Materials Science & Engineering C 92 (2018) 745–756

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Reduction-sensitive polypeptide nanogel conjugated BODIPY-Br for NIR imaging-guided chem/photodynamic therapy at low light and drug dose

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ARTICLE INFO

Keywords:
Reduction-sensitive
Polypeptide
Nanogel
Near-infrared (NIR) imaging
Photodynamic therapy (PDT)
Chemotherapy

ABSTRACT

A heavy atoms modified reaction-active photosensitizer NHS-BODIPY-Br possessing efficient near infrared (NIR) fluorescence emission and singlet oxygen generation properties has been synthesized, which can be used for synchronous NIR imaging and photodynamic therapy (PDT). A reduction-sensitive PEGylated polypeptide nanogel was prepared by ring-opening polymerization (ROP) of L-cystine-*N*-carboxy anhydride. Poly (ethylene glycol) methyl ether was used as initiator and hydrophilic segment, the as-prepared nanogel was conjugated with the NHS-BODIPY-Br molecule by chemical linkage, and it can be directly used as a macrophotosentizer for NIR imaging-guided PDT. In addition, the nanogel also showed good encapsulating ability for doxorubicin (DOX). In the presence of glutathione (10 mM), the obtained NIR nanogel showed obvious reduction-induced drug release behavior. In vitro tests on internalization of the NIR nanogel by HepG2 cells indicated its efficiency in detecting cancer cells. Meanwhile, MTT assays performed on HepG2 cells confirmed that the cancer cells growth could be obviously suppressed (almost all cells) when exposed to an extremely low energy light (25 mW/cm^2, 10–15 J/cm^2) and low dose of DOX (3–5 μg/ml), indicates an efficient NIR image-guided chem/photodynamic therapy.

1. Introduction

Photodynamic therapy (PDT) has become an intriguing treatment modality since the first PDT drug was approved in 1999 due to its minimal invasiveness and repeatability without induction of resistance [1–3]. Essentially, PDT is a tumor-localizing therapeutic procedure which consists of three individually nontoxic components: photosensitizer, light with a specific wavelength, and oxygen. In typical PDT, local generation of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$) with light irradiation, can ablate the tumor by vascular shutdown, direct killing of tumor cells or systemic inflammation [4–6].

More importantly, imaging guided PDT is highly desired for improving the theraputic accuracy [7]. For example, it can be used in fluorescence-guided resection of glioblastoma multiforme to treat a variety of disseminated tumors for its repeatable treatments without induction of resistance [8]. Most photosensitizers can integrate fluorescent imaging with oncotherapy for its potential fluorescence emission performance [9, 10]. Among which, BODIPY shows its superiority to other PSs for excellent optical properties in the vis/NIR region, especially enhance quantum yields of singlet oxygen via the heavy atom effect, and bond connectivity by introduction of reactive functional groups [11–13].

It has been widely reported that PDT is generally used in the adjuvant treatment of cancers [14, 15], and combination therapies of PDT with chemotherapy are particularly promising [16–19], However, the hydrophobicity of the traditional antineoplastic drugs and photosensitizers makes it necessary to integrate different anticancer agents with a combined nanocarrier platform, especially at low light and drug dose [20–22]. Stimuli-responsive nanocarriers have been a hot topic in biomedicine field for their abilities in prolonging half-life of drug in plasma and enhancing accumulation in solid tumors via the enhanced permeability and retention (EPR) effect [23, 24]. Over the past decades, a myriad of tumor micro-environment responsive drug delivery systems (DDSs) have been designed to make full use of the endogenous and exogenous stimuli of tumor, aiming to maximize the therapy efficacy and minimize the side effects. Generally, temperature, pH and redox gradient are the main characteristics differentiating the normal and tumor tissues, and can be used to trigger drug release [25–27]. Among them, the reduction-responsive nanoparticles relying on the remarkable differences in glutathione (GSH) concentration were proved to be outstanding in drug delivery and cancer therapy.

