Materials Science & Engineering C 84 (2018) 21–31

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

# Thermo-sensitively and magnetically ordered mesoporous carbon nanospheres for targeted controlled drug release and hyperthermia application

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ARTICLE INFO

*Keywords:*
Ordered mesoporous carbon nanospheres
Themo-sensitivie
Magnetic
Controlled release
Hyperthermia
Doxorubicin hydrochloride

ABSTRACT

A multifunctional nanoplatform based on thermo-sensitively and magnetically ordered mesoporous carbon nanospheres (TMOMCNs) is developed for effective targeted controlled release of doxorubicin hydrochloride (DOX) and hyperthermia in this work. The morphology, specific surface area, porosity, thermo-stability, thermo-sensitivity, as well as magnetism properties of TMOMCNs were verified by high resolution transmission electron microscopy, field emission scanning electron microscopy, thermo-gravimetric analysis, X-ray diffraction, Brunauer–Emmeltt–Teller surface area analysis, dynamic light scattering and vibrating sample magnetometry measurement. The results indicate that TMOMCNs have an average diameter of ~146 nm with a lower critical solution temperature at around 39.5 °C. They are superparamagnetic with a magnetization of 10.15 emu/g at 20 kOe. They generate heat when inductive magnetic field is applied to them and have a normalized specific absorption rate of 30.23 W/g at 230 kHz and 290 Oe, showing good potential for hyperthermia. The DOX loading and release results illustrate that the loading capacity is 135.10 mg/g and release performance could be regulated by changing pH and temperature. The good targeting, DOX loading and release and hyperthermia properties of TMOMCNs offer new probabilities for high effectiveness and low toxicity of cancer chemotherapy.

## 1. Introduction

Chemotherapy is becoming one of the most important cancer treatments as the proliferation and metastasis of cancer cells can be prevented by using chemical drugs. However, as a result of low selectivity, chemical drugs inevitably cause untoward effects on normal cells [1]. For example, the anticancer drug doxorubicin hydrochloride (DOX), which has a broad spectrum anticancer activity, can kill cancer cells by directly embedding in DNA, and also induce serious adverse reactions such as bone marrow suppression and cardiac toxicity because of its low specific selectivity [2]. In order to overcome its shortcoming, pharmacy researchers are focusing on the design of new medicine formulation to improve the efficacy and reduce toxic side effects. Therefore, the concept of drug delivery systems was proposed to deliver DOX at the right place and suitable time with expected dose, in order to achieve maximum efficacy and minimal side effects [3].

Ordered mesoporous carbon nanospheres (OMCNs) have attracted enormous attention in drug delivery fields because of their good biocompatibility, stability and high specific surface area [4]. As potential candidate drug vehicles, OMCNs show excellent drug loading capacity owing to their high porosity and high pore volume. Drug molecules are adsorbed and stored not only onto the pore surface, but also throughout the interconnected pore structure. Moreover, OMCNs are succeptible to modification with abundant bonding sites by amino, carboxyl and thiol groups, which can effectively govern the diffusion of drugs. To date, several OMCNs-based delivery systems for transporting DOX have been explored [5–7]. For example, Zhou et al. [5] developed an OMCNs-based delivery system with DOX loading capacity of 41.0 mg/g and

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https://doi.org/10.1016/j.msec.2017.11.033
Received 21 July 2017; Received in revised form 23 October 2017; Accepted 24 November 2017
Available online 26 November 2017
0928-4931/ 

cumulative release of 70% in 15 h, exhibiting good inhibition for cancer cells. Although OMCNs show favorable DOX loading performance, drug leakage and fast release always take place during their delivery, as the DOX loading ability is only relied on the physical adsorption interactions between OMCNs and DOX, leading to low efficiency and high toxicity. Therefore, lacking of targeted and controlled release properties limits further applications of DOX delivery system based on OMCNs [8].

