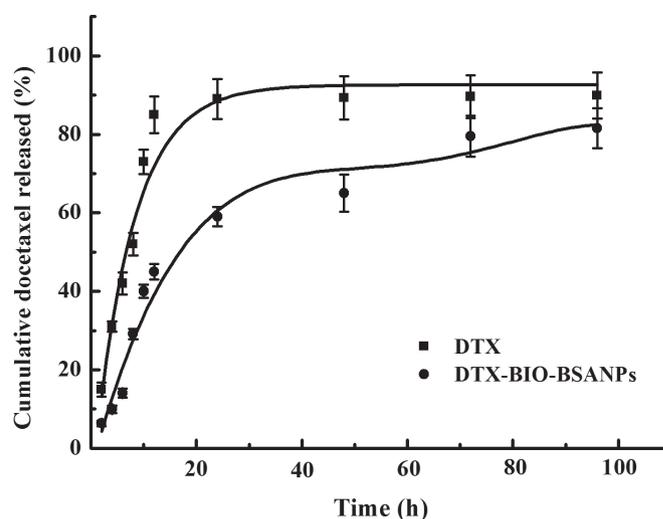
**FIGURE 2** - The X-ray diffraction spectrum of docetaxel and freeze-dried DTX-BIO-BSA-NPs. A, docetaxel; B, docetaxel-loaded biodegradable bovine serum albumin nanoparticles with biotin decoration and trehalose. C, docetaxel-loaded biodegradable bovine serum albumin nanoparticles with biotin decoration; D, docetaxel-loaded biodegradable bovine serum albumin nanoparticles.



**FIGURE 3** - The differential scanning calorimetry of docetaxel and freeze-dried DTX-BIO-BSA-NPs. DTX, docetaxel; DTX-BIO-BSA, docetaxel-loaded biodegradable bovine serum albumin nanoparticles with biotin decoration; DTX-BIO-BSA+trehalose (TRE), docetaxel-loaded biodegradable bovine serum albumin nanoparticles with biotin decoration and trehalose.

2016). The *in vitro* drug release profiles were represented as a cumulative percentage of drug released with respect to time. The *in vitro* drug release profiles of the DTX-BIO-BSA-NPs in PBS (pH7.4) in the first 4 days are illustrated

in Figure 4. The docetaxel release from the DTX-BIO-BSA-NPs is a complex process, which could be attributed to docetaxel diffusion in the bovine serum albumin carrier followed by BSA degradation. As shown in Figure 4, the free drug was found to release completely within 12 hours, reflecting the diffusion profile of free drug through the dialysis membrane. Similar results were reported by Liu and co-workers (Liu *et al.*, 2016). In comparison, the drug release from DTX-BIO-BSA-NPs presented sustained release pattern which demonstrated the potential for prolonging therapeutic exposure time. For example, an initial slow release from DTX-BIO-BSA-NPs of 29.1% in the first 8 hours can be observed. In the following 28 hours, burst release increased release to 59.0%, in a sustained manner, which provides the possibility to continuously fight against cancer cells, resulting in decreased cancer cell viability. The cumulative release of DTX-BIO-BSA-NPs (81.6%) was less than that of docetaxel (89.9%) after 4 days, attributed to the docetaxel being well encapsulated in the rigid core of DTX-BIO-BSA-NPs. A cumulative release of 81.6% after 4 days shows that the nanoparticle formulation reaches almost full release of docetaxel. The generally sustained and controlled release profile of docetaxel facilitates the application of nanoparticles for the delivery of anticancer drugs (Gan, Chien, Feng, 2010).



**FIGURE 4** - *In vitro* docetaxel release profile from DTX-BIO-BSA-NPs and docetaxel (Taxoere™). Phosphate-buffered saline (0.1 M, pH=7.4) was selected as the release medium. The nanoparticle dispersion was put in an orbital shaker and shaken at 120 rpm at 37 °C. High-performance liquid chromatography was performed to measure the released drug concentration.

