Applied Surface Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

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

Synthesis and characterization of diselenide linked poly(ethylene glycol) nanogel as multi-responsive drug carrier

Balkew Zewge Hailemeskel[a], Kefyalew Dagnew Addisu[a], Adhimoorthy Prasannan[a], Shewaye Lakew Mekuria[a], Chen-Yu Kao[b], Hsieh-Chih Tsai[a,*]

[a] Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, Section 4, #43, Keelung Road, Taipei 106, Taiwan, ROC
[b] Graduate Institute of Biomedical Engineering, National Taiwan University of Science and Technology, Section 4, #43, Keelung Road, Taipei 106, Taiwan, ROC

ARTICLE INFO

Article history:
Received 28 July 2017
Received in revised form 3 December 2017
Accepted 7 December 2017
Available online xxx

Keywords:
Selenium
Cancer
Polymer
Nanogel
Drug

ABSTRACT

Diselenide containing polymeric-nanoparticles considered as stimuli-responsive due to their low bond energy of Se—Se linkage with stimuli-sensitive properties Se—Se bond. Recently, diselenide bond containing amphiphilic block copolymers based nanoparticles become emerging materials as drug carrier. In this work, biocompatible, redox and gamma (γ)-ray sensitive diselenide-linked polymeric-nanogel (PEGSeSe)n was developed from alpha, omega-Bis-Bromo poly(ethylene glycol) (Br-PEG-Br) for anticancer drug carrier. A stimuli-responsive anti-cancer drug, doxorubicin (Dox) releasing of the nanogel was evaluated using an ultraviolet-visible spectrophotometer. The drug loading efficiency of the nanogel was found to be 32.7%. Redox-stimuli-drug release study was conducted in the presence of 0.1% (w/w) hydrogen peroxide (H_2O_2), and 1 mg/mL of glutathione (GSH) in phosphate-buffered saline (PBS) pH of 7.4 at 37 °C, as well as 5 Gy γ-ray was applied for understand their radiation responsive release of Dox. The drug release profiles indicated that fast release were observed during the initial few hours and maximum release 54% were shown after 48 h and 52.5% after 24 h in the presence of GSH and H_2O_2 respectively, and gradually drug release were decreased as the time increases. Interestingly, γ-ray irradiation drug release also showed that maximum of 46% the drug released within 12 h after irradiation. Based on the obtained results, it can be concluded that the synthesized nanogel could be a promising redox and radiation-sensitive nanocarrier biomaterial for high loading amount of anticancer drug and could be used for anticancer drugs delivery in redox environment and a low dose of γ-ray radiation.

1. Introduction

Selenium is an essential microelement that plays a crucial role in human beings [1]. Selenium-containing compounds have shown great potential application including therapeutic agents in cancer [2], and the synthesis of organic compounds [3]. Moreover, it is an important part of the antioxidant enzyme that can control the high level of free radicals which can harm cells and results in the development of different chronic diseases [4–6]. Recently, diselenide bond containing polymeric biomaterials have attracted great attention due to their dynamic covalent bond nature, various physical and chemical stimuli-responsive properties [4,7]. Dynamic covalent bonds such as disulfide bonds, acylhydrazone bonds, imine bonds, hexatomic ring formed via Diels–Alder cycloaddition reaction, and Se—X bonds (Se—Se, Se—N) are reversibly formed covalent bonds having the properties of cleavable, reform of the bond or metathesis under a certain conditions which make them having wide application in self-healing materials, responsive systems, and fabricating polymers [8–10]. Due to the weaker electronegativity and the bigger atomic size of selenium than sulfur, the bond energies of Se—Se (172 kJ mol^{-1}) and C—Se (244 kJ mol^{-1}) are lower than the bond energies of S—S (240 kJ mol^{-1}) and C—S (272 kJ mol^{-1}).The lower bond energy of Se—Se and C—Se bonds make them stimuli responsive to mild condition [8,11]. Therefore, selenium-containing polymers are among of the appropriate candidates for stimuli-responsive polymers like sulfur-containing polymers.

