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Fabrication of folic acid decorated reductive-responsive starch-based microcapsules for targeted drug delivery via sonochemical method

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ABSTRACT

In this present study, original, biodegradable, biocompatible, non-immunogenic and non-poisonous folic acid (FA) decorated reductive-responsive starch-based microcapsules (FA-RSMCs) were designed and fabricated via the sonochemical method for targeted delivery and controlled release of hydrophobic drugs. A green hydrophobic fluorescent dye, Coumarin 6 (C6), was encapsulated into the FA-RSMCs as a substitute of hydrophobic drugs. The as-synthesized C6-loaded FA-RSMCs were characterized by DLS, SEM and TEM. The results indicated that the obtained C6-loaded FA-RSMCs had an appropriate size range and could enter into the bloodstream with their splendid stability. Moreover, C6-loaded FA-RSMCs showed a high selectivity to Hela cells, and a brilliant targeted drugs delivery and reductive-responsive release ability for hydrophobic drugs, particularly for hydrophobic anti-cancer drugs.

1. Introduction

Nowadays, chemotherapy is a major way to heal the cancers, but it still faces a big problem of lacking selectivity between normal cells and tumor cells. As a result, the rapid proliferating cells such as bone marrow will be affected by the harmful drugs (Kedar, Phutane, Shidhaye, & Kadam, 2010). Thus, effective and specific targeted drug delivery and controlled release system has been intensively encouraged to investigate in the last few years (Li, Yang et al., 2015). This system is a main application form in drug delivery and controlled release, while the drugs are often loaded in the particles by means of dissolving, bonding and wrapping (Brannon-Peppas, 1994). It is demonstrated in a number of appealing studies that the advantages of the drug delivery system are shown as the two following aspects: i) Indirectly changing the solubility of drugs and improving the stability of drugs; ii) Having the property of stimuli-responsive targeted drug delivery by modifying functional groups and substances, avoiding drugs releasing at healthy tissues (Mura, Nicolas, & Couvreur, 2013; Simoes, Moreira, Fonseca, Duzgunes, & de Lima, 2004). Particularly, the study on the reduction-oxidation drug delivery system has been an attractive topic because of the obvious imparity of extra/intracellular redox environment, which is induced by a certain amount of glutathione (GSH) in the cancer cells (Mura et al., 2013). Moreover, several fascinating researches and reviews have highlighted that folic acid (FA) is a great affinity ligand of the folate receptors (FRs), which has been identified as a tumor marker (Li, Liu et al., 2015). Our work is inspired by the reviews that we can decorate FA onto the drug carriers to improve the anti-tumor therapeutic efficiency and combine the redox difference of extra/intracellular environment to realize the reductive-responsive targeted drug delivery (Li, Wang, Du, Shi, & Cui, 2018).

As drug carriers, microcapsules (MCs) are considered as a kind of effective drug carriers because of their excellent drug loading ability. Recently, the investigations and applications of MCs have attracted a great deal of attentions of researchers because that MCs can greatly improve the stability of drugs, reduce drug toxicity, control drug release rate, and even have stimuli-responsiveness (Dong et al., 2017; Shimanovich, Bernardes, Knowles, & Cavaco-Paulo, 2014). So far, there are many materials used for fabricating various kinds of MCs, such as bovine serum albumin (BSA), chitosan (CS), liposomes, polymer/drug conjugates, starch and so on (Cui, Guan et al., 2017; Rapoport, 2007). Conspicuously, starch is one of natural polymers with a perfect level of biodegradability and biocompatibility, and it also has the character of non-immunogenic and non-poisonous (Wang, Chen, Luo, & Fu, 2016). Until now, there are many interesting approaches to prepare starch-