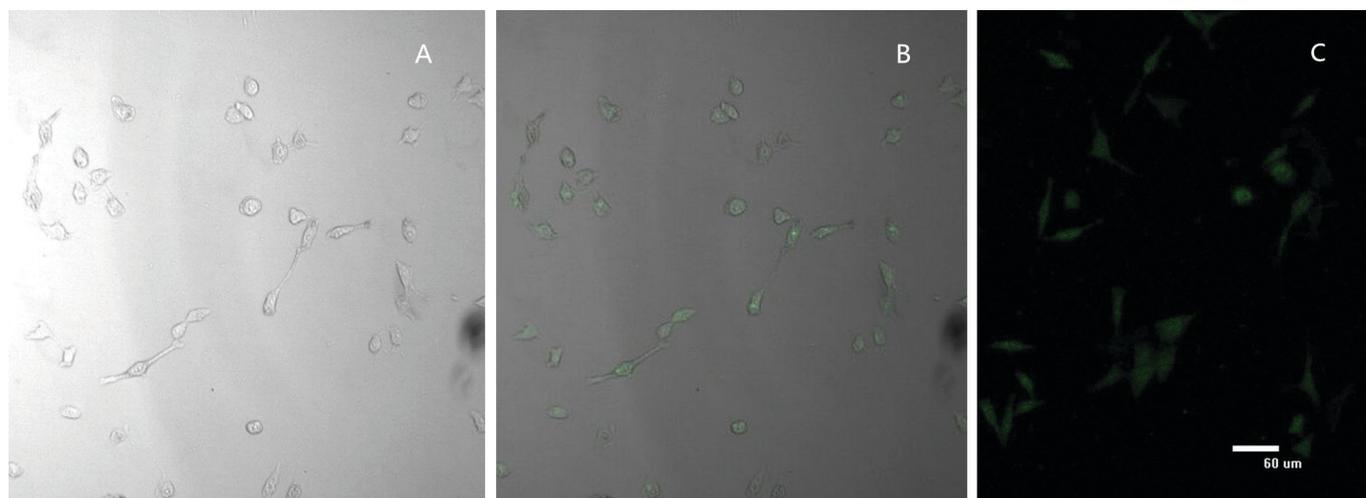
### *In vitro* cellular uptake

The therapeutic effects of the drug-loaded

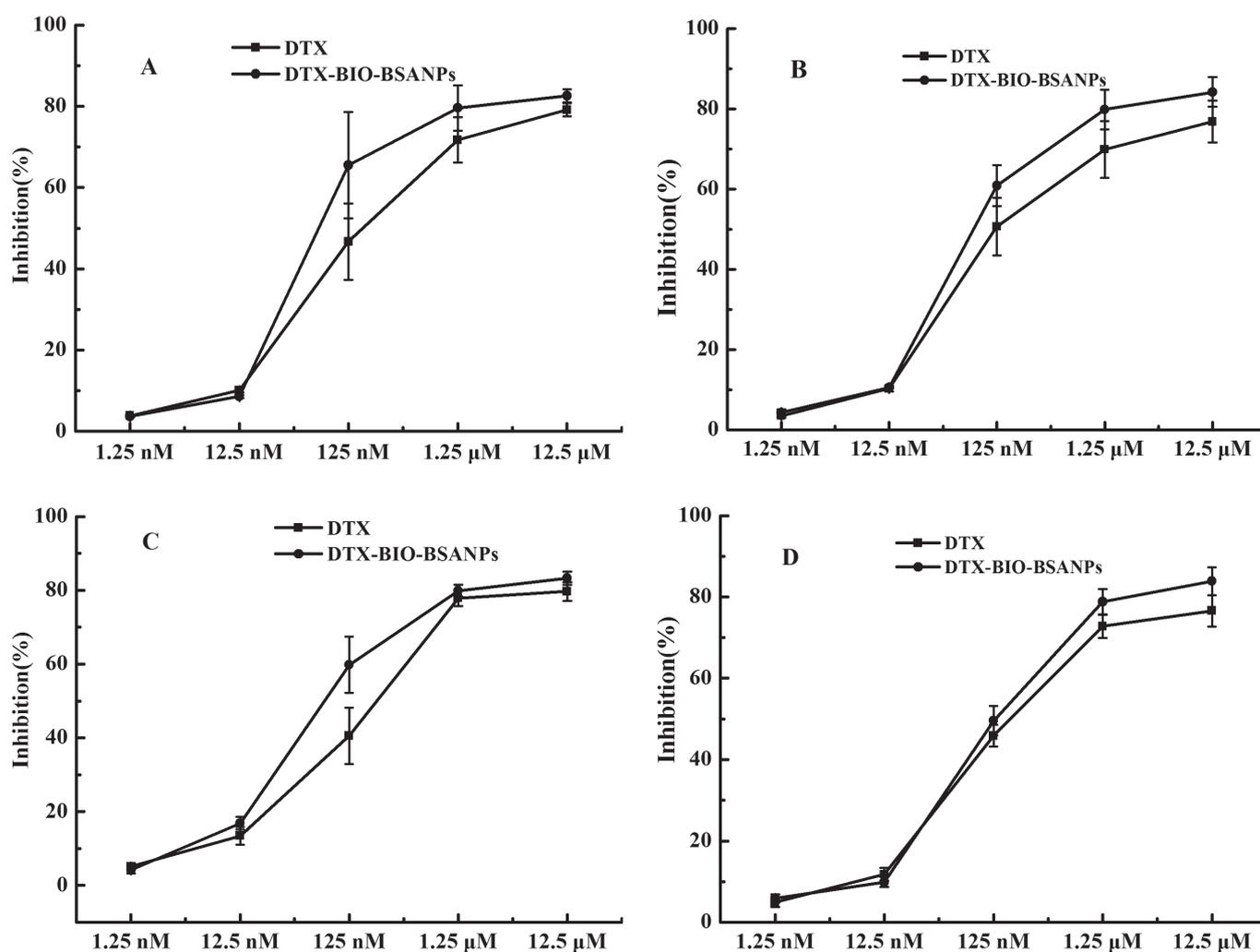
nanoparticles would depend on internalization and sustained retention of the nanoparticles by the diseased cells. The *in vitro* research could provide some preliminary evidence to display the advantages of the nanoparticle formulation in comparison with the free drug. The cellular uptake of DTX-BSA-NPs and DTX-BIO-BSA-NPs labeled by fluorescein isothiocyanate (a fluorescent probe) was examined to demonstrate internalization of the nanoparticles into cells and the targeting effects. Internalization of the DTX-BIO-BSA-NPs was visualized by confocal laser scanning microscopy. In Figure 5 (A-C), the fluorescence from the nanoparticles internalized in cells is shown. Figure 5 (B and C) shows the images of the cells incubated for 4 h with DTX-BSA-NPs and DTX-BIO-BSA-NPs. A significant fluorescence difference between DTX-BSA-NPs and DTX-BIO-BSA-NPs can be observed. The results show that the biotin-conjugated nanoparticles (DTX-BIO-BSA-NPs) had better target delivery than nanoparticles without biotin conjugation (DTX-BSA-NPs). High-affinity biotin-binding protein was characterized in human breast cancer cell line MCF-7, and biotin-avidin binds to the membrane fraction that cross reacts with the anti-intestines-specific membrane antigen antibody. The results of Nateghian *et al.* (2016) demonstrated that biotin-decorated docetaxel-loaded human serum albumin conjugates were more powerful in the reduction in tumor size and increased the survival rate when compared to free docetaxel. Therefore, we firmly believe that the DTX-BIO-BSA-NPs with smaller hydrodynamic size would have higher cellular uptake efficiency.

