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Solvothermal synthesis of cobalt ferrite nanoparticles loaded on multiwalled carbon nanotubes for magnetic resonance imaging and drug delivery

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ABSTRACT

Multiwalled carbon nanotube (MWCNT)/cobalt ferrite ($CoFe_2O_4$) magnetic hybrids were synthesized by a solvothermal method. The reaction temperature significantly affected the structure of the resultant MWCNT/$CoFe_2O_4$ hybrids, which varied from 6 nm $CoFe_2O_4$ nanoparticles uniformly coated on the nanotubes at 180 °C to agglomerated $CoFe_2O_4$ spherical particles threaded by MWCNTs and forming necklace-like nanostructures at 240 °C. Based on the superparamagnetic property at room temperature and high hydrophilicity, the MWCNT/$CoFe_2O_4$ hybrids prepared at 180 °C (MWCNT/$CoFe_2O_4$-180) were further investigated for biomedical applications, which showed a high T_2 relaxivity of 152.8 Fe mM^{-1} s^{-1} in aqueous solutions, a significant negative contrast enhancement effect on cancer cells and, more importantly, low cytotoxicity and negligible hemolytic activity. The anticancer drug doxorubicin (DOX) can be loaded onto the hybrids and subsequently released in a sustained and pH-responsive way. The DOX-loaded hybrids exhibited notable cytotoxicity to HeLa cancer cells due to the intracellular release of DOX. These results suggest that MWCNT/$CoFe_2O_4$-180 hybrids may be used as both effective magnetic resonance imaging contrast agents and anticancer drug delivery systems for simultaneous cancer diagnosis and chemotherapy.

1. Introduction

In recent years, carbon nanotubes (CNTs) have attracted increasing attention in biomedical fields due to their unique structure and properties, including high aspect ratios, large surface areas, nanosized stability and rich surface chemical functionalities. They are particularly attractive as transporter candidates for the delivery of biomolecules and drugs. By appropriate functionalization, CNTs have been used to construct drug delivery systems (DDSs) for transporting plasmid DNA [1,2], siRNA [3,4], proteins [5,6] and drugs [7–9] into different types of cells.

Suitable approaches to loading drugs onto functionalized CNTs are indispensable to the fabrication of favorable DDSs. The loading of the drugs using CNTs as carriers has been achieved either by covalent or noncovalent interaction methods. Some anticancer drugs, such as 10-hydroxycamptothecin [10], cisplatin [8] and methotrexate [11], can be conjugated to the side walls of CNTs covalently. However, such chemical conjugation alters the chemical entities of the drugs, especially if they are covalently conjugated by nonbiodegradable linkages, which might result in lower drug efficacy and relevant side effects [12]. The physical adsorption of the drugs onto CNTs by specific interaction is preferred to the chemical conjugation technique, because there are no changes in the chemical entities and the resultant controlled drug release [12–14]. Some cancer chemotherapy agents with extended π-structures larger than one aromatic ring and a degree of solubility in aqueous solution, such as the anthracycline doxorubicin (DOX), can be conveniently loaded onto the side walls of CNTs due to the strong supramolecular π–π stacking interaction. DOX has been adsorbed on the surface of functionalized CNTs in this way, and the resultant CNT–DOX DDSs have shown high cytotoxicity to cancer cells [13,14].

Further research into the applications of CNTs in the living body and the associated toxicological hazards are still needed [15–18]. This will require non-anatomical in vivo observations of the motion and accumulation of CNTs. Magnetic resonance imaging (MRI) is currently one of the most powerful diagnosis tools used in medical research because of its excellent temporal and spatial

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resolution, the lack of exposure to radiation, the rapid in vivo acquisition of images and the long effective imaging window [19,20]. By labeling an MRI contrast agent onto CNTs, the MRI technique can be used for noninvasive in vivo observations of the behavior of CNTs. Recent progress has shown the potential of Gd^{3+}-containing CNTs and CNT/iron oxide nanoparticle complexes as MRI contrast agents [21–23]. If we can attach an MRI contrast agent onto CNTs in a suitable way, the obtained CNT-based hybrids could be used as an MRI contrast agent and a drug carrier simultaneously.

Cobalt ferrite ($CoFe_2O_4$), belonging to the family of spinel-type ferrites, has already been proposed for biomedical applications, especially for MRI contrast enhancement and hyperthermic treatments, because of its high magnetic anisotropy and saturation magnetization, which give rise to suitable magnetic behavior at room temperature [24–28]. There are few papers concerning the preparation of magnetic $CoFe_2O_4$ nanoparticles attached on the outside surfaces of CNTs. Liu et al. [29] deposited magnetic ferrite MFe_2O_4 (M=Fe, Co, Ni) nanoparticles on CNTs by high-temperature hydrolysis of metal chlorides, sodium acetate and concentrated-acid-oxidized CNTs in triethylene glycol solution. Jiang et al. [30] synthesized multiwalled carbon nanotube (MWCNT)/$CoFe_2O_4$ magnetic hybrids in ethylene glycol by a solvothermal treatment of a mixture of metal chloride, sodium acetate, polyethylene glycol and poly(sodium 4-styrenesulfonate)-wrapped CNTs. These reports involved the heavy pretreatment of CNTs in the synthesis and the bio-applications were unfortunately not well explored. In this contribution, we develop a solvothermal method to synthesize MWCNT/$CoFe_2O_4$ hybrids using diethylene glycol (DEG) and diethanolamine (DEA) as solvents and complexing agents. The reaction was highly efficient, and the MWCNTs were used without intensive oxidation treatment by concentrated acid or surface modification by polyelectrolytes. To explore the bio-application potential of the resultant hybrids, the cytotoxicity, hemolytic activity, and in vitro MRI negative contrast enhancement effect of the MWCNT/$CoFe_2O_4$ hybrids prepared at 180 °C (MWCNT/$CoFe_2O_4$-180) were studied. Moreover, a large amount of anticancer drug DOX was loaded onto the MWCNT/$CoFe_2O_4$-180 hybrids by physical adsorption, and the cytotoxic effect of the drug-loaded hybrids on HeLa cells was examined. To our knowledge, this is the first example of using MWCNT/$CoFe_2O_4$ hybrids for MRI contrast enhancement investigation and drug delivery.

