Sensors & Actuators: B. Chemical 273 (2018) 264–275

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Simultaneous fluorescence imaging monitoring of the programmed release of dual drugs from a hydrogel-carbon nanotube delivery system

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ARTICLE INFO

Keywords:
Multispectral fluorescence imaging
Dual drug delivery
Programmed release
Hydrogel-carbon nanotubes
Combination chemotherapy

ABSTRACT

Multispectral fluorescence imaging, from cellular imaging to in vivo imaging, represents a significant advancement for drug delivery research. Tracking multi-drug release using real-time imaging in vivo can reveal the release processes for guiding the design of an ideal drug delivery system to implement combination chemotherapy. Herein, a sustained and programmed drug delivery system was developed based on a PCL-PEG-PCL thermosensitive hydrogel combined with chitosan-multiwalled carbon nanotubes using doxorubicin (DOX) and rhodamine B (RB) as the model drugs. Dual drug delivery was monitored by fluorescence imaging at the cellular level, and in vivo fluorescence signals in nude mice were tracked by a multispectral fluorescence imaging system. The results demonstrated that the release of these two drugs can be dynamically tracked in vitro and in vivo, respectively using fluorescence imaging techniques. After loading onto carbon nanotubes, DOX exerted a significantly slower release rate compared with RB in the hydrogel due to the dual release of DOX. The hydrogel-carbon nanotube delivery system achieved programmed release of DOX and RB, which affirmed that it can be a potential drug delivery system with programmed release for combined administration.

1. Introduction

Chemotherapy is one of the main clinical treatments for primary and metastatic cancer. However, the clinical outcomes following chemotherapy with a single drug may be limited and insufficient due to the complex and refractory nature of cancer. The undesirable outcome of mono-chemotherapy is related to common issues including drug resistance, dose-limiting toxicity, tumor heterogeneity, insufficient curative effect, and tumor relapse [1]. With the increased understanding of tumor biology and the mechanisms of chemotherapy, combination therapy containing two or more therapeutic agents has increasingly become a standard treatment to solve the insufficiency of mono-therapy [2,3]. Combination chemotherapy has been applied in the clinic to combat a variety of cancers for decades and plays an important role in cancer chemotherapy [2,4,5]. Nevertheless, the clinical outcomes of combination chemotherapy often do not attain their anticipated effect because the component drugs cannot achieve the desired spatiotemporal delivery and distribution according to their clinical requirements, hampering the success of their combined use. Efficient drug carriers could deliver different drugs to the correct site at appropriate intervals due to the inherent differences in physicochemical, pharmacokinetic and pharmacological properties among drug components [2,6]. Therefore, it is imperative to design an ideal drug delivery system to implement combinational strategies for on-demand drug therapy.

Combination drug carriers can achieve desired spatiotemporal drug release that single drug carriers cannot achieve. A hydrogel combined with a nanomaterial drug delivery system may afford suitable drug release for multi-drugs based on the different properties of the hydrogel and nanoparticles [7,8]. Hydrogel-based drug delivery systems can locally release a large amount of drugs in pathological tissues and reduce adverse effects in normal tissues with excellent hydrophilicity and biocompatibility [9,10]. Nano-carriers can effectively deliver the drug to the targeted site and improve therapeutic effects with beneficial cellular uptake and bioavailability, and few adverse side-effects [11–13]. The hydrogel-nanomaterials system can avoid the fast flexibility and rapid phagocytosis of a nano-delivery system and extend the drug release process with dual delivery from the hydrogel and nanomaterials [14–16]. The combination of nanomaterials with a hydrogel drug delivery system has demonstrated unique advantages for long-term sustained drug delivery [5,17]. The single hydrogel-based drug

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https://doi.org/10.1016/j.snb.2018.06.064
Received 12 September 2017; Received in revised form 4 June 2018; Accepted 13 June 2018
Available online 13 June 2018

carrier can achieve relatively more rapid drug release by passive diffusion or hydrogel degradation than the hydrogel-nanomaterial system. The differences in drug release can meet the requirements for combination chemotherapy by the drug loading on the hydrogel or nanomaterials respectively [15,17]. Thus, the hydrogel-nanomaterials system can achieve the desired spatiotemporal delivery and distribution of multiple drugs for on-demand combination chemotherapy [8,18].

The assessment of in vivo drug delivery is essential to reveal the pharmacokinetic properties and the mechanisms of chemotherapy. In situ medical imaging technologies provide a strategic advantage in research on in vivo drug delivery because they are amenable to real-time, nondestructive, longitudinal and quantitative analysis [19–22]. However, it is still difficult for conventional imaging techniques to simultaneously track the release of two or more drugs because of the lack of an imaging distinction from the drugs. Noticeably, fluorescence imaging plays an important role in the tracking of the release of multiple drugs based on multispectral imaging separation techniques, which is unique and advanced in comparison with other imaging modalities [23–25]. Multispectral fluorescence imaging can distinguish and separate different fluorescence signals with a multicolor composite image in view of the difference fluorescence drugs or labels [26–28]. Although these techniques have been widely used in biomedical research at the cellular level, including confocal laser scanning imaging and flow cytometry [29–31], in vivo multispectral fluorescence imaging remains a challenging task because of the complexity of biological tissues. Recently, some drug delivery systems have been successfully investigated in vivo using multispectral fluorescence imaging, including monitoring of the biological fate of the carrier and tracking drug release simultaneously [27,28,32,33]. For instance, a pH-sensitive dual fluorescent drug delivery system was simultaneously tracked in vivo for passive tumor targeting based on an N-(2-hydroxypropyl)methacrylamide copolymer [27]. In our previous reports, several doxorubicin-loaded hydrogel systems were reported to track the release of fluorescent drug and the degradation of the fluorescent carriers with multispectral fluorescence imaging [28,33]. Nevertheless, there are still few reports regarding the in vivo monitoring of the simultaneous release of multiple drugs for combination chemotherapy. The imaging visualization of the spatiotemporal delivery and distribution of multiple drugs from the drug delivery system will be critical for guiding combination chemotherapy.

