Journal of Colloid and Interface Science 440 (2015) 179–188

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

One-step preparation of hydrophilic carbon nanofiber containing magnetic Ni nanoparticles materials and their application in drug delivery

Chih-Jen Wang [a], Tse-Ching Chen [b], Jarrn-Horng Lin [c], Pei-Rong Huang [d], Hsing-Jui Tsai [a], Ching-Shiun Chen [a,*]

[a] Center for General Education, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan, ROC
[b] Department of Pathology, Chang Gung Memorial Hospital, 5, Fusing St., Kwei-Shan, Tao-Yuan 333, Taiwan, ROC
[c] Department of Materials Science, National University of Tainan, 33, Sec. 2, Shu-Lin St., Tainan 700, Taiwan, ROC
[d] Department of Molecular and Cellular Biology, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan, ROC

ARTICLE INFO

Article history:
Received 14 August 2014
Accepted 31 October 2014
Available online 8 November 2014

Keywords:
Magnetic Ni
Carbon nanofibers
Doxorubicin
Drug delivery
CO_2 hydrogenation

ABSTRACT

A one-step process for the synthesis of hydrophilic carbon nanofibers (CNFs) through CO_2 hydrogenation on Ni—Na/Al_2O_3 was developed for the loading and targeted delivery of the anticancer drug doxorubicin (DOX). CNFs that were synthesized on Ni—Na/Al_2O_3 for 9 h at 500 °C exhibited an adequate magnetic response and a large content of hydrophilic oxygen-containing functional groups on the carbon surface, resulting in excellent colloidal solution. The CNF material exhibited a highly efficient capacity for DOX adsorption, particularly at pH 9.0. The loading and release of DOX was strongly pH dependent, possibly due to electrostatic and π–π stacking interactions between DOX and CNF sample. The Langmuir isotherm and pseudo second-order kinetics of DOX-loaded CNFs were well-modeled for the process of DOX adsorption. DOX-loaded CNF targeted cancer cells more selectively and effectively than free DOX and exhibited a marked tendency to kill HeLa cancer cells and reduced toxicity to normal human primary fibroblast (HPF) cells.

1. Introduction

Nanomaterial-based drug carriers have attracted increasing attention at the interface of nanotechnology and biomedicine because these carriers can be efficiently loaded and enable the targeted delivery and controlled release of drugs [1–20]. During the past decade, nanometer-sized magnetic nanoparticles (NPs), such as iron oxide NPs, have been the subject of extensive research for many applications, including biomedical probes, drug delivery, contrast agents for magnetic resonance imaging and separation of biochemical products [11–20]. For practical applications, the direct use of magnetic NPs is hampered by their limited water solubility and aggregation in physiological environments. Several polymer materials have been employed to modify the surface of iron oxide NPs to improve their hydrophilic properties, biocompatibilities and blood circulation times [11–20]. Due to their ultrahigh surface area, intrinsic stability and structural flexibility, single-walled carbon nanotubes (SWCNTs) can also function as carriers of biologically relevant molecules. SWCNTs can serve as delivery vehicles to effectively shuttle various biomolecules, such as drugs, proteins and plasmid DNA, across membranes into living cells [1]. However, complicated treatments, such as oxidation by concentrated acids and conjugation with polyethylene glycol (PEG) molecules, are required to enhance the aqueous solubility of hydrophobic SWCNTs to enable their use as drug carriers [1–10].

