Acta Biomaterialia 9 (2013) 9434–9441

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Covalent attachment of Mn-porphyrin onto doxorubicin-loaded poly(lactic acid) nanoparticles for potential magnetic resonance imaging and pH-sensitive drug delivery

Lijia Jing [a,1], Xiaolong Liang [b,1], Xiaoda Li [a], Yongbo Yang [a], Zhifei Dai [b,*]

[a] School of Life Science and Technology, Harbin Institute of Technology, Harbin 150001, China
[b] Department of Biomedical Engineering, College of Engineering, Peking University, Beijing 100871, China

ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form 31 July 2013
Accepted 12 August 2013
Available online 17 August 2013

Keywords:
Theranostic agent
MRI
Mn-porphyrin
Doxorubicin
pH-sensitive drug delivery

ABSTRACT

In this paper, theranostic nanoparticles (MnP-DOX NPs) were fabricated by conjugating Mn-porphyrin onto the surface of doxorubicin (DOX)-loaded poly(lactic acid) (PLA) nanoparticles (DOX NPs) for potential T_1 magnetic resonance imaging and pH-sensitive drug delivery. An in vitro drug release study showed that the release rate of DOX from MnP-DOX NPs was slow at neutral pH but accelerated significantly in acidic conditions. It was found that MnP-DOX NPs could be easily internalized by HeLa cells and effectively suppressed the growth of HeLa cells and HT-29 cells due to the accelerated drug release in acidic lysosomal compartments. Magnetic resonance imaging (MRI) scanning analysis demonstrated that MnP-DOX NPs had much higher longitudinal relaxivity in water (r_1 value of 27.8 mM^{-1} s^{-1} of Mn^{3+}) than Mn-porphyrin (Mn(III)TPPS3NH$_2$; r_1 value of 6.70 mM^{-1} s^{-1} of Mn^{3+}), behaving as an excellent contrast agent for T_1-weighted MRI both in vitro and in vivo. In summary, such a smart and promising nanoplatform integrates multiple capabilities for effective cancer diagnosis and therapy.

1. Introduction

Imaging guided tumor ablation has turned out to be one of the most exciting and challenging strategies for cancer treatment [1]. Chemotherapy is a conventional treatment used for a wide range of cancers. However, most chemotherapeutic agents usually have relatively short half-lives and create undesirable side-effects in vivo as a result of the toxicity to healthy tissues, so there has been a significant interest in the development of nanocarriers for controlled drug delivery. Among the imaging technologies, magnetic resonance imaging (MRI), because of its advantages of noninvasiveness, high spatial resolution and three-dimensional imaging, has been widely used for early diagnosis of cancer [2–7]. Recently, nanoplatforms integrating the functions of MRI and drug delivery have been developed for the treatment of cancer to improve therapeutic efficacy [8–12].

The sensitivity of MRI can be enhanced greatly using paramagnetic metal complexes (such as Gd-diethylenetriaminetetraacetic acid and Gd-tetraazacyclododecanetetraacetic acid) [13,14] or super-paramagnetic iron oxide (SPIO) nanoparticles (NPs) [15,16] as contrast agents. Manganese is an essential element for humans. It is less toxic than other paramagnetic metal ions and has relatively high electronic spins and a fast water exchange rate [17]. Mn-porphyrins have been investigated as new potential molecular MRI probes [18,19]. Mn-porphyrins show high stability compared to other metalloporphyrins, usually having much higher longitudinal relaxivities [20]. In addition, Mn-porphyrins are intended to be used as targeted MRI agents because of their tumorous "preferential uptake" property [20,21]. Compared with SPIO NPs, small molecule paramagnetic metal complexes have more advantages due to high contrast ability and easy urinary excretion [1]. However, there are still some problems that need to be overcome. For example, the renal toxicities of widely used paramagnetic gadolinium-based complexes have raised our concerns [22–24]. In addition, nearly all paramagnetic metal complexes have short half-lives in vivo, leading to limited imaging time and poor imaging outcomes [25–27].

Nanocarriers have attracted intensive interest for their use in delivering small molecule MRI contrast agents in order to increase their half-life and target efficiency [28–30]. Owing to the outstanding biocompatibility, good biodegradability and easy functionalization with a wide range of different substances such as ligands for active targeting, poly(lactic acid) (PLA) nanoparticles have been widely investigated as efficient carriers of various contrast agents and therapeutic agents with different solubilities [31–33]. However, the most rigorous bottleneck is that paramagnetic metal complexes confront the deteriorations of their r_1 relaxivity when encapsulating inside nanocarriers because of the poor interaction

* Corresponding author. Tel.: +86 13366214460.
E-mail address: zhifei.dai@pku.edu.cn (Z. Dai).
[1] These authors contributed equally to this work.

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.08.018

with water protons [28]. Herein, a novel nanotheranostic system was fabricated by encapsulating doxorubicin (DOX) as a chemotherapeutic agent in PLA NPs followed by covalently attaching Mn-porphyrin of Mn(III)TPP3NH$_2$ as a contrast agent onto the surface of PLA NPs for MRI and chemotherapy of cancer (Fig. 1). The physicochemical properties of the obtained Mn-porphyrin modified and DOX-loaded PLA NPs (MnP-DOX NPs) were characterized in terms of morphology, size distribution and drug loading content. In vitro release profiles of MnP-DOX NPs were examined in both acidic and neutral environments. The in vitro anticancer activity of MnP-DOX NPs was determined using a human cervical cell line (HeLa cells). MRI contrast behavior of MnP-DOX NPs was evaluated both in vitro and in vivo.