Herein, a sustained and programmed drug delivery system was developed based on a PCL-PEG-PCL thermosensitive hydrogel combined with multiwalled carbon nanotubes (CNTs) using doxorubicin (DOX) and rhodamine B (RB) as model drugs. Unlike other dual drug delivery systems, DOX and RB co-loaded dual drug delivery systems, on the basis of their suitable fluorescence, can be tracked in real time using in vivo fluorescence imaging. The PCL-PEG-PCL thermosensitive hydrogel combined with a multiwalled carbon nanotubes system has been used for drug delivery with good biocompatibility as shown in our previous report [34]. In this hydrogel-carbon nanotube system, DOX was loaded in the carbon nanotubes, and then combined with RB into the hydrogel network, which can exert programmed drug delivery from dual carriers (Fig. 1). The optical properties of these two model drugs were investigated using UV–vis and fluorescence spectroscopy in vitro. Dual drug delivery was monitored by fluorescence imaging at the cellular level. Importantly, the programmed drug delivery of two drugs was tracked real time using in vivo fluorescence imaging of nude mice as models based on multispectral fluorescence imaging skills. The CNTs/hydrogel system can potentially be a programmed release drug delivery system for combination chemotherapy using two drugs. It can reveal the spatiotemporal delivery and distribution of two drugs from the drug delivery system by monitoring the unique drug fluorescent signals. Simultaneous in vivo fluorescence imaging tracking may provide a basis for guiding on-demand combination chemotherapy.

2. Materials and method

2.1. Materials

Polyethylene glycol (PEG, Mn = 1000) was obtained from Merck & Co., Inc. (Germany), which was vacuum-dried at 60 °C for 12 h before use. ε-Caprolactone (ε-CL) and stannous octoate ($Sn(Oct)_2$) were purchased from Aladdin Industrial Corporation (Shanghai, China), and ε-CL was purified by vacuum distillation. Multiwalled carbon nanotubes (CNTs) were purchased from DK nano technology Co., Ltd. (Beijing, China). Chitosan (CS) was provided by Haidebei Marine Bioengineering Co. Ltd. (Jinan, China). Doxorubicin hydrochloride (DOX, > 98%) was purchased from Dakub Meilun biology technology Co., Ltd. (Dalian, China). Rhodamine B (RB) was provided by Bodi Chemical Industry Co. Ltd. (Tianjin, China). Chloral hydrate (> 99%, pharmaceutical grade) was purchased from Yulong Algae Co., Ltd. (Qingdao, China). MTS was purchased from Promega Company. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) solution was purchased from Beyotime Biotech Institute. All other reagents were analytic reagent (AR) grade and used without further purification.

Balb/c nude mice (6–7 weeks old, 18–20 g) were used in these studies. All animal procedures were conducted following the protocol approved by the Institutional Laboratory Animal Ethics Committee, and all animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, People's Republic of China. Animals were housed in cages with free access to food and water.

2.2. Preparation of RB-DOX-CNTs/hydrogel

The PCL-PEG-PCL copolymer was synthesized by the ring-opening copolymerization of ε-CL initiated by PEG with stannous octoate acting as a catalyst as previously described [35]. DOX was loaded in the CNTs with non-covalent bonding, using chitosan as a dispersant [36]. First, DOX (1 mg) and CNTs (2 mg) were added to a sodium chloride injection solution (1 mL) with stirring for 16 h at room temperature in darkness. After another 10 h of stirring with the addition of chitosan (8 mg), the mixture was dialyzed (MWCO: 3.5 kDa) against PBS solution (pH 7.4) to remove the unbound DOX. The amount of unbound DOX was determined to calculate the drug loading efficiency by measuring the characteristic absorbance of DOX at 490 nm. Encapsulation efficiency and drug-loading capacity were calculated according to the equations in our previous report [36]. Afterward, the RB solution and DOX-CNT solution were mixed with the PCL-PEG-PCL copolymer to prepare the RB-DOX-CNTs/hydrogel.

2.3. Characterization of RB-DOX-CNTs/hydrogel

The morphological characterization of CNTs/hydrogel was performed using transmission electron microscopy (JEM-1010; JEOL, Tokyo, Japan). The sol-gel phase transformation of CNTs/copolymer solution was performed using the tube-inversion method. The mixture was placed in a small bottle at a concentration of 40%, and heated in a water bath from 10 °C to 40 °C, with a heating rate of 1 °C min^{-1}. The bottle was photographed at 20 °C or 37 °C, respectively, to record the status of the gel formation.

To determine the rheological properties of the CNTs/hydrogel, a rheological measurement was conducted using a rheometer (MCR 302, Anton Paar Instrument, Austria). The aqueous CNTs/copolymer solution was placed between a cone plate geometry (25 mm diameter) and a parallel plate with a gap of around 1 mm. Data were collected at a frequency of 1 Hz at 1% strain at a heating rate of 2 °C min^{-1} and a temperature range of 10–60 °C.