Here, a reactive NHS-BODIPY-Br photosensitizer with excellent imaging and PDT properties was prepared, and then bond connected to

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https://doi.org/10.1016/j.msec.2018.07.034
Received 29 September 2017; Received in revised form 4 June 2018; Accepted 12 July 2018

a reduction-responsive nanogel (mPEG-PCys) containing disulfide bonds synthesized via ring-opening polymerization (ROP) of a-amino acid *N*-carboxyanhydrides (NCA). Also doxorubicin hydrochloride (DOX) was entrapped into the above nanogel to test the synergistic effects of PDT and chemotherapy (Scheme 1). The disulfide bonds were easily cleaved by GSH (10 mM) stimulation to release DOX while singlet oxygen would further kill tumor cells with light irradiation. The MTT experiments upon HepG2 cells proved the BODIPY and DOX carrying nanogel a great promising potential for synchronous tracking and further combination therapy.

2. Experimental section

2.1. Materials

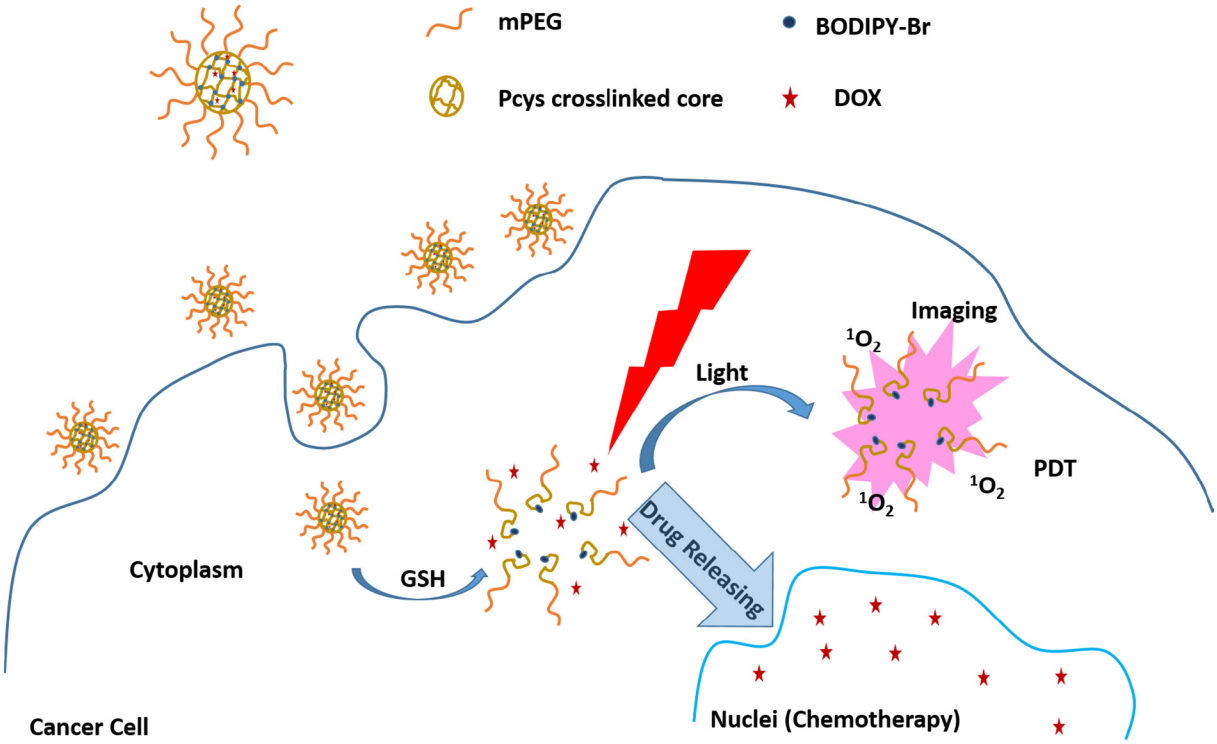
All reagents (AR purity) purchased from Aladdin Corporation (China) were used without any further purification. Organic solvents were purchased from Sinoreagent Corporation. Dichloromethane (CH_2Cl_2), n-hexane and DMF were dried with CaH_2 before use. Fetal bovine serum (FBS), dulbecco modified eagle medium (DMEM), methyl thiazolyl tetrazolium (MTT), 4′,6-diamidino-2-phenylindole (DAPI), propidium iodide (PI) and fluorescein diacetate (FDA) were obtained from Sangon Corporation, China. Dialysis bags (cutoff M_w = 3000/1000) were purchased from Bomei Biotechnology Corporation. Ultrapure water was prepared applying a Milli-Q Synthesis System (18.2 MΩ, Millipore, USA).