As for stimuli-responsive polymer, the synthesis of different selenium containing amphiphilic copolymer structures such as side-chain selenium containing polymers [12], main-chain

* Corresponding author.
E-mail address: h.c.tsai@mail.ntust.edu.tw (H.-C. Tsai).

https://doi.org/10.1016/j.apsusc.2017.12.058
0169-4332/

monoselenide containing polymers [13,14], main chain-diselenide containing polymers [15], hyperbranched polydiselenide polymers [16], hyperbranched polyselenide polymers [17],and dendrimers [18] were reported. For biocompatible and biodegradable stimuli-responsive aggregates, irradiation is among various stimuli that have been used widely because of it require no addition chemicals and its convenience of operation. Near infrared (NIR) and ultraviolet-visible (UV/vis) light have been used in drug delivery system to destroy the responsive aggregates and release the encapsulated functional molecules [19–21]. Xu et al. reported that metathesis reaction undergoes between two different diselenide containing symmetric compounds (R1SeSeR1 and R2SeSeR2) to form an asymmetric product (R1SeSeR2) with irradiation of visible light in the absence of any catalysts [9,22]. Yang et al. reported γ-radiation could induce gel–sol transition and distraction of the network structure of hydrogel based on a diselenide-containing amphiphilic polymer [23].The disassembly of selenium-containing polymeric aggregates in response to changes of stimuli under appropriate physiological conditions with their promising application for controlled drug release and synthetic enzyme mimics were investigated [9,24]. The stimuli-responsive nanogel drug delivery system realizes that the encapsulated drugs would not be released from the drug carrier systems before reaching the disease foci ("zero premature release") in response to either internal local microenvironment difference or external stimuli [25].

In the biological system, redox potential is one of the standard sensitive stimuli that results from the significant variation in the concentration of reduced GSH between the intracellular($\sim$2 – 10mM) and extracellular matrix ($\sim$2 – 10μM) compartment. Furthermore, tumor tissues have at least four-fold higher GSH concentration over that of the normal tissues and are even higher in multidrug-resistant cancer cells so that drug release behaviors in the cytoplasmic matrix in response to redox stimuli become effective [26–29]. Also, researches have shown that tumor cells typically have higher levels of reactive oxygen species (ROS) such as H_2O_2, hydroxyl radical ($HO^{\bullet}$), hydroperoxy radical ($^{\bullet}HO_2$) and superoxide anion radical than normal cells [30,31]. The differences in ROS levels between the tumor cells and the normal cells are because of dysregulation of redox balance in tumor cells that take place when intracellular production of ROS increases or levels of antioxidant agents become reduced [2,32,33]. The presence of higher concentration of Glutathione, higher levels of (ROS) in the tumor cell and redox stimuli responsiveness of the diselenide bond have been used to propose the diselenide containing biocompatible polymers for redox-responsive drug carriers for cancer treatment.

Xu et al. reported the synthesis of a dual-redox-responsive diselenide-containing multi-cleavable polymer micelles and redox-responsive disassembly of the aggregate which promoted the release of encapsulated molecules by the addition of reductants or oxidants [14,24,34]. Zhang et al. reported low dose of γ-radiation (5 Gy) which is equivalent to the radiation dose applied during a single radiotherapy treatment can destroy anticancer drug loaded diselenide-containing block co-polymers aggregates in aqueous solution and the release of drug [35]. Thus, the release of the encapsulated drugs indicated that diselenide bond containing aggregates have potential application for the combination of chemotherapy and radiotherapy.

