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ScienceDirect
Biomaterials 27 (2006) 5725–5733
Biomaterials
www.elsevier.com/locate/biomaterials

Synthesis and in vitro anti-cancer evaluation of tamoxifen-loaded magnetite/PLLA composite nanoparticles

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Received 16 May 2006; accepted 13 July 2006
Available online 7 August 2006

Abstract

The present study deals with the synthesis and characterization of tamoxifen-loaded magnetite/poly(L-lactic acid) composite nanoparticles (TMCN), and their in vitro anti-cancer activity against MCF-7 breast cancer cells. The composite nanoparticles with an average size of ~200 nm, were synthesized via a solvent evaporation/extraction technique in an oil/water emulsion. The superparamagnetic property (saturation magnetization value of ~7 emu/g) of the TMCN is provided by Fe_3O_4 nanoparticles of ~6 nm encapsulated in the poly(L-lactic acid) matrix. The encapsulation efficiency of the Fe_3O_4 and tamoxifen as a function of the concentration in the organic phase was investigated. The uptake of TMCN and tamoxifen by MCF-7 was estimated from the intracellular iron concentration. After 4 h incubation of MCF-7 with TMCN, significant changes in the cell morphology were discernible from phase contrast microscopy. Cytotoxicity assay shows that while the Fe_3O_4-loaded poly(L-lactic acid) composite nanoparticles exhibit no significant cytotoxicity against MCF-7, ~80% of the these cells were killed after incubation for 4 days with TMCN.

Keywords: Composite nanoparticles; Magnetic drug targeting; Controlled release; Tamoxifen; MRI

1. Introduction

Tamoxifen is the most widely used drug for the treatment of estrogen receptor-positive breast cancer and is the only drug approved for the prevention of breast cancer in healthy women at high-risk of breast cancer [1,2]. It acts as an anti-estrogen by binding to the estrogen receptor. The tamoxifen-estrogen receptor complex binds with DNA and can alter or block subsequent mRNA transcription leading to cellular apoptosis [3]. Promising success rates of tamoxifen in the treatment of advanced breast cancer have been reported [4,5]. However, tamoxifen also has estrogenic effects in the uterus and the most significant side effect of tamoxifen treatment appears to be increased risks of endometrial cancer. Other side effects include liver cancer, pulmonary emboli, venous thrombosis and ocular side effects such as retinopathy and corneal opacities [6].

These side effects were reported to be dose and concentration dependent [7], and an increased risk of endometrial cancer has been associated with duration of treatment and accumulated dose [8–10]. The activity and side effects of tamoxifen may also be attributed to its biologically active metabolites and their accumulation in target tissues [11]. Through drug targeting, these negative side-effects can be minimized by maintaining the drug concentration in other non-target organs and tissues at below certain minimal levels. Magnetically-guided particles are regarded to have excellent potential as drug targeting carriers due to its non-invasive character and high targeting efficiency [12]. By application of an external magnetic field, magnetic drug carriers could be retained to achieve very high concentrations of the chemotherapeutic agent near the target site for a given period of time without any toxic effects to normal surrounding tissue or to the whole body.

The first clinical cancer therapy trial using magnetic targeting carriers was performed by Lubbe et al. for the treatment of advanced solid cancer [13–15]. In contrast to systemic chemotherapy, they found a much higher drug

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0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.07.014

concentration in the tumor and the peritumoral area by using only 50% and 20% of the normal dose [16]. FeRx (San Diego, CA, USA) also carried out Phase I/II clinical trials with its Magnetic Targeted Carrier technology [17–19]. In these carriers, the chemotherapeutic agents were adsorbed on the surface of the carriers and quickly released in 1–2 h [16,17]. Recently, magnetite nanoparticles covered with a layer of biodegradable polymer shell or evenly distributed in the matrix of polymer nanoparticles have been reported as potential drug targeting vehicles [20–24]. The magnetite/polymer composite nanoparticles have demonstrated lower in vivo toxicity than magnetite [25,26].

In the present study, we describe the synthesis of tamoxifen-loaded magnetite/poly(L-lactic acid) (PLLA) composite nanoparticles (TMCN) and the evaluation of its cytotoxicity against MCF-7 breast cancer cells. With its superparamagnetic property, these nanoparticles can also be used as contrast agents for magnetic resonance imaging (MRI), with which the distribution of TMCN can be visualized in vivo [27,28]. PLLA is a biodegradable polyester that is widely used in drug delivery application and the encapsulation of tamoxifen in the polymer matrix can extend the release profile over that achievable from the adsorption of the drug on the magnetic particles surface. Moreover, nanoparticulate carrier systems formulated with tamoxifen can increase drug concentration in tumors through enhanced permeability and retention (EPR) effect [29–33].

2. Materials and methods

2.1. Materials

Benzyl ether, 1,2-hexadecanediol, oleic acid, oleylamine, iron (III) acetylacetonate were purchased from Aldrich Chemical Co. Poly(vinyl alcohol) (PVA) with MW of 30,000–70,000, PLLA with MW of 85,000–160,000, RPMI-1640 medium, fetal bovine serum, L-glutamine, penicillin-streptomycin solution, tamoxifen (trans-2-[4-(1,2-diphenyl-1-butenyl)phenoxyl]-*N*,*N*-dimethylethylamine), methylthiazolyldiphenyl-tetrazolium bromide (MTT), phosphate buffered saline (PBS, pH 7.4), trypsin-EDTA solution were purchased from Sigma. Dimethyl sulfoxide (DMSO), ethanol, hexane, dichloromethane (DCM) and methanol were purchased from either Fisher Scientific or Aldrich and used as received. De-ionized (DI) water (Millipore) was used throughout the experiment. MCF-7 breast cancer cells were purchased from ATCC.