Numerous magnetic transition metals, such as Ni or Fe, have been shown to be efficient catalysts for the production of SWCNTs from the high-pressure decomposition of carbon monoxide (HiPco) process [21–25]. Raw CNT materials are unsuitable as drug carriers because they are extremely hydrophobic, and the carbon surface must be treated with concentrated acids (HNO_3 or H_2SO_4) to generate carboxylic acid and carboxylate groups. However, the treatment of CNTs with concentrated acids may lead to the removal of metal NPs from the CNT surface. Thus, it has been assumed that the combination of hydrophilic SWCNTs with magnetic NPs is inherently difficult. The preparation of hydrophilic

* Corresponding author. Fax: +886 32118700.
E-mail addresses: scottpw@hotmail.com (C.-J. Wang), ctc323@adm.cgmh.org.tw (T.-C. Chen), janusjhlin@mail.nutn.edu.tw (J.-H. Lin), d9201110@stmail.cgu.edu.tw (P.-R. Huang), rachealjui@hotmail.com (H.-J. Tsai), cschen@mail.cgu.edu.tw (C.-S. Chen).

http://dx.doi.org/10.1016/j.jcis.2014.10.073
0021-9797/

CNTs containing Fe_3O_4 involving the synthesis of the magnetic Fe_3O_4 NPs on CNTs decorated with surfactant has been reported [26,27]. The literature reported that the fibrous-structured Fe_3O_4/silica mesoporous material has been synthesized in one-step process [28]. This material providing ca. 340 m^2/g surface area could adsorb doxorubicin on surface used for drug delivery. The Multifunctional mesoporous silica-coated superparamagnetic manganese ferrite ($MnFe_2O_4$) nanoparticles were developed for applying on targeted drug delivery and magnetic resonance imaging [29].

However, there have been no reports of the one-step synthesis of oxidized carbon nanomaterials with magnetic metal NPs, and straightforward synthesis of hydrophilic carbon nanomaterials with magnetic metal NPs is challenging. To combine the advantages of magnetic metal NPs and carbon nanomaterials, we report here a novel synthesis of carbon nanofiber (CNF)-coated Ni metal that uniquely combines magnetic Ni NPs and hydrophilic carbon nanomaterials. In this work, hydrophilic, strongly magnetic Ni—Na@CNFs with strong magnetization were directly synthesized by CO_2 hydrogenation on Ni—Na/Al_2O_3 and loaded with the anticancer drug doxorubicin (DOX). The various Ni—Na@CNF materials were characterized to identify their oxygen-containing functional groups, total surface area, zeta potentials and magnetization. The adsorption kinetics and equilibrium adsorption of DOX on suitable Ni—Na@CNFs are discussed in detail. The DOX-loaded Ni—Na@CNFs exhibited significant efficiency in inducing HeLa cancer cell death and a marked reduction in toxicity against normal human primary fibroblast (HPF) cells. This is the first report of the combination of magnetic NPs with CNFs for use as drug carriers for targeted delivery.

2. Materials and methods

2.1. Preparation of catalyst and CNFs

The catalyst used in this study was a commercially available 12 wt% Ni/Al_2O_3 catalyst manufactured by Süd-Chemie Catalysts, Inc. (catalyst # FCR-42). A Ni/Al_2O_3 sample containing 3 wt% Na was prepared by adding the required volume of aqueous $NaNO_3$ to the Ni/Al_2O_3 catalyst without any pretreatment. The samples were subsequently air-dried at 80 °C for 10 h. The catalysts were then calcined in air and reduced under H_2 at 500 °C for 5 h prior to use. All CNF syntheses were performed in a fixed-bed reactor at atmospheric pressure. A total of 50 mg of catalyst was used for each carbon deposition reaction. The CNFs were generated at 500 °C by feeding a stream of H_2/CO_2 (1:1) into the reactor at 100 mL/min [30].