Although there are several works on the synthesis of stimuli-responsive diselenide containing nanocarrier aggregates, many of them were fabricated from the hydrophilic/hydrophobic polymer combination. In this present study, redox and γ-ray responsive diselenide linkage containing nanocarrier aggregate has been synthesized only from Br-PEG-Br (MW 6000 Da) and disodium diselenide (Na_2Se_2) in aqueous solution as depicted Scheme 1.The formation of spherical aggregates most possibly due to the cross-linking of selenium atom with its neighboring selenium and the hydrophobic diselenide bond surrounded with the hydrophilic part polyethylene glycol which can be used as an anti-cancer drug carrier in a controlled manner.

In this work, reductant (GSH), oxidant (H_2O_2) and a low dose of γ-radiation (5 Gy) were used to destroy the drug-loaded aggregates formed by diselenide-containing polyethylene glycol in aqueous solution. The encapsulated drug has been released based on the sensitive diselenide bonds. Therefore, this study demonstrates that diselenide linkage containing poly ethylene glycol aggregate is potentially multi stimuli-responsive biomaterial for the use of anti-cancer drug carrier.

2. Experimental

2.1. Materials

Br-PEG-Br (MW = 6kDa), Sodium borohydride ($NaBH_4$, 99%), gray selenium powder (Se), H_2O_2, (35% w/w), GSH, 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide (MTT), D_2O and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Cellulose dialysis membrane (MWCO: 1 and 6–8 kDa) were purchased from CelluSept T1 (Braine l'Alleud, Belgium). Sterilized phosphate-buffered saline (PBS), Dulbecco's modified eagle medium (DMEM), sodium pyruvate, fetal bovine serum (FBS), trypsin and L-glutamine were purchased from Gibco (Carlsbad, CA). Doxorubicin hydrochloride (Dox.HCl) was purchased from Cayman Chemical Co., Ltd. Double distilled water was used throughout the experiment. All other chemicals and reagents were of analytical grades, were purchased from commercial sources.

2.2. Preparation of disodium diselenide (Na_2Se_2)

An aqueous solution of Na_2Se_2 was prepared by the reaction of gray Selenium powder and sodium borohydride according to an earlier reported procedure [12,36]. 70 mg (0.89 mmol) of gray selenium powder was added to 4 mL of aqueous solution containing 70 mg (1.89 mmol) sodium borohydride at room temperature with stirring under nitrogen atmosphere. The initial reaction occurred vigorously with the release of H_2 gas within 5 min, and then an additional 70 mg (0.89 mmol) of selenium powder was added. The reaction mixture was then warmed with stirring for 10 min to complete the dissolution and reaction of the selenium. Finally, a reddish aqueous solution of $Na_2Se_2$was formed.

2.3. Preparation of diselenide linked polyethylene glycol (PEGSeSe)n nanogel

Diselenide linked polyethylene glycol polymer was prepared through substitution reaction of a good leaving group containing Br-PEG-Br with Na_2Se_2. Briefly, 100 mg (0.017 mmol) of Br-PEG-Br, MW = 6 kDa was dissolved in 5 mL of double distilled water, and excess amount (2 mL) of the synthesized solution of Na_2Se_2 was injected under nitrogen. The reaction was carried out at a temperature of 50 °C in a water bath for 12 h. Then the solution was dialyzed for 72 h with the dialysis membrane molecular weight cutoff of 6000–8000 against double distilled water within 4 h exchanging interval. Finally, (PEGSeSe)n solution was filtered with a syringe filter pore size of 0.45 μm and lyophilized to obtain the sponge-like (PEGSeSe)n nanogel product which was stored for further use.

2.4. Characterization

X-ray photo- electron spectroscopy (XPS) measurements were carried out on a British VG Scientific ESCALAB 250 spectrometer fitted with XR5M monochromatic X-ray Gun. Raman spectra

Please cite this article in press as: B.Z. Hailemeskel, et al., Synthesis and characterization of diselenide linked poly(ethylene glycol) nanogel as multi-responsive drug carrier, Appl. Surf. Sci. (2017), https://doi.org/10.1016/j.apsusc.2017.12.058