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based MCs, including polyol method, coprecipitation technique, organic solvent heating technique, sonochemical method and so on (Saikia, Hussain, Ramteke, Sharma, & Maji, 2014). Among the numerous approaches, sonochemical method is a convenient and efficient approach to synthesize starch-based MCs. Sonochemical method has simple operation conditions that using some convenient equipment under mild reaction conditions and short reaction time without additional high temperature and pressure, nevertheless, the obtained product is excellent with both characters of targeted drug delivery and stimuli-responsive controlled release (Wu, Zhu, Cao, & Chen, 2010; Xu, Zhao, Wang et al., 2016). During the process of sonochemical method, hydrophobic drugs dispersed in oil can be loaded into MCs directly with effectively protection and without destruction by ultrasound (Francesko et al., 2014). Furthermore, it should be noticed that materials with hydrosulfuryl groups (-SH) could be cross-linked by sonochemical method and formed disulfide bonds, which would be broken when it received a stimulus of reductive signal, showing an excellent reductive-responsiveness (Li, Xu et al., 2015; Li, Yang et al., 2015). Up to now, many excellent chemists and scientific literatures have initiated starch-based MCs (Tan et al., 2017; Zhang et al., 2013, 2017), whereas the characters of targeted drug delivery and reductive-responsive drug release can be combined by synthesizing starch-based MCs modified with multifunctional groups and substances exclusively.

In this work, we designed and synthesized an original, biodegradable, biocompatible, non-immunogenic and non-poisonous molecular targeted carrier (FA decorated starch-based MCs) through the sonochemical method for targeted delivery and reductive-triggered release of hydrophobic drugs. The targeting molecule FA was decorated onto the wall materials of starch-based microcapsules, and a hydrophobic green fluorescent dye Coumarin 6 (C6), used as a substitute of hydrophobic drugs, was encapsulated into the MCs. Through the following analysis, the as-prepared FA decorated reductive-responsive starch-based microcapsules (FA-RSMCs) showed a good selectivity to Hela cells, and a perfect reductive-triggered drug release property. The size, morphology, stability and loading capacity of FA-RSMCs were satisfied for the demands of blood flowing. Therefore, the synthesized FA-RSMCs could be potential intellective drug carriers for targeted delivery and controlled release of hydrophobic anti-tumor drugs for the cancer therapy.

2. Experimental section

2.1. Materials

Starch [$(C_6H_{10}O_5)_n$] and epichlorohydrin (C_3H_5ClO, > 99.5%) were purchased from Tianjin Tiantai Chemical Reagents Company (Tianjin, China). Sodium hydroxide (NaOH, > 96%) and hydrochloric acid (HCl, 36%–38%) were purchased from Beijing Chemical Reagent Company (Beijing,China). L-Glutathione (GSH, > 98%), Folic acid (FA, > 97%), Nhydroxysuccinimide (NHS, > 97%) and coumarin 6 (C6) were purchased from Sinopharm Chemical Reagent Limited Corporation (Shanghai, China). 1-ethyl-3-(3-dimethyl-amine propyl) carbodiimide hydrochloride (EDC, > 98%) and cysteine (Cys, > 98%) were purchased from Aladdin Industrial Corporation (Shanghai, China). Dimethyl sulfoxide (DMSO, > 99%) was purchased from Tianjin Guangfu Chemical Reagents Company (Tianjin, China). Ethylenediamine ($C_2H_8N_2$, > 99%) was purchased from Xilong Chemical Engineering Reagent Company (Ningbo, China). Phosphate buffers solution (PBS) was prepared by us. All other chemicals were of analytical grade and were used without further purification.

2.2. Synthetic procedures

2.2.1. Preparation of aminated starch (Starch-NH_2)

Aminated starch was synthesized by following a previous report with a few changes (Saikia, Das, Ramteke, & Maji, 2017). The starch (1 g) was added in 50 ml NaOH aqueous solution (0.05 M) at 80 °C. When the starch was all dissolved, epichlorohydrin (0.1 ml) was added to the solution and kept for 7 h under constant stirring by using a magnetic stirrer. Then the pH of the solution was adjusted to be 6–7 by adding HCl aqueous (0.01 M). To this, ethylenediamine (1 ml) was added to the solution, and then precipitated with excess ethanol, collected by centrifugation (8000 rpm, 8 min), and washed with ethanol. This purification process was repeated three times. At last, samples were lyophilized by drying frozen aqueous polymer solutions for 24 h, and then stored at 4 °C for later use.

