Journal of the Taiwan Institute of Chemical Engineers 000 (2018) 1–8

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

A pH stimuli thiol modified mesoporous silica nanoparticles: Doxorubicin carrier for cancer therapy

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ARTICLE INFO

Article history:
Received 13 January 2018
Revised 26 February 2018
Accepted 26 March 2018
Available online xxx

Keywords:
Mesoporous silica nanoparticles
Thiol modified silica nanoparticles
pH-responsive
Doxorubicin hydrochloride
Drug nanocarrier

ABSTRACT

Mesoporous silica nanoparticles (MSN) have been attracted in the field of biomedicine due to their versatile properties as a drug nanocarrier. In this research, MSN and thiol modified MSN (mMSN) were synthesized to be the drug nanocarriers, by the sol–gel technique for the efficient delivering doxorubicin hydrochloride (DOX). These carriers were characterized by Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FESEM), SA3100 analyzer (Brunauer–Emmett–Teller), Zetasizer and Fourier transform infrared spectroscopy (FTIR). FESEM observation revealed that these particles have uniform sizes and the zeta potential analysis confirmed the existence of surface charge and stability of the particles. Brunauer–Emmett–Teller showed that MSN and mMSN are characteristic type IV N_2 adsorption/desorption patterns. The *in vitro* drug release profile studies have profound that the drug-loaded carriers were pH dependent and the drug-loaded mMSN (DOX@mMSN) displayed a faster drug release at the acidic pH with tumor cells than the pH with normal cells. Cell cycle arrest and ROS generation were analyzed by Fluorescence-activated cell sorting. Assay on the cytotoxicity against the HeLa cells showed a better antitumor effect with DOX@mMSN compared to DOX@MSN. This study attested that pH-responsive thiol modified drug nanocarrier can have the potential cancer therapy.

1. Introduction

Cancer is noxious to the human life and there is a continuous increment with the incidence and mortality of cancer [1]. Cancer is non-metastatic and for the primary treatment surgery and radiotherapy have been considered as the choices. On the other hand, in case of metastatic cancers, anticancer drugs (chemotherapy, hormone, and biological therapies) are in practice. The disadvantage of chemotherapeutic drugs is, it cannot differentiate the cancer cells against the normal cells, and it inhibits the proliferation of the growth of both cancer and normal cells [2]. To overcome this primary issue, nanotechnology-based drug delivery systems (NDDS) are the preference over the conventional chemotherapy. The traditional chemotherapy often lacks the potential to target the specific tumor sites, leading to be decreased in efficacy and causes the side-effects [3]. The nanotherapeutic approaches have unique potential, which can overcome the limitations originated from chemotherapy. Nanotherapeutic approaches favor with the easier modification of particle surface, increase the stability of anticancer drugs under the human physiological condition, decrease the resistance of P-glycoprotein (P-gp) expressing cells and decrease then on-specific toxicity [4].

Nanoparticles have been designed for targeted drug deliveries are nanospheres, nanocapsules, micelles, liposomes and dendrimers. These nanocarriers help to address the cytotoxic effects to the normal cells to some extent [5]. Due to the distinctive features of inorganic nanoparticles, such as stability, mechanical strength and resistance to microbes have contributed to the therapeutic applications. The reported particles include mesoporous silica [6–8], superparamagnetic iron oxide [9], polymer nanoparticles [10,11], and gold nanoparticles [12]. Among them, mesoporous silica nanoparticles (MSNs) have been utilized much to be a novel drug carrier. MSNs have excellent features such as a large surface area, drug loading capacity, capability of encapsulate the

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https://doi.org/10.1016/j.jtice.2018.03.048
1876-1070/

hydrophilic and hydrophobic drugs, amenable for surface functionalization chemistry and the pore size can be tunable. MSNs were considered as drug delivery vehicles owing to their surface area with silanol groups on the pore and a low toxicity [13]. Moreover, MSN was reported to be biodegradable and biocompatible [14]. As compared to the liposomes, dendrimers and niosomes, MSNs are more stable against the external factors, which cause degradation and mechanical stress due to the occurrence of a strong Si–O bond [15]. The external stimuli such as pH-responsive, redox potential, temperature or enzymes were shown to be used for the controlled release of loaded drug and guaranteed for drug carrier without leakages [16,17]. Among all the external stimuli, pH activation has a higher potential and able to control the drug release precisely [18]. Most of the cancers were found to be associated with abnormal cellular pH. As tumor tissues have a lower pH than normal human tissues (pH 7.4), the pH inside the endosomes is much lower (pH 4.5–5). These factors help in the controlled release of drugs as pH stimuli and assist the cancer therapy [19].

Doxorubicin (DOX) is a chemotherapeutic drug under the class anthracycline, has shown to be effective and treat varieties of tumor [20–23]. It has a mechanism to inhibit the topoisomerase II-DNA complex by the process of intercalation and stops the cellular replication [24]. It results in causing the side effects such as fatigue, nausea and fatal cardiotoxicity [25]. These side effects can be minimized by the controlled targeted drug delivery by the nanocarriers. Earlier literature have been reported for the application of pH-controlled release of DOX loaded MSN [16,25]. Authors have demonstrated that MSN has improved the solubility and able to control the release of the hydrophobic drugs, such as the camptothecin and paclitaxel [14,26]. The hydrophilic molecules andadenosine triphosphate have able to control the drug release by altering the pore size of microporous and mesoporous materials. It has been reported that the dual function of the carboxyl modified MSN enhances the pH-response controlled release and cellular uptake of DOX [17]. Thiols (SH) and amines (NH_2) are two commonly used reactive functional groups for the bioconjugation reaction. Thiol-modified MSN are often used in drug delivery applications because of their compatibility with typical bioconjugation techniques to initiate additional functionality into the particles [27]. The strong binding affinity of MSN to thiols can be used to conjugate with DOX for targeted drug delivery system. In addition, The γ-PGA coated MSN and thiol functionalized MSN with DOX as a model drug enhanced the cellular uptake [28,29].

