Biomaterials 35 (2014) 3885–3894

Contents lists available at ScienceDirect

Biomaterials

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

Biodegradable polymeric vesicles containing magnetic nanoparticles, quantum dots and anticancer drugs for drug delivery and imaging

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ARTICLE INFO

Article history:
Received 15 December 2013
Accepted 16 January 2014
Available online 1 February 2014

Keywords:
Biodegradable polymer
Multifunctional nanoparticles
Anticancer drug delivery
Busulfan
Fluorescence imaging
Magnetic resonance imaging

ABSTRACT

We have developed biodegradable polymeric vesicles as a nanocarrier system for multimodal bioimaging and anticancer drug delivery. The poly(lactic-*co*-glycolic acid) (PLGA) vesicles were fabricated by encapsulating inorganic imaging agents of superparamagnetic iron oxide nanoparticles (SPION), manganese-doped zinc sulfide (Mn:ZnS) quantum dots (QDs) and the anticancer drug busulfan into PLGA nanoparticles via an emulsion-evaporation method. T_2^*-weighted magnetic resonance imaging (MRI) of PLGA–SPION–Mn:ZnS phantoms exhibited enhanced negative contrast with r_2^* relaxivity of approximately 523 s^{-1} mM^{-1} Fe. Murine macrophage (J774A) cellular uptake of PLGA vesicles started fluorescence imaging at 2 h and reached maximum intensity at 24 h incubation. The drug delivery ability of PLGA vesicles was demonstrated *in vitro* by release of busulfan. PLGA vesicle degradation was studied *in vitro*, showing that approximately 32% was degraded into lactic and glycolic acid over a period of 5 weeks. The biodistribution of PLGA vesicles was investigated *in vivo* by MRI in a rat model. Change of contrast in the liver could be visualized by MRI after 7 min and maximal signal loss detected after 4 h post-injection of PLGA vesicles. Histological studies showed that the presence of PLGA vesicles in organs was shifted from the lungs to the liver and spleen over time.

1. Introduction

Functional nanoparticles used in the treatment of cancer attract extensive attention due to their intrinsic physical properties, long blood circulation time, specific targeting capability, enhanced intracellular uptake and manipulation of molecular behavior on the nanometer scale [1–4]. So far, studies on the treatment of cancer and other diseases using nanomaterials have mainly focused on therapy by locally produced cytotoxic heat [5,6], targeted drug delivery [7,8] or a combination of these two strategies [9–11]. In particular, several nano-structured particles based on polymers [12], lipids [13], inorganic materials [14] or natural materials [15] have been investigated for therapeutic purposes, mainly as drug delivery vehicles. With appropriate encapsulation, drugs are more stable in a physiological environment and the kinetics of the drugs can be more carefully controlled [16]. Furthermore, targeted drug delivery can be developed to improve chemotherapy in cancer treatment, not only by reducing the adverse effects in non-target organs but also by enhancing the therapeutic efficacy in the targeted organ [17].

A colloidal system based on biodegradable polyester nanoparticles (such as polylactic acid (PLA)), polylactic-*co*-glycolic acid (PLGA) and polycaprolactone (PCL) nanoparticles represents one of the most promising candidates for *in vivo* diagnosis and treatment for cancers, from preclinical development to clinical translation [1,18]. The use of these amphiphilic polymers results in the formation of nanoparticles with a hydrophobic core and a hydrophilic shell. The core–shell structure allows them to encapsulate and carry poorly water-soluble drugs [19] and to release these drugs at a sustained rate in the optimal range of drug concentration [20]. They

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http://dx.doi.org/10.1016/j.biomaterials.2014.01.041

can be further functionalized with polyethylene glycol (PEG) to avoid nonspecific absorption by proteins and fast clearance by the immune system [21,22] as well as equipped with targeting ligands for delivery of drugs to specific pathological sites [12]. Another important aspect is the encapsulation of inorganic nanoparticles together with anticancer drugs into the core of polymeric nanoparticles for localized contrast enhancement in different medical visualization techniques, which makes them superior in physical and chemical properties to commercial imaging agents [23,24], thereby providing precise diagnosis and evaluation of therapeutic efficacy.

