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Redox/pH dual stimuli-responsive degradable Salecan-*g*-SS-poly(IA-*co*-HEMA) hydrogel for release of doxorubicin

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ABSTRACT

Salecan is a novel water-soluble extracellular β-glucan and possesses excellent physicochemical and biological properties. Here, redox/pH dual stimuli-responsive hydrogel based on Salecan grafted with itaconic acid (IA) and 2-hydroxyethyl methacrylate (HEMA) were prepared using disulfide-functionalized crosslinker *N,N-bis*(acryloyl)cystamine (BAC) for controlled drug delivery. The introduction of carboxylic groups endows the system with pH-sensitive character, swelling behavior of the hydrogel was conducted by changing the pH and Salecan content. It was demonstrated that DOX was efficiently loaded into the hydrogels and released in a controlled fashion via pH-control and swelling-shrinking mechanism. More importantly, DOX-loaded hydrogels showed dose dependent cytotoxicity toward A549 cell, and efficient cell killing was observed. Furthermore, a key point of this study was that the presence of disulfide linkage in system favored the degradation of hydrogels in the reductive environment. These results highlight the potential of Salecan-*g*-SS-poly(IA-*co*-HEMA) hydrogel as a novel system for the controlled release of anti-cancer drugs.

1. Introduction

Hydrogels are defined as cross-linked, three-dimensional and hydrophilic polymer networks that can absorb large amounts of water while maintaining their insoluble property. One of the more recent trends in hydrogels research has been directed at stimuli-responsive hydrogels, which show great potential in various pharmaceutical and biomedical applications (Tang, Du, Hu, Shi, & Kennedy, 2007; Zhou et al., 2008). The advantage offered by stimuli-responsive hydrogels is that they have the ability to change their volumes and shape reversibly under external stimuli, such as pH, temperature, light, electric/magnetic field, as well as specific molecular recognition (Dragan & Apopei, 2011, 2013; Himmelein, Lewe, Stuart, & Ravoo, 2014; Owino, Arotiba, Baker, Guiseppi-Elie, & Iwuoha, 2008; Ozay, Ekici, Baran, Aktas, & Sahiner, 2009; Su et al., 2014; Zhu & Zheng, 2006). Among these systems, pH-responsive hydrogels have been exclusively studied in the design of delivery carriers because they can utilize the variations of pH in different tissues and cell organelles as triggering agents in drug delivery (Rana et al., 2011; Wang, Nie, Chang, Sun, & Yang, 2013).

The successful use of stimuli-responsive hydrogels in controlled drug release greatly relies on their physical and chemical properties including swelling behavior, pore structure, degradation rate and interaction with bioactive drug molecules (Koetting & Peppas, 2014). Generally speaking, drug release from hydrogel carriers could be driven by three forces: diffusion, the interaction between drugs and hydrogel matrix, and degradation or erosion of the hydrogel itself (Lin, Lee, Chen, & Chen, 1987). Among them, controllable degradability is an important qualification because degraded polymers are easily excreted by the human body. Moreover, an ideal hydrogel carrier should not only provide a slow release in a sustained manner but also protect the drug from degradation before reaching the diseased area (Yu, Fan, Huang, & Chen, 2011). Extensive attention has lately been focused on the introduction of disulfide-functionalized crosslinkers to use redox potential for tailoring the degradability of the hydrogels. In this respect, the disulfide can be cleaved orthogonally by reducing reagent such as dithiothreitol (DTT) and glutathione (GSH) through thiol-disulfide exchange reactions (Meng, Hennink, & Zhong, 2009). This is a very useful trigger for the biodegradation and the resultant crosslinked hydrogels could be degradable to non-toxic, water-soluble compounds.

There is a considerable interest in fabricating hydrogel drug carriers using natural polymers such as polysaccharides due to their non-toxicity, biodegradability and biocompatibility (Dinu,

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Cocarta, & Dragan, 2016; Kono, Otaka, & Ozaki, 2014; Kong, Kim, & Park, 2016). Unfortunately, the major drawbacks that still remain with these polysaccharide-based hydrogels are their weak mechanical strength, and the burst release of drugs is hard to avoid when they are used as drug carriers. Graft copolymerization is an intuitively appealing technique to address these problems (Mukhopadhyay et al., 2014; Sanoj Rejinold, Chennazhi, Nair, Tamura, & Jayakumar, 2011). In graft copolymerization, various amounts of guest monomers could be attached onto the polysaccharide backbone and benefit this host polymer with some special characteristics, such as moderate swelling capacity and stimuli-responsive features, which are useful for drug release systems (Singh, Kaur, & Kennedy, 2015; Hamidian & Tavakoli, 2016).

Salecan, produced by the fermentation of a salt-tolerant new strain, Agrobacterium sp. ZX09, is a novel water-soluble extracellular β-glucan (Cas.No.1439905-58-4). It is a linear (1 → 3)-β-D-glucan comprising β-1-3-linked glucopyranosyls with a small number of α-1-3-linked which was reported in 2010 (Xiu et al., 2010). As a novel microbial polysaccharides, Salecan has excellent rheological properties and biological activities, as well as edible safety. Previous studies had shown that Salecan can be utilized in both the food and medical fields (Chen et al., 2011; Chen, Wang et al., 2012; Xiu, Zhan et al., 2011; Xiu, Zhou et al., 2011; Zhang et al., 2013; Zhou et al., 2013). In addition, Salecan contains large amounts of hydrophilic hydroxyl functional groups, which makes it amenable to chemical modification. A series of Salecan-based hydrogels were successfully prepared by our group recently, these novel hydrogels are of great potential to be applied to tissue engineering and control release systems (Hu, Feng, Wei et al., 2014; Hu, Feng, Xei et al., 2014; Hu et al., 2015; Qi, Hu et al., 2015; Qi, Wei et al., 2015; Wei, Hu et al., 2015; Wei, Qi et al., 2015).