2. Experimental section

2.1. Reagents and instrumentation

MWCNTs with an outer diameter of 10–30 nm and an average length of 0.5–2 μm, kindly provided by Shenzhen Nanotech Port Co. Ltd., were treated with 20% HNO_3 aqueous solution according to a previously published method [31]. $FeCl_3{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, DEG, DEA, sodium hydroxide and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. Doxorubicin hydrochloride (DOX·HCl) was provided by Beijing Hua Feng United Technology Co., Ltd. All the chemicals were used without further purification except the MWCNTs. Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm.

Transmission electron microscope (TEM) images, selected area electron diffraction (SAED) and energy-dispersive X-ray spectroscopy (EDS) data were obtained with a JEOL JEM-2100 high-resolution transmission electron microscope (HR-TEM). Scanning electron microscope (SEM) images were taken on a JEOL JSM-6460 electron microscope. X-ray diffraction (XRD) patterns were determined by a Rigaku DMAX 2000 diffractometer equipped with Cu K_α radiation (λ = 0.15405 nm) (40 kV, 40 mA). Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Avatar 370 FTIR spectrophotometer using KBr pellets. Thermal gravimetric analyses (TGA) were carried out with a Perkin–Elemer Pyris 1 DTA–TGA instrument under a flow of air at a heating rate of 10 °C min^{-1}. Magnetic measurements were performed using a commercial superconducting quantum interference device (SQUID) magnetometer (MPMS XL, Quantum Design). The ultraviolet–visible (UV–vis) absorption spectra were obtained with a UV-7502PC spectrophotometer.

2.2. Preparation of MWCNT/$CoFe_2O_4$ hybrids

MWCNT/$CoFe_2O_4$ hybrids were prepared by a solvothermal co-precipitation method. Briefly, $FeCl_3{\cdot}6H_2O$ (1.5 mmol) and $CoCl_2{\cdot}6H_2O$ (0.75 mmol) were dissolved in 10 ml of DEG at 90 °C in an oil bath. After 30 min of stirring, 2.5 ml of DEA was injected into the above hot solution. Then NaOH (6 mmol) dissolved in 5 ml of hot DEG was also introduced into the mixture. After another 10 min of stirring, 15 mg of purified MWCNTs fully dispersed in 10 ml of DEG were charged, followed by 30 min of vigorous stirring. The mixture was then transferred to a Teflon-lined autoclave of 50 ml capacity, and the sealed autoclave was maintained at 180–240 °C for 8 h. Finally, the hybrids were isolated by centrifugation, rinsed repeatedly with ethanol, and then dried under vacuum.

2.3. Cell culture

HeLa and L929 cell lines were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The cells were cultured in regular growth medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were maintained at 37 °C in a humidified and 5% CO_2 incubator. They were routinely harvested by treatment with a trypsin–ethylene diamine tetraacetic acid (EDTA) solution (0.25%).

2.4. In vitro cytotoxicity of MWCNT/$CoFe_2O_4$-180 hybrids

Cells seeded in a 96-well cell culture plate at a density of 1×10^4 cells per well were cultured in 5% CO_2 at 37 °C for 24 h. Then the medium was replaced with a fresh medium containing the hybrids (0–200 μg ml^{-1}) and further incubated for 24 h. Finally, the cell viability was assessed by the 3-(4,5-dimethylthiazol)-2-diphenyltetrazolium bromide (MTT) assay. The cytotoxicity was expressed as the percentage of cell viability compared to that of untreated control cells.

2.5. Hemolysis assay

Human red blood cells (HRBCs) stabilized with EDTA were kindly provided by Shanghai Blood Center. The HRBCs were obtained by removing the serum from the blood by centrifugation and suction. The cells were washed five times with sterile isotonic phosphate-buffered saline (PBS) solution, then diluted to 1/10 of their volume with PBS solution. Next, 0.3 ml of the diluted HRBC suspension was mixed with: (i) 1.2 ml of deionized water as a positive control; (ii) 1.2 ml of PBS as a negative control; and (iii) 1.2 ml of MWCNT/$CoFe_2O_4$-180 hybrids at concentrations of 50, 100, 200, and 400 μg ml^{-1}. The mixtures were shaken gently, then left to stand for 2 h at room temperature. Afterwards, the samples were centrifuged and the absorbance of the supernatants was recorded from 500 to 650 nm. The hemolysis percentages of the samples were calculated by dividing the difference in absorbance at 541 nm between the sample and the negative control by the difference in absorbance between the positive and negative controls, and multiplying the resulting ratio by 100.