To improve the targeted and controlled release properties of OMCNs nano-carriers, many efforts have been undertaken to develop multifunctional OMCNs with stimuli responsiveness, which could target and control the DOX release by actively or passively responding to the outside environment, such as magnetic field [9], temperature [10], and pH [11]. Multifunctional OMCNs drug carriers provide new perspectives for drug delivery, controlled release and targeted treatment in the research of cancer chemotherapy [12]. Strategies for developing multifunctional OMCNs drug carriers are proposed as follows: first, introduction of $Fe_3O_4$ nanoparticles into OMCNs to impart magnetic targeting properties, which could rapidly realize targeted release of DOX at cancer tissue and induced hyperthermia by an alternating magnetic field [13]; second, grafting of thermo-sensitive polymer layer around the surfaces of magnetic OMCNs (MOMCNs) to bestow thermosensitivity [14], by which a DOX storage gate as well as a release switch is created to response to temperature for the controlled release of DOX upon volume phase transition of thermo-sensitive polymer induced by hyperthermia effect; third, design of a pH-responsive DOX delivery system, in which -$NH_2$ groups are protonated under acidic conditions to become hydrophilic and desalinated under base conditions to become hydrophobic [15]. The peripheral tissue of cancer generally show lower pH value (5.5–6.0) compared with normal cells as more acidic metabolites are produced in the metabolism of cancer cells [15]. Thus, by means of different affinity between DOX carrier and tissue fluid under different pH, the DOX release could be further controlled.

Herein, in order to improve the targeted and controlled release of DOX delivery system based on OMCNs, thermo-sensitively and magnetically OMCNs were constructed for targeted and controlled release of DOX and hyperthermia in this work. The DOX-loading system is targeted at cancer tissue by magnetic field at first, and then controlled release of DOX is realized by the endogenous pH stimuli of cancer tissues and the extraneous temperature stimuli that are induced by hyperthermia. It is expected to increase the efficacy of DOX and decrease the side effects, thus overcoming the limitations of conventional cancer treatment methods. First, OMCNs were prepared by soft template method in which α-$Fe_2O_3$ nanoparticles were used as catalyst. To obtain a targeted DOX delivery system with hyperthermia effect, MOMCNs were synthesized through impregnation method. Subsequently, silane modified MOMCNs (SMOMCNs) were obtained to introduce C=C double bonds on the surfaces of MOMCNs. Then, thermo-sentitive polymer poly(N-isopropylacrylamide) (PNIPAM), whose lower critical solution temperature (LCST) is quite close to physiological temperature of human being, was grafted by radical polymerization to obtain thermo-sensitive MOMCNs (TMOMCNs). Finally, the loading and controlled release of DOX *in vitro* and the hyperthermia were investigated, as shown in Fig. 1.

## 2. Materials and methods

### 2.1. Materials

Pluronic poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (F127) was supplied by Sigma-Aldrich Company Ltd., China. DOX was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd., China. *N*-isopropylacrylamide (NIPAM) and *N*,*N*′-methylenebisacrylamide (MBA) were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Phosphate buffer (PBS, pH = 7.4) was supplied by Wuhan Doctor Bioengineering Co., Ltd., China. Ammonia solution (25–28 wt%), formaldehyde (37–40 wt%), hydrochloric acid (36%), phenol, phosphoric acid and ammonium persulfate (APS) were purchased from Tianjin Guangfu Technology Development Co., Ltd., China. Ferric nitrate nonahydrate ($Fe(NO_3)_3{\cdot}9H_2O$) was supplied by Tianjin Fuchen Chemical Reagents Plant, China. Acetic acid, γ-methacryloxy propyl trimethoxyl silane (MPS), sodium hydroxide (NaOH) and ethanol were purchased from Tianda Chemical Reagent Factory, Tianjin, China. All chemicals and reagents were of analytical grade and were used as received without further purification. α-$Fe_2O_3$ nanoparticles were prepared by our previous work [16]. Distilled water was used during the experiment.