The present work is concerned with studying the graft polymerization of itaconic acid (IA) and 2-hydroxyethylmethacrylate (HEMA) onto Salecan in aqueous media using a disulfide crosslinker *N,N'-bis*(acryloyl)cystamine (BAC) for producing a novel redox/pH dual stimuli-responsive hydrogel as a drug carrier. Doxorubicin hydrochloride (DOX), an amphiphilic anticancer drug, was chosen as a model drug due to its potency and a broad spectrum of activity against diverse cancer types. To our knowledge, this is the first report on the preparation and characterization of Salecan-*g*-SS-poly(IA-*co*-HEMA) (PIH) hydrogel for release of doxorubicin. Specially, the negatively charged hydrogels can be availably used for positively charged DOX loading through electrostatic bonding to the drug, which could be influenced by the pH value, leading to a relatively slow and pH-controlled drug release. The effects of Salecan and BAC concent on DOX release were also discussed in detail. More importantly, the disulfide-crosslinked polymer network was designed to become the origin of reduction-sensitivity. The degradation of hydrogel could be tailored by changing the concentration of DTT. In addition, the cytotoxic effect of the blank and DOX-loaded hydrogel against COS-7 and A549 cells was examined. The key objective of this study is to find the potential application of Salecan-g-SS-PIH hydrogels in intelligent drug release systems.

2. Experimental

2.1. Materials

Salecan was made by the Center for Molecular Metabolism, Nanjing University of Science & Technology. Itaconic acid (IA), 2-Hydroxyethyl methacrylate (HEMA), ammonium persulfate (APS) and dithiothreitol (DTT) were purchased from Aladdin Industrial Corporation (Shanghai, China). *N,N'-bis*(acryloyl)cystamine (BAC) was obtained from Alfa Aesar. Doxorubicin hydrochloride (DOX) was offered by Dalian Meilun Biology Technology Co., Ltd (Dalian, China). African green monkey kidney cells (COS-7 cells) and human lung adenocarcinoma (A549) cells were obtained from Medical School of Southeast University (Nanjing, China). An MTT cell proliferation and cytotoxicity detection kit was purchased from Nanjing KeyGen Biotech Co., Ltd (Nanjing, China).

2.2. Hydrogel preparation

A series of Salecan-g-SS-PIH hydrogels used in this study were prepared according to the following procedure. The feed compositions of the hydrogels are listed in Table 1. Briefly, a calculated amount of Salecan solution (2%, w/v) was added to a 50 mL four-neck fitted with a reflux condenser, a mechanical stirrer, a thermometer and an argon line. After being purged with argon for 30 min to eliminate the dissolved oxygen from the system, the solution was heated to 70 °C, and then an appropriate amount of initiator APS was introduced and the solution was continuously stirred at 70 °C for 20 min to generate radicals. The solution was allowed to cool at room temperature, then 55% (w/v) monomer solution (IA + HEMA) and 5% (w/v) BAC/methanol solution were added and the final volume of the solution was made up to 13.5 mL with deionized water. The mixture was stirred continuously under an argon atmosphere at room temperature for 10 min and then all transferred into a circular glass mold. The glass mold was sealed and placed in a water bath at 50 °C for 24 h.

After polymerization, the samples were carefully taken out from the mold and purified by soaking in a large excess of deionized water for 1 week. During this time, the water was refreshed every 6 h to remove any unreacted monomers and impurities within the hydrogel matrix.

3. Characterization

3.1. Stability of Salecan in hydrogels

To determine the concentration of Salecan in the washing solutions of Salecan-g-SS-PIH hydrogels, phenol/sulfuric acid assay was performed (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Briefly, 1 mL of 6% (w/w) phenol in water was mixed into 2 mL of washing solutions in a test tube, and then 5 mL of concentrated sulfuric acid was added and agitated for 10 min to obtain a homogeneous solution. The resulting mixture was kept still for 15 min at room temperature prior to measuring the absorbance at 490 nm. The concentration of Salecan in the washing solution was determined using a standard curve as follows: $y = 10.993x + 0.0785$, $R^2 = 0.9992$ (Table S1 of Supporting information).

3.2. Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra were obtained on a Nicolet IS-10 FT-IR spectrometer equipped with an attenuated total reflectance accessory (ATR). The absorption spectra of the dry samples were recorded in the frequency range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} for 16 scans.

3.3. X-ray diffraction analysis (XRD)

Wide-angle X-ray diffraction patterns of Salecan, freeze-dried SPIH2 and PIH hydrogels were recorded using a DMAX-2200 X-ray diffractometer operated at 30 kV and 20 mA with Cu Kα radiation ($\lambda = 0.154$ nm) in a range of $2\theta = 10–60^{\circ}$.

3.4. Thermal gravimetric analysis (TGA)

Thermogravimetric analysis (TGA) of Salecan and the dried hydrogel samples was performed using a TA Model Q 600 thermal