### Antiproliferative efficacy of DTX-BIO-BSA-NPs against MCF-7 cells, SGC7901 cells, LS-174T cells and A549 cells *in vitro*

As shown in Figure 6, the antiproliferative effect of both DTX-BIO-BSA-NPs and free docetaxel against all tested cells were no more than 15% at 12.5 nm concentration, and about 80% at 1.25  $\mu\text{M}$  concentration, indicating that both cytotoxicity profiles were similar. The drug concentration at which 50% of cells have been killed,  $\text{IC}_{50}$  values, of docetaxel and DTX-BIO-BSA-NPs was respectively,  $0.43 \pm 0.04 \mu\text{M}$  and  $0.26 \pm 0.07 \mu\text{M}$  for MCF-7 cells ( $p < 0.05$ , docetaxel vs. DTX-BIO-BSA-NPs),  $0.46 \pm 0.04 \mu\text{M}$  and  $0.23 \pm 0.06 \mu\text{M}$  for SGC7901 cells ( $p < 0.01$ ),  $0.34 \pm 0.04 \mu\text{M}$  and  $0.21 \pm 0.04 \mu\text{M}$  for LS-174T cells ( $p < 0.05$ ), and  $0.42 \pm 0.04 \mu\text{M}$  and  $0.27 \pm 0.03 \mu\text{M}$  for A549 cells ( $p < 0.01$ ). Consistent with Mirzaie *et al.* (2016), the cytotoxicity of DTX-BIO-BSA-NPs was significant higher than that of docetaxel. Overall, the results clearly demonstrate that DTX-BIO-BSA-NPs are more effective against cancer cells than free docetaxel. The higher cytotoxicity of DTX-BIO-BSA-NPs can be attributed to synergistic combination of different mechanisms. DTX-BIO-BSA-NPs were adsorbed onto the cell surface leading to an increase in drug concentration near the cell membrane, thus generating a concentration gradient that promotes the drug influx into the cell (Fonseca, Simoes, Gaspar, 2002). Cancerous cells exhibit enhanced endocytic activity and internalize the polymeric nanoparticles in the interior of the cell leading to an increase in drug concentration. In addition, free DTX molecules, are transported out by P-glycoprotein



**FIGURE 5** - Confocal laser scanning microscopy images show the internalization of fluorescent nanoparticles in cells. (A) MCF-7 cells with no drug incubation. (B) MCF-7 cells incubated with DTX-BSA-NPs for 2 h. (C) MCF-7 incubated with DTX-BIO-BSA-NPs for 2 h. DTX-BIO-BSANP, docetaxel-loaded biodegradable bovine serum albumin nanoparticles with biotin decoration; DTX-BSA-NPs, docetaxel-loaded biodegradable bovine serum albumin nanoparticles.



**FIGURE 6** - DTX-BIO-BSA-NPs and docetaxel induces growth inhibition. The MCF-7 (A), SGC7901 cells (B), LS-174T cells (C) and A549 cells (D) were exposed to DTX-BIO-BSA-NPs (●) and docetaxel (■) at concentrations ranging from 1.25 nM-12.5 μM and incubated for 48 h. The inhibitory ratio was measured by MTT assay. Data are represented as means ± SD of three independent experiments with six replicates each.

(P-gp) pumps while drug-loaded nanoparticles are taken up by cells through an endocytosis pathway. Thus, it results in a higher cellular uptake of the entrapped drug, thereby enabling them to escape the effect of P-gp pumps (Panyam, Labhassetwar, 2003).

As a nanoparticle formulations are expected to reduce the side effects caused by docetaxel and improve its cellular uptake, the docetaxel-loaded nanoparticles DTX-BIO-BSA-NPs could be an ideal anticancer medicine for the targeted therapy.

## CONCLUSION

In this study, nanoparticles of biotin-conjugated water-insoluble docetaxel-loaded bovine serum albumin have been successfully synthesized and characterized.

The nanoparticles showed appropriate particle size, desired surface properties, high stability in favor of cellular uptake, and can be targeted specifically to cancer cells. DTX-BIO-BSA-NPs also exhibited enhanced antiproliferative effect in comparison to free docetaxel against tumor cells. The results of this study bolsters the validity of a novel DTX-BIO-BSA-NPs formulation prepared by desolvation technique using bovine serum albumin as carrier for cancer therapy, and represents a promising approach for nanoparticulate delivery systems for cytostatic agent delivery.

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