2.2.2. Preparation of FA coupled aminated starch (FA-Starch-NH_2)

FA-Starch-NH_2 with primary amino group partially modified by FA was synthesized by the method consistent with our research group (Xu, Zhao, Yang et al., 2016). Briefly, 50 mg FA was dispersed in 10 ml DMSO. After dissolving completely of FA, an amount of EDC (25 mg) and NHS (50 mg) was added into the solution and kept stirring for 1 h under dark condition to obtain the activated FA ester. Meanwhile, Starch-NH_2 (0.4 g) was dissolved in 50 ml PBS solution (pH 7.4), and then the activated FA ester was drop-wise added into the above-mentioned aqueous solution slowly and kept magnetic stirring under dark condition at room temperature for 24 h to prepare FA-Starch-NH_2 with primary amino group partially modified by FA. Then, the obtained solution was dialyzed (molecular mass cut-off 8–14 kDa) several times against distilled water for 2 days under dark condition to purify FA-Starch. Later, the resulting products were centrifuged (3000 rpm, 5 min) to remove the indissoluble part. At last, the aqueous part was lyophilized by drying frozen aqueous polymer solutions for 24 h, and then stored at 4 °C for later use.

2.2.3. Preparation of thiolated FA-Starch-NH_2 (FA-Starch-SH)

The thiolated FA-Starch-NH_2 was synthesized by the method which was mentioned in our previous work (Cui, Dong et al., 2017). FA-starch-NH_2 (0.3 g) was dissolved into 50 ml PBS solution (pH 7.4). Then, Cys (0.1 g), NHS (0.1 g), EDC (0.05 g) were added into the solution above and kept magnetic stirring under dark condition at room temperature for 24 h. This reaction was under N_2 atmosphere to prevent the oxidation of -SH. Subsequently, the obtained solution was dialyzed (molecular mass cut-off 8–14 kDa) several times against distilled water for 2 days in the dark to purify FA-Starch-SH (Scheme 1 A). Later, the obtained products were centrifuged (3000 rpm, 5 min) to remove the indissoluble part. Finally, the aqueous part was lyophilized by drying frozen aqueous polymer solutions for 24 h, and then stored at 4 °C for later use.

2.2.4. Preparation of C6-loaded FA decorated stimuli-responsive starch-based MCs (FA-RSMCs)

The procedure for preparing C6-loaded FA-RSMCs was illustrated in Scheme 1B. First, a green fluorescence dye C6 was dispersed into soybean oil as a model hydrophobic drug. After that, FA-Starch-SH PBS solution (5 ml, 0.8 wt%, pH 7.4) and soybean oil (1 ml) were put into a cylindrical vessel. Then an ultrasonicator probe (SCIENTZ-PD, Ningbo Scientz Biotechnology Co., Ltd., China) was inserted into the vessel with the tip placed at the oil-water interface. The whole vessel was maintained in an ice-water bath, and temperature of solution was controlled below 30 °C during the ultrasound treatment. The mixture was sonicated (500 W/cm^2, 20 kHz) for 5 min. at the mode of ultrasonication 2 s and pause 2 s. After ultrasonication, the solution became a light green suspension. The resulting suspension with C6-loaded FA-RSMCs was stored in a refrigerator for next experiments.

2.2.5. Controlled release of C6-loaded FA-RSMCs

A 6 ml of as-synthesized C6-loaded FA-RSMCs suspension was injected into a dialysis bag (molecular mass cut-off 8–14 kDa). Next, three bags with C6-loaded FA-RSMCs were immersed into three vessels with 200 ml PBS (pH 7.4), 200 ml PBS with GSH (10 μM) and 200 ml PBS