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A double-targeted magnetic nanocarrier with potential application in hydrophobic drug delivery

Guobin Ding, Yi Guo*, Yanyun Lv, Xiaofeng Liu, Li Xu*, Xuezhong Zhang

Key laboratory for Molecular Enzymology and Engineering, the Ministry of Education, College of Life Science, Jilin University, Changchun 130012, China

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ABSTRACT

A double-targeted magnetic nanocarrier based with potential applications in the delivery of hydrophobic drugs has been developed. It consists of magnetite (Fe_3O_4) nanoparticles encapsulated in self-assembled micelles of the amphiphilic copolymer MPEG–PLGA [methoxy poly (ethylene glycol)-poly (D,L-lactide-co-glycolide)], and was fabricated using the solvent-evaporation technique. The magnetic nanocarrier has a very stable core–shell structure and is superparamagnetic. Its cytotoxicity was evaluated using the MTT assay with three cell lines—HeLa, MCF-7, and HT1080; it exhibited no cytotoxicity against any tested line at concentrations of up to 400 μg/mL after incubation for 24 h. Its cellular uptake was studied by Prussian blue staining and by fluorescence microscopy after encapsulating a fluorescent probe (hydrophobic quantum dots) into the nanocarrier. Finally, the magnetic targeting property of the magnetic nanocarrier was confirmed by an in vitro test. Overall, the results obtained demonstrate the potential of the double-targeted nanocarrier for the intracellular delivery of hydrophobic drugs.

1. Introduction

Cancer is one of the most feared and deadly human diseases [1–3]. Conventional chemotherapy involves delivering toxic anticancer agents to cancerous and healthy tissues or organs in a non-selective fashion, giving rise to systemic toxicity and serious side effects. Moreover, the poor water solubility of many anticancer drugs greatly impairs their effectiveness in chemotherapy [4–6]. The efficacy of chemotherapy could potentially be greatly improved by the development of a targeted drug carrier that would enhance the effective water solubility of hydrophobic anticancer drugs and selectively deliver them to malignant tissues, thereby increasing the curative effectiveness of the cancer treatment while sparing normal tissues from the side effects of the chemotherapeutic agent.

Considerable attention has been paid to polymeric micelles in recent years because of their numerous biomedical applications [7], especially as drug delivery vehicles [8]. Amphiphilic block copolymers can spontaneously self-assemble into supramolecular core–shell polymeric micelle structures with a diameter of 10–100 nm in aqueous solutions. The micelles' hydrophobic cores can act as reservoirs for hydrophobic agents; their hydrophilic shells consist of a brush-like protective corona that stabilizes their structure in aqueous solution [5,9,10]. Such nanoscale polymeric micelles present various advantages as drug delivery vehicles [9,11–13], including the chemical flexibility of core–shell structure, their high stability in aqueous solution, and their ability to solubilize water-insoluble drugs and increase the amount of the incorporated agent delivered to the cells, thereby increasing its efficacy. Moreover, they are less prone to uptake by the reticuloendothelial system and are passively targeted to solid tumors by the enhanced permeability and retention (EPR) effect that stems from tumor-associated leaky vasculature and poor lymphatic drainage [1,14–16].

In addition to encapsulating hydrophobic drugs, it has also been shown that polymer micelles can be used to assemble nanoparticles for optical imaging, magnetic resonance imaging (MRI), and targeted drug delivery [17,18]. For example, superparamagnetic iron oxide nanoparticles have been encapsulated into polymer micelles that were then used as a contrast agent for ultrasensitive MRI [19,20]. However, the stability and cellular internalization of the iron oxide nanoparticle-loaded micelles have not yet been studied in detail. The micelles' stability can greatly affect their encapsulation efficiency and drug release behavior, while cellular internalization is a critically important factor in intracellular drug delivery and many of the other biomedical applications of colloidal particles [21,22].

In this paper, we describe the development and characterization of a magnetic micellar nanocarrier based on the amphiphilic copolymer MPEG–PLGA and magnetite (Fe_3O_4) nanoparticles, and discuss its potential for double-targeted hydrophobic drug delivery that takes advantage of both the EPR effect and magnetic guidance. The magnetic nanocarrier was prepared by incorporating

* Corresponding authors. Tel.: +86 431 85155226; fax: +86 431 85155226.
E-mail addresses: xuli@jlu.edu.cn (L. Xu), guoyi@jlu.edu.cn (Y. Guo).

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clusters of magnetite nanoparticles into the cores of the micelles that are spontaneously formed by the MPEG–PLGA copolymer, and its physicochemical properties such as morphology, particle size, stability, and magnetic behavior were determined. The cytotoxicity of the magnetic nanocarrier was estimated by means of an in vitro MTT assay using three cell lines—a human cervical cancer cell line (HeLa), a human breast cancer cell line (MCF-7), and a human fibrosarcoma cell line (HT1080). The cellular uptake of the magnetic nanocarrier was monitored by Prussian blue staining and fluorescent microscopy. The magnetic nanocarrier could accumulate at the targeted area rapidly under a magnetic field. Overall, our results indicate that magnetic nanocarriers of this kind are stable and safe, with potential applications as double-targeted drug delivery vehicles.