2.2. Characterization of CNFs

Soft XAS measurements were taken at the BL20A1 station at the National Synchrotron Radiation Research Center (NSRRC), Taiwan, in the total electron yield mode for carbon and oxygen K-edge spectra; each measurement was taken in an ultra-high vacuum (UHV) chamber with a base pressure of 1×10^{-10} Torr. The chemical composition and oxidation state of the catalyst surface were examined by X-ray photoelectron spectroscopy (XPS). XPS data were obtained using a Thermo VG-Scientific Sigma Probe spectrometer at the Precision Instrument Center of the College of Engineering at the National Central University, Taiwan. The spectrometer was equipped with an Al Kα X-ray source (1486.6 eV; 1 eV = 1.602×10^{-19} J) operated at 108 W and a hemispherical analyzer operated at a pass energy of 50 eV. The instrument was typically operated with an analysis chamber pressure of approximately 1×10^{-9} Torr. The binding energies of the catalyst samples were referenced to the C 1s line (284.6 eV) of the carbon overlayer. Fourier transform infrared (FTIR) analysis of the Ni—Na@CNF samples was performed using a Nicolet 5700 FTIR spectrometer, which was operated at 1 cm^{-1} resolution with 256 scans. Zeta potentials were measured at 25 °C. The temperature-programmed desorption (TPD) experiment was performed in a 100 mL/min He stream at atmospheric pressure in a conventional flow system. The temperature was increased from 25 to 800 °C at a rate of 10 °C/min over the course of the TPD process. All signals were measured with a VG Smart IQ + 300D mass spectrometer. The temperature was measured with a K-type thermocouple inserted into the catalyst bed, and the desorbed products were admitted into the vacuum chamber through a leak valve, using an air stream as the carrier gas. The operating pressure in the chamber was approximately 3×10^{-7} mbar, and the base pressure in the chamber was approximately 3×10^{-10} mbar.

2.3. Drug loading and release

The loading of DOX onto the Ni—Na@CNFs was achieved by mixing varying quantities of DOX with 25 mg of Ni—Na@CNFs in 40 mL of PBS-buffered aqueous solutions in various pH values at room temperature, followed by stirring for 6 h. UV–Vis spectroscopy was used to confirm the concentration of free DOX in the aqueous phase over the course of the adsorption. UV–Vis spectra were collected with a Shimadzu UV-1800 spectrophotometer. To examine DOX release, 10 mg of DOX-loaded Ni—Na@CNFs was suspended in PBS-buffered solution at room temperature. The concentration of released DOX in PBS-buffered aqueous solutions at pH 7 and pH 5 was measured by UV–Vis spectroscopy at various time intervals.

2.4. Cell viability tests

HeLa cancer cells and normal HPF cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C in a 5% CO_2 environment. Next, approximately 3×10^5 cells were seeded in tissue culture flasks. For MTS assays, the cells were seeded in 96-well plates at a density of 3×10^3 cells per well in 200 μL of culture medium. For experimental purpose, the different concentrations of DOX/Ni—Na@CNFs (3.0, 1.5 and 0.75 μg/mL) were prepared from mixing desired DOX and 0.5 mg/mL Ni—Na@CNF colloidal solution in PBS-buffered solutions at pH 9.0. The resulting DOX-CNF suspension was stored at 4 °C until use. The HeLa and HPF cells were then incubated with either free DOX or different concentrations of DOX/Ni—Na@CNFs (3.0, 1.5 and 0.75 μg/mL). After continuous incubation for 24, 48 or 72 h, the relative cell viability was evaluated by MTS assay. All cell viability tests were performed in the identical culture medium at pH 7.0.

3. Results and discussion

3.1. Characterization of Ni—Na@CNFs

The IR spectra of the CNFs synthesized with reaction times of 3, 9 and 15 h, denoted by S-3 h, S-9 h and S-15 h, respectively, are shown in Fig. 1. These spectra indicate a complex mix of carbon oxidation states. Intense peaks were observed at 3440 cm^{-1} for ν(O—H) and 1640 cm^{-1} for ν(C═O) of ketone/quinone groups. The weak shoulder band at 1710 cm^{-1} was attributed to the ν(C═O) of a carboxyl group. Fig. 2 shows the C and O K-edge near-edge X-ray absorption fine structure (NEXAFS) for the CNFs and graphite samples. The surface-sensitive total electron yield (TEY) modes of NEXAFS were employed to characterize the