In the present investigation, we report the development of a multifunctional polymeric drug delivery system aiming to deliver anti-cancer drugs and to enable *in vivo* and *in vitro* imaging in order to study the cell uptake as well as biodistribution of polymeric nanoparticles by magnetic resonance imaging (MRI) and histopathology. This drug delivery system consisted of PLGA nanoparticles encapsulating two hydrophobic inorganic nanocrystals, superparamagnetic iron oxide nanoparticles (SPION) for *in vivo* MRI [25] and cadmium-free manganese-doped zinc sulfide (Mn:ZnS) quantum dots (QDs) for fluorescence *in vitro* imaging. The PLGA vesicles (i.e., PLGA–SPION–Mn:ZnS) were also loaded with the chemotherapeutic drug busulfan [26], which is used in high doses as a conditioning agent prior to stem cell transplantation. Our aim was to construct a drug delivery system able to efficiently entrap and release lipophilic anticancer drugs and track cellular uptake *in vitro* as well as the biodistribution *in vivo* via noninvasive MRI. Such a vehicle is of significant value for diagnosis and therapy of cancer, where simultaneous drug delivery and therapeutic efficacy follow up is needed.

2. Materials and methods

2.1. Chemicals

Poly(lactic-*co*-glycolic acid) (PLGA) with the brand name PURASORB® PDLG 5002A (molecular weight ca. 15 kDa), terminated with carboxylic acid and having a ratio of 50/50 for DL-lactide/glycolide, was obtained from Purac Biomaterials, Gorinchem, the Netherlands. Sodium oleate, ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), n-hexane, octyl ether, dichloromethane, manganese chloride ($MnCl_2$), stearic acid (SA), tetramethylammonium hydroxide (TMAOH), zinc acetate dihydrate ($ZnAc_2$), sulfur, oleylamine (OLA), octadecene (ODE), 1-dodecanethiol, and PVA were purchased from Sigma Aldrich, Munich, Germany and used without any further purification.

2.2. Synthesis of SPION, Mn:ZnS QDs, and PLGA–SPION–Mn:ZnS nanoparticles

Monodisperse SPION were synthesized by thermal decomposition of a Fe-oleate complex in octyl ether at approximately 297 °C in the presence of oleic acid according to a previously reported method [27]. The Fe_3O_4 nanocrystals were stabilized with oleic acid and dispersed in dichloromethane at a concentration of 9.1 mg/mL Fe. Mn:ZnS QDs were synthesized by a nucleation-doping strategy [28]. First, manganese stearate ($MnSt_2$) was prepared by dropwise addition of methanolic $MnCl_2$ solution into a mixture of SA and TMAOH in methanol [29]. A mixture of $MnSt_2$ and 1-dodecanethiol in ODE was then degassed at 100 °C for 15 min, followed by the addition of sulfur and $ZnAc_2$ in sequence at 250 °C. The Mn:ZnS nanoparticles thus obtained were washed against acetone and finally re-dispersed in dichloromethane. Dichloromethane solutions of PLGA, SPION and Mn:ZnS were mixed with PVA aqueous solution (1:20 oil to water ratio) using a probe-type sonicator to form an emulsion, which was agitated overnight to evaporate the organic solvent and washed against de-ionized (DI) water (15 MΩ cm) to collect PLGA–SPION–Mn:ZnS nanoparticles. The PBS suspension of these particles was deposited on a copper grid and positively stained for TEM examination using a 2% aqueous solution of phosphotungstic acid ($H_3PW_{12}O_{40}$).

2.3. Characterization of nanoparticles

The morphology and elemental composition of SPION, Mn:ZnS, and PLGA–SPION–Mn:ZnS nanoparticles were characterized by JEM-2100F field emission transmission electron microscope (FE-TEM) operating at an accelerating voltage of 200 kV. The hydrodynamic size of the particles was measured by dynamic light scattering (DLS) (Delsa™Nano particle size analyzer, Beckman Coulter, Brea, CA, USA). The magnetization measurements were performed using a vibrating sample magnetometer (VSM-NUOVO MOLSPIN, Newcastle-upon-Tyne, UK). The optical absorbance and fluorescence intensity of Mn:ZnS and PLGA–SPION–Mn:ZnS nanoparticles were measured by Lambda 900 UV–Vis–NIR spectrometer (Perkin Elmer, Waltham, MA, USA) and LS 55 Fluorescence spectrometer (Perkin Elmer, Waltham, MA, USA), respectively. Electron paramagnetic resonance (EPR) measurement of Mn:ZnS was done on a Bruker ELEXSYS EPR spectrometer (X-band, 9–10 GHz) at 113 K (Bruker, Billerica, MA, USA). Concentrations of iron, manganese and zinc in samples were measured by Thermo Scientific iCAP 6500 inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Thermo Fisher Scientific, Kungens Kurva, Sweden).