2. Materials and methods

2.1. Materials

D,L-Lactide (≥99.5 mol%) and Glycolide (≥99.5 mol%) were purchased from Beijing GLACO Ltd. Poly(ethylene glycol) methyl ether (average Mn ~2000) and benzyl ether (99%) were purchased from Aldrich. Iron (III) acetylacetonate ($Fe(acac)_3$) (≥97%), 1,2-dodecanediol (≥90%), and oleylamine (≥70%) were obtained from Fluka. Stannous octoate (Tin(II) 2-ethylhexanoate, ~95%) and thiazolyl blue tetrazolium bromide (MTT, ~98%) were purchased from Sigma. Analytical grade oleic acid was purchased from Institute of Tianjin Guangfu Fine Chemicals. Hydrophobic quantum dots (QDs) dispersed in toluene were provided by the college of chemistry at Jilin University, having been synthesized using a method similar to that previously reported [23]. All other organic solvents used were of analytical grade. High-purity water with a resistivity of 18.2 MΩ cm was obtained using a Milli-Q system.

2.2. Instrumentation

Gel permeation chromatography (GPC) was used to determine the molecular weight and polydispersity index (PDI) of the polymers prepared; the measurements were conducted on a Waters HPLC system equipped with a refractive index detector (model 410). Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 mL/min. The sample was filtered through a 0.45 μm micropore film before measurement. ^{1}H NMR spectra were recorded on Bruker Avance 500 MHz spectrometer at room temperature with tetramethylsilane as the internal standard, using deuterated water (D_2O), chloroform ($CDCl_3$), or DMSO-d6 as the solvent. TEM analysis was performed on a JEOL JEM-1200EX model transmission electron microscope using an operating voltage of 100 kV. For negative staining, a dispersion of the magnetic nanocarrier micelles was dropped onto a carbon-coated copper grid, dried in air at room temperature, stained with 2 wt% sodium phosphotungstate aqueous solution for 40 s, and imaged within 24 h of staining. Powder X-ray diffraction (XRD) data were collected on a Siemens D5005 diffractometer with Cu Kα radiation (λ = 1.5418 Å). The magnetic properties were studied using a physical property measurement system (PPMS, Quantum Design) at 300 K. The hydrodynamic diameters of the magnetic nanocarrier in aqueous solution were determined using a Brookhaven ZetaPALS dynamic light scattering instrument with a BI-90Plus digital autocorrelator at a wavelength of 656 nm at 25 °C. The iron content of the magnetic nanocarrier was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 3300 DV, PerkinElmer).

2.3. Synthesis of the MPEG–PLGA copolymer

The MPEG–PLGA copolymer was synthesized by ring-opening polymerization of D,L-lactide and glycolide in the presence of MPEG [24]. Briefly, MPEG (Mn = 2000 g/mol) (1 mmol), D,L-lactide (34.7 mmol), glycolide (8.7 mmol) and stannous octoate (60 mg) were added to freshly distilled toluene in a three-necked flask. The mixture was heated to reflux with magnetic stirring under a flow of nitrogen for 18 h and then cooled to room temperature. A mixture of *n*-hexane and diethyl ether (v/v = 4:1) was poured into the reaction mixture to precipitate the polymer product, which was separated from the supernatant by decantation. The obtained polymer product was redissolved in dichloromethane and filtered, and the filtrate was slowly added to vigorously stirred water at 60 °C. After the evaporation of the dichloromethane from the emulsion, the resulting aqueous solution was lyophilized to yield the product as a white powder.

2.4. Preparation of magnetite nanoparticles

Magnetite nanocrystals were prepared by thermal decomposition, using a previously reported method [25]. 0.3532 g of iron (III) acetylacetonate, 1.0117 g of 1,2-dodecanediol, 1.022 mL of oleic acid, 0.99 mL of oleylamine, and 10 mL of benzyl ether were mixed in a three-necked flask with magnetic stirring under a flow of nitrogen. The mixture was gradually heated to 200 °C under a nitrogen atmosphere for 2 h and then, under a blanket of nitrogen, heated to reflux (~300 °C) for 1 h. The resulting black mixture was cooled to room temperature and 20 mL of ethanol was added under ambient conditions. A black material (the Fe_3O_4 nanoparticles) precipitated and was separated by centrifugation.

2.5. Preparation of the magnetic nanocarrier

The magnetic nanocarrier was prepared using a modified variant of a previously reported solvent-evaporation method [26]. The following procedure is representative: 10 mg of MPEG–PLGA copolymer and 2 mg of magnetite nanoparticles were dissolved in 1 mL THF. The resulting solution was added dropwise to 10 mL of high-purity water under vigorous ultrasonic agitation. The container was then opened to air overnight, allowing the slow evaporation of the THF and the formation of micelles. Finally, the micelle solution was subjected to successive filtrations, first through a filter with a pore size of 0.45 μm and then through another with a pore size of 0.22 μm, to eliminate polymer and magnetite aggregates. Empty micelles were prepared using the same procedure without the addition of magnetite nanoparticles.

2.6. Micellization behavior of the MPEG–PLGA copolymer

The freeze-dried empty micelle powder prepared by lyophilizing empty copolymer micelles in aqueous solution was redispersed in DMSO-d6 or D_2O and analyzed by ^{1}H NMR.

2.7. Determination of the magnetic nanocarrier's magnetite content

A solution of magnetic nanocarrier micelles was frozen and lyophilized to yield solid micelle samples. In order to completely digest the freeze-dried powder and release the iron for analysis, the dried samples were weighed, suspended in 6.65% nitric acid, and heated until the solid sample dissolved. The Fe_3O_4 nanoparticle content was calculated as the ratio of the mass of iron oxide in the sample to the total mass of the freeze-dried magnetic nanocarrier powder.