2.2. Preparation of tamoxifen-loaded magnetic carrier

Oleic acid-stabilized 6 nm Fe_3O_4 magnetic nanoparticles were prepared via a high temperature reaction of iron acetylacetonate in phenyl ether in the presence of alcohol, oleic acid and oleylamine according to a reported method [34]. The particles are quite uniform and can disperse uniformly in organic solvents. The Fe_3O_4-containing polymeric nanoparticles were prepared by the solvent evaporation/extraction technique in o/w emulsion with PLLA as the encapsulation material. The TMCN were prepared using the following procedure: 100 mg of PLLA, 20 mg of 6 nm Fe_3O_4 and 7.5 mg of tamoxifen were dissolved in 8 ml dichloromethane and vortexed for 10 min to make the organic phase. The organic phase was then poured into 50 ml of stirred aqueous solution containing 1% PVA as emulsifier. The mixture was sonicated for 120 s with an ultrasonic processor (Sonics, VCX 130 PB). The formed o/w emulsion was then stirred at room temperature overnight with a magnetic stirrer to evaporate the organic solvent. The TMCN were collected by centrifugation at 10,000 rpm for 10 min and washed three times with DI water. The nanoparticles were resuspended with 10 ml water and freeze-dried (Edwards freeze dryer, ESM 1342) for 2 days. The amounts of Fe_3O_4 and tamoxifen were varied to obtain different TMCN. The PLLA nanoparticles without and with Fe_3O_4 (PLAN and FeLN, respectively) were prepared in the same manner except that only 100 mg PLLA and 20 mg Fe_3O_4 were used in the organic phase for preparing FeLN, and only 100 mg PLLA was used for PLAN. All experiments with tamoxifen were carried out under subdued light as the drug is photosensitive.

2.3. Characterization of the magnetic carrier

2.3.1. Particle size and surface properties

The size distribution of the nanoparticles were determined by dynamic light scattering (DLS) method using a 90 Plus particle size analyzer from Brookhaven Instruments at 25 °C and at a 90° detection angle. The freeze-dried nanoparticles were suspended in DI water and sonicated for 10 min to form a uniform dispersion. The morphology of the nanoparticles was imaged using a field emission scanning electron microscope (JEOL JSM-6700F), while the distribution of Fe_3O_4 nanoparticles in the magnetic carriers was observed using transmission electron microscope (TEM, JEOL JEM-2010F) at an accelerating voltage of 200 kV.

The zeta potential of the nanoparticles suspended in DI water (after sonication) was determined using the Zeta Plus analyzer (Brookhaven Instruments). For particle size and surface charge measurements, the mean value of five readings was reported. The surface composition of the TMCN was determined with X-ray photoelectron spectroscopy (XPS) on an AXIS HSi spectrometer (Kratos Analytical Ltd.) [35].

2.3.2. Fe_3O_4 loading and tamoxifen encapsulation efficiencies

The loading of Fe_3O_4 nanoparticles incorporated in the magnetic carrier was determined by thermogravimetric analysis (TGA) carried out with a TGA 2050 Thermogravimetric Analyzer (TA Instruments). Samples weighing between 5 and 15 mg were heated from 30 to 700 °C at a heating rate of 10 °C/min in air. Measurement of magnetization of the nanoparticles was carried out with a Vibrating Sample Magnetometer (VSM, DMS 1600).

The amount of tamoxifen incorporated in the nanoparticles was determined in triplicate by HPLC assay. A known amount of the nanoparticles (2 mg) was re-dissolved in DCM (2 ml) and 5 ml of acetone was then added. A stream of nitrogen was introduced to evaporate the DCM. The solution was then made up to 5 ml with additional acetone to compensate for the amount evaporated. The solution was filtered through a 0.45-μm membrane filter before HPLC assay using a Hewlett-Packard 1100 HPLC system. A reverse phase C_{18} column (Zorbax, Hewlett-Packard) was used as the stationary phase and 1.0% (v/v) triethylamine in DI water/methanol (11:89, v/v) was used as the mobile phase. The injection volume was 20 μl and the flow rate of the mobile phase was 0.8 ml/min. The column effluent was monitored at 265 nm with a UV detector. The amount of tamoxifen in the nanoparticles was determined from the calibration curve of the drug in acetone. The encapsulation efficiency of the drug was calculated as the mass ratio of the amount of the drug entrapped in nanoparticles to that used in the nanoparticle preparation.

2.4. In vitro tamoxifen release studies

The in vitro release of the tamoxifen from the nanoparticles was carried out in PBS (pH 7.4) containing 0.5% (w/v) sodium lauryl sulfate (SLS). SLS was used to increase the solubility of tamoxifen in the buffer solution and prevent adsorption of the tamoxifen on the surface of the tube [29]. About 3 mg of the freeze-dried, drug-loaded nanoparticles were suspended in 10 ml SLS-PBS in a 15-ml centrifuge tube and kept in a GFL shaker at 150 rpm and 37 °C. After a particular time interval, the tubes were taken