2.4. In vitro phantom magnetic resonance imaging

Phantoms (10 mL) of PLGA–SPION–Mn:ZnS nanoparticles with 0.05 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, and 1 mM of iron were made by mixing the nanoparticle suspension with agarose gel (3 wt%) in DI water at 85 °C and letting it cool down naturally overnight in 50 mL Eppendorf centrifuge tubes. The phantoms were placed in the extremity coil of a 3 T MRI scanner (Siemens Trio, Siemens, Erlangen, Germany). A gradient echo T_2^* sequence with a fixed repetition time (TR) of 2000 ms and 12 TEs of 2–22.9 ms was used for MR imaging to obtain T_2^*-weighted images. Circular ROIs (region of interest) were placed manually on the images and the negative logarithmic values of the signal intensities at different TEs were plotted versus the respective TE values. The T_2^* relaxation time was calculated as the slope of a semi-log plot of the signal intensities versus the TEs. In phantoms with a high concentration of iron oxide, the calculations were based on fewer TEs excluding those TEs where full transaxial relaxation had already occurred.

2.5. In vitro cellular uptake and fluorescence imaging

To evaluate the effects of cellular uptake for PLGA–SPION–Mn:ZnS nanoparticles, we used the murine J774A macrophage cell line (European Type Tissue Culture Collection, CAMR, Salisbury, UK). These cells were obtained as a kind gift from Professor Carmen Fernandez, Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden. First, the J774A cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) (Invitrogen™, Life Technologies, Carlsbad, CA, USA) in a 50 cm^2 tissue culture flask (Costar, Corning, NY, USA). The cultures were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide. J774A cells were then cultured in 8-chamber polystyrene vessel tissue culture treated glasses at a density of 5×10^5 cells/chamber, at 37 °C, for 12 h in an atmosphere containing 5% carbon dioxide to allow cell attachment. Thereafter, the cell culture medium was aspirated from each chamber and substituted with the medium alone (negative control) or the same medium containing PLGA–SPION–Mn:ZnS nanoparticles at concentrations of 1000, 100, 50, 25, 12.5, 6.25 and/or 3 μg/mL. Chambers were then incubated at 37 °C for 1, 2, 4 and 24 h in an atmosphere containing 5% carbon dioxide. The uptake experiment was terminated at each time point by aspirating the test samples, removing the chamber and washing the cell monolayers with ice-cold PBS three times. Each slide was then fixed with methanol–acetone (1:1, v/v), followed by examination under a Nikon Eclipse i80 fluorescence microscope (Nikon, Tokyo, Japan) at a wavelength of 520 nm.

2.6. In vitro drug release

For *in vitro* busulfan release experiments, 30 mg busulfan was dissolved in dichloromethane solution containing PLGA, SPION and Mn:ZnS QDs, and then emulsified with PVA at a total volume of 6 mL. After evaporation of organic solvent and centrifugation to wash off unloaded drugs, PLGA vesicles containing drugs were transferred into a cellulose permeable membrane bag with a molecular weight cut-off (MWCO) of 12–14 kDa to dialyze against PBS solution at 37 °C. Entrapment efficiency of busulfan in PLGA–SPION–Mn:ZnS nanoparticles was calculated as [(mass of the total drug − mass of free drug) × 100%/mass of total drug]. Three parallel release experiments were conducted and samples were taken at specific time points. Concentrations of busulfan released in dialysis media, left in dialysis bag or left in centrifuged supernatant were measured by gas chromatography (SCION 436-GC; Bruker, Billerica, MA, USA) with electron capture detector (ECD) according to a method reported previously by Hassan et al. [30]. The release percentage of loaded busulfan is averaged from the three parallel experiments with error bars representing standard deviation.

2.7. In vitro degradation of PLGA vesicles

The synthesized PLGA vesicles were placed in a cellulose permeable membrane bag (MWCO 12–14 kDa) and dialyzed against 1 L PBS (pH 7.4) at 37 °C. At predetermined time intervals, a 5 mL aliquot of PBS solution was withdrawn and fresh PBS was added into the dialysis solution. The concentration of lactic acid released in PBS was measured by high-performance liquid chromatography (HPLC). The HPLC system consisted of a Gilson autoinjector (100 μL loop), an LKB HPLC pump 2150 (Pharmacia Inc., Sweden), an LDC analytical spectromonitor 3200 UV detector (Riviera Beach, FL, USA) and a CSW 32 chromatography station integrator. Separation was performed on a Zorbax SB-CN column (4.6 mm × 150 mm; 5 μm) from Agilent Technologies (Santa Clara, CA, USA), and the column was maintained at room temperature during analysis. The mobile phase was composed of $NH_4H_2PO_4$ (0.1 M,