2.2. Characterization

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 300 NMR spectrometer using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-$_{d6}$) containing 0.03 v/v% tetramethylsilane (TMS) as the solvents. UV–Vis spectra were measured on a UV1700PC ultraviolet spectrophotometer (Shanghai AuCy Scientific Instrument Co. Ltd.). Fluorescence spectra were obtained on an F97pro fluorescence spectrophotometer (Shanghai Lengguang Industrial Co. Ltd.) with an excitation (10 nm) and emission (10 nm) slit width. FT-IR spectra were carried out on a Bruker EQUINOX 55 spectrometer via potassium bromide (KBr) method. LC-20AD Gel permeation chromatography (GPC, Shimadzu) equipped with Shodex KD-804 column, refractive index detector (RID-10A) and fluorescence detector (SPD-20A) were used to measure the molecular weights of polymers, using DMF as the mobile phase (5 mg/mL) at 60 °C. To measure the size and size distribution of the nanogel, dynamic light scattering (DLS, Malvern Zetasizer Nano ZS90) and transmission electron microscopy (TEM, JEOL-2010 microscope, 200 kV) tests were carried out at room temperature. MTT absorbance was obtained with a Bio-rad iMark microplate reader. Olympus U-HGLGPS fluorescence microscope was used to observe and get cell fluorescence images.

2.3. Synthesis of photosensitizer BODIPY-NHS (5)

1 (350 mg, 1.3 mmol) and 4-formylbenzoic acid (92 mg, 0.61 mmol) were dissolved in TFA (5 mL) under nitrogen atmosphere and stirred at 50 °C for 2 h. Then $POCl_3$ (1.5 mL) was added and stirred for another 30 min. After cooling to room temperature, the mixture was precipitated into cold water and collected by filtration without further purification to get compound **2**. Under a nitrogen atmosphere, the dried **2** (340 mg, 0.61 mmol) was dissolved in DMF (5 mL), EDCI (594 mg, 3.1 mmol), *N*-hydroxysuccinimide (356 mg, 3.1 mmol), and DMAP (35 mg, 0.31 mmol) were then added. After stirring at 25 °C for 15 h, the mixture was precipitated into cold water and crude compound **3** was collected by filtration. To a solution of crude **3** in dichloromethane (20 mL), triethylamine (1 mL) and BF3·OEt_2 (1 mL) were added. The mixture was stirred at 25 °C overnight under nitrogen atmosphere, and then washed with water and dried over Na_2SO_4. The solvent was evaporated to obtain a green solid and purified by column chromatography using 80% chloroform/hexane to get NHS- BODIPY **4** (130 mg, 30.2%). ^{1}H NMR($CDCl_3$, 300 MHz) δ ppm: 2.96 (s, 4H), 3.88 (s, 6H), 6.74 (s, 2H), 6.99 (d, J = 12.5 Hz, 4H), 7.42 (s, 2H), 7.66 (d, J = 12.5 Hz, 4H), 7.78 (d, J = 8.5 Hz, 2H), 8.30 (d, J = 8.5 Hz, 2H). MS-ESI (m/z): calcd for $C_{38}H_{26}BF_2N_3O_6S_2$, 733.13; found, 733.13 $[M]^+$.



Scheme 1. Reduction sensitive nanogel for NIR imaging guided photodynamic therapy and chemotherapy combination.