### 2.2. Thermo-sensitively and magnetically ordered mesoporous carbon nanospheres

#### 2.2.1. Ordered mesoporous carbon nanospheres

OMCNs were prepared by soft template method reported in the literature [17], with α-$Fe_2O_3$ nanoparticles as catalyst. The procedures were as follows: 0.6 g of phenol was dissolved in 2.1 mL of formaldehyde, and then 15 mL of 0.1 mol/L NaOH solution and certain amount of α-$Fe_2O_3$ nanoparticles were added, followed by sonicating for 10 min until the α-$Fe_2O_3$ nanoparticles were completely dispersed. After the mixture were reacted at 70 °C for 30 min, 0.96 g of F127 dissolved in 15 mL of distilled water was added and stirred at 66 °C for 2 h, and 50 mL of distilled water was added to dilute the mixture and the resultant mixture was continually stirred at 66 °C for 17 h. After that, 10 mL of above precursor solution and 30 mL of distilled water were put into a 100 mL teflon-lined stainless autoclave, heated at 130 °C for a period of time, and then cooled to room temperature. The products were collected by centrifugation, washed with distilled water and ethanol for several times, and dried for 10 h. The carbonization was performed at 700 °C in $N_2$ atmosphere for 1 h. Finally, the black powder was rinsed by distilled water, purified by removing magnetic components that were produced by α-$Fe_2O_3$ nanoparticles and dried to obtain OMCNs.

#### 2.2.2. Magnetic ordered mesoporous carbon nanospheres

MOMCNs were prepared using the modified impregnating method as described previously [18]. Theoretical content of the magnetic nanoparticles could be calculated from $Fe(NO_3)_3{\cdot}9H_2O$ and pore structure properties of OMCNs. If all of the mesopore were impregnated by Fe $(NO_3)_3{\cdot}9H_2O$, the mass of $Fe(NO_3)_3{\cdot}9H_2O$ and the content of magnetic nanoparticle could be calculated according to the following equation:

$$m(Fe_2(NO_3)_3{\cdot}9H_2O) = m(C) * V_t(C) * \rho(Fe(NO_3)_3{\cdot}9H_2O) \quad (1)$$

where $m(Fe(NO_3)_3{\cdot}9H_2O)$ and m(C) present the mass of $Fe(NO_3)_3{\cdot}9H_2O$ and OMCNs, respectively; $V_t(C)$ is the pore volume of OMCNs (~0.64 $cm^3/g$), which is obtained from Table 1; $\rho(Fe(NO_3)_3{\cdot}9H_2O)$ is the density of $Fe(NO_3)_3{\cdot}9H_2O$ (1.684 $g/cm^3$); M ($Fe(NO_3)_3{\cdot}9H_2O$) and M ($Fe_2O_3$) are the mole mass of $Fe(NO_3)_3{\cdot}9H_2O$ and $Fe_2O_3$, respectively (g/mol). When 0.30 g of OMCNs was used to prepare MOMCNs, the dosage of $Fe(NO_3)_3{\cdot}9H_2O$ is 0.32 g.

Therefore, 0.3 g of OMCNs and 0.32 g of $Fe(NO_3)_3{\cdot}9H_2O$ were dispersed into 5 mL of ethanol and sonicated for 15 min, and 200 μL of 0.2 mol/L HCl solution was added to inhibit the hydrolysis of Fe $(NO_3)_3{\cdot}9H_2O$. The dispersion was sealed and stirred at 25 °C for 1 h, then open stirred until ethanol was evaporated completely. After being dried at 60 °C under vacuum for 3 h, the powder was put into a little open glass bottle, which was then placed into a 100 mL teflon-lined stainless autoclave containing 10 mL of 14 wt% ammonia solution. The glass bottle was used to separate the powder from ammonia solution. After sealing, the autoclave was heated to 60 °C for 3 h to hydrolyze ferric nitrate to ferric hydroxide *in situ* and then cooled to room temperature. The blackish product was filtered and rinsed by water to remove byproducts, and then annealed for 30 min at 500 °C under $N_2$ atmospheres with a heating rate of 10 °C/min to obtain MOMCNs.