In the present research, pH-responsive MSN nanocarrier and thiolated MSN (mMSN) were prepared. These nanocarriers were characterized and loaded with the weaker base drug, DOX. The potential of mMSN was compared with MSN and the results were studied under i*n vitro* drug release based on pH condition. Further, cytotoxicity, cell arrest and ROS analyses were carried out to test the nanocarrier efficacy. This study shows the simple and easier modification methods for stimuli responses to DOX delivery assisted by MSN and mMSN as a nanocarrier, to be beneficial for cancer therapy.

2. Materials and methods

2.1. Materials

Tetraethoxysilane (TEOS) and cetyltrimethylammonium bromide (CTAB) and 3-mercaptopropyltrimethoxysilane (MPTMS) were purchased from AlfaAesar. Doxorubicin hydrochloride (DOX) and Thiazolyl Blue Tetrazolium Bromide (MTT) were purchased from Sigma-Aldrich. The HeLa and HaCat cell lines were obtained from American Type Culture Collection (ATCC). The MCF-7 cell line was procured from Sigma-Aldrich. Millipore water was used throughout the experiments.

2.2. Synthesis of mesoporous silica nanoparticles (MSN) and thiol modified silica nanoparticles (mMSN)

MSN was synthesized by the modified sol–gel technique developed by Tang et al. [29]. In a typical synthesis, 0.2 g CTAB was dissolved in a mixture of 96 mL deionized water, which was kept at constant stirring at 80 °C, followed by the addition of 0.7 mL of 2 M NaOH. The resultant solution was kept at constant stirring at 80 °C for 30 min. After the solution became homogeneous, 1.4 mL of tetraethylorthosilicate (TEOS) was added drop wise into the solution under vigorous stirring. After 2 h, the obtained precipitation was centrifuged, washed once with ethanol and twice with deionized water, dried at 60 °C in a hot air-oven. The dried sample was redispersed in 40 mL of ethanol and 4.5 mL (12 M) HCl, kept in a shaker for 24 h to remove the CTAB template. Then, the solution was centrifuged, washed once with ethanol and twice with deionized water, dried at 60 °C in a hot air-oven. The dried sample was kept in a muffle furnace for 5–6 h at 650 °C to obtain a white powder. The synthesis of mMSN was carried out using the same procedure, but after adding TEOS it was added with 0.2 mL of MPTMS.

2.3. Characterization of MSN and mMSN

The morphological and elemental analyses were performed using transmission electron microscopy (TEM) (TECNAI G2 BioTWIN, Hillsboro, OR, USA) and field emission scanning electron microscopy (FESEM) equipped with EDS (Quanta FEG 200, Hillsboro, OR, USA). Samples were loaded on a carbon-coated copper grid and allowed to dry in a hot air-oven at 50 °C for an hour before the observation. All samples were examined at a voltage of 20 kV. The surface area and pore size distributions were analyzed using a SA3100 analyzer (Beckman Coulter, USA). The specific surface area was evaluated from the nitrogen adsorption data over the relative pressure ranged from 0.05 to 0.1 using the Brunauer–Emmett–Teller (BET) method. Pore size distributions were determined from the adsorption branches of isotherms using the Barrett–Joyner–Halenda (BJH) method. Zeta potential was measured by Zetasizer Nano ZS (Malvern Instruments, UK). Measurements were taken using a He–Ne laser of 633 nm at 25 °C. Prior to the measurements, samples were stabilized overnight at room temperature and sonicated. Measurements were calculated by the mean of triplicates. Fourier transform infrared spectroscopy (FTIR) spectra were obtained by using FTIR spectrophotometer (Agilent Technologies, USA). The sample (5 mg) was mixed with 95 mg of KBr and compressed into a pellet using a hydraulic press (Specac, USA). The spectra were recorded at the range of 4000–400 cm^{-1} and corrected against the reference, KBr.

2.4. Drug loading and in-vitro drug release study

The DOX loading and *in vitro* DOX release studies were performed based on the modified method by Xie et al. [30]. Typically, 25 mg of MSN was dispersed in 12.5 mL PBS (pH 8) containing 12.5 mg of DOX. In the absence of the light, the solution was stirred for 24 h. Then, the solution was centrifuged and the supernatant was collected. The DOX concentration was calculated using UV-spectrometer at 483 nm, based on the measurements from the original solution and supernatant.

Loading content (%) = [(Initial amount of DOX − supernatant free amount of DOX)/Weight of the nanoparticles] × 100

Entrapment efficient (%) = [(Initial amount of DOX − supernatant free amount of DOX)/Initial amount of drug] × 100

The DOX release was obtained by immersing the drug-loaded particle (1 mg/mL) in PBS different pH (pH 5 and 7.4), in the

Please cite this article as: P. Velusamy et al., A pH stimuli thiol modified mesoporous silica nanoparticles: Doxorubicin carrier for cancer therapy, Journal of the Taiwan Institute of Chemical Engineers (2018), https://doi.org/10.1016/j.jtice.2018.03.048