2. Materials and methods

2.1. Chemicals and materials

Poly(DL-lactic acid) with a group of carboxylic acid in the terminal (DL-PLA-COOH, 80 k MW, inherent viscosity 0.2–0.5 dl g^{-1}) and polyvinyl alcohol (PVA, 86–89% hydrolyzed, 20 k MW) were obtained from Shandong Medical Instrumental Institute and Alfa Aesar, respectively. DOX was obtained from Beijing Huafeng United Technology Co. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxy-succinimide (NHS) were purchased from Sigma–Aldrich. Fetal bovine serum (FBS) was obtained from Zhejiang Tianhang Biological Technology Co. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from AMRESCO Inc. RPMI 1640 was obtained from Thermo Fisher Scientific Inc. Dimethyl sulfoxide (DMSO) was obtained from Sigma–Aldrich. Dialysis membrane was obtained from Spectrum Laboratories Inc. (molecular weight cut-off 12,000–14,000). 2-(4-aAmidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) was obtained from Sigma–Aldrich. De-ionized water (DI water, 18.2 MΩ cm) from Milli-Q Gradient System was used in all the preparations. All other chemicals and reagents were of analytical grade.

2.2. Synthesis of Mn(III)TPPS3NH$_2$

Mn(III)TPPS3NH$_2$ was synthesized partially according to a procedure previously described [18,19] and the synthetic route is shown in Fig. S.1. The synthesis of Mn(III)TPPS3NH$_2$ was described as follows: benzaldehyde and pyrrole were first reacted in dimethylformamide (DMF) with the existence of $AlCl_3$ to give compound 1, which was nitrated by fuming nitric acid to obtain compound 2. Then, the nitro group of compound 2 was reduced to an amino group to yield compound 3 under the catalysis of HCl--$SnCl_2$, followed by reacting with concentrated sulfuric acid to obtain compound 4 with three sulfonic acid groups. Finally, compound 4 was metallated with manganese acetate in DMF to obtain the target compound Mn(III)TPPS3NH$_2$ (compound 5). Chemical structures of compound 4 and compound 5 were analyzed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy in deuterated dimethyl sulfoxide (Supplementary Figs. S.2 and S.3).

2.3. Preparation of DOX-loaded PLA nanoparticles

The DOX-loaded PLA nanoparticles (DOX NPs) can be easily generated from PLA, DOX and polyvinyl alcohol (PVA) by following a nanoemulsion method [32,34]. To optimize drug loading content (DLC) and encapsulation efficiency (EE) of DOX NPs, DOX and PLA at different weight ratios (1:5, 1:8, 1:10 and 1:20) were mixed with excess triethylamine, followed by dissolving in 20 ml of dichloromethane (DCM), respectively. Then each organic phase was filtered and mixed with 40 ml 2% (w/v) PVA aqueous continuous phase; later the mixture was emulsified by continuous probe sonication for 1 min with a 1.27 cm (1/2 inch) diameter titanium alloy horn (Sonicator 4000, Misonix) under 100% output amplitude setting. After evaporation of the organic solvent, the prepared nanoparticles were purified by centrifugation at 15,000 rpm and washed three times by DI water, and the obtained DOX NPs were lyophilized (–(−55 °C, 72 h) by using a freeze dryer (TFD5505, Ilshin Lab, Korea).

2.4. Preparation of MnP-DOX NPs

100 mg DOX NPs were dispersed in 10 ml DI water, followed by the addition of 5 mg (5.4 μmol) Mn(III)TPPS3NH$_2$, 3 mg (15.7 μmol) EDC and 2 mg (17.4 μmol) NHS. The resulting solution was stirred at room temperature for 4 h. Then, the particles were collected by centrifugation at 15,000 rpm for 30 min and washed with DI water three times. Then, the obtained MnP-DOX NPs were lyophilized (–(−54 °C, 48 h) using a freeze dryer.

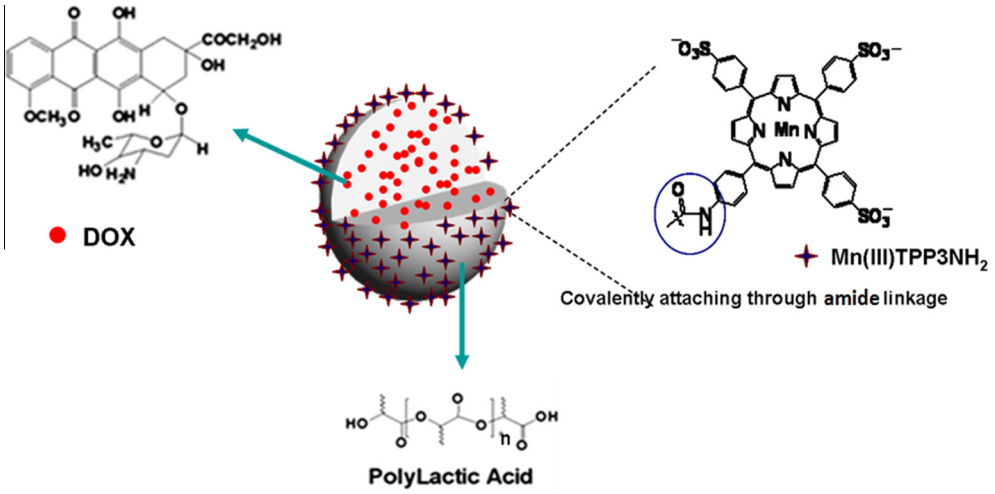

Fig. 1. Illustration of Mn-porphyrin modified PLA NPs containing DOX for MRI and chemotherapy of cancer.