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Fabrication and evaluation of the novel reduction-sensitive starch nanoparticles for controlled drug release

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ABSTRACT

A novel type of reduction-sensitive starch nanoparticles was prepared *via* the reversed-phase microemulsion method by using crosslinker, *N*,*N*-bisacryloylcystamine (BAC) with the disulfide linkages, which was specifically cleaved by dithiothreitol (DTT). Starch nanoparticles had a spherical morphology with a small size of 40 nm in the optimal condition. The influences of process parameters (starch amount, surfactant amount and oil/water (O/W) ratio) on the size of starch nanoparticles were studied by dynamic light scattering (DLS). BAC crosslinked starch nanoparticles were degraded into oligomers with the reducing agent of DTT due to the cleavage of the disulfide linkages. A model drug 5-aminosalicylic acid (5-ASA) could be loaded efficiently into starch nanoparticles and the *in vitro* drug release behaviors were also studied. The results suggested that the disulfide crosslinked starch nanoparticles exhibited an accelerated drug release behavior in the presence of DTT. *In vitro* methyl thiazolyl tetrazolium (MTT) assays indicated that BAC crosslinked starch nanoparticles had a good biocompatibility when cocultured with human HeLa cancer cells. Hence, with excellent biocompatibility and biodegradability, and rapid drug release in response to DTT, BAC crosslinked starch nanoparticles showed a great potential as a biomaterial carrier for the application of drug controlled release. In contrast to BAC crosslinked starch nanoparticles, *N*,*N*-methylenebisacrylamine (MBA) crosslinked starch nanoparticles were prepared as the control without the disulfide linkages.

1. Introduction

Recently, polymer nanoparticles have attracted significant interest in polymer chemistry, pharmaceutics and biomaterial science for biomedical application and controlled release [1]. A number of nontoxic, biocompatible and biodegradable natural polymers including polylactic acid (PLA) [2], chitosan [3] and starch [4,5] have been utilized as promising biomaterials for drug-carrier applications. Among these known carbohydrate polymers, starch has been used in the fields of drug delivery [6,7] and biocatalysts [8], because of its advantages, such as improving drug solubility and stability, decreasing drug toxicity and side effects [9], and having excellent biocompatibility and storage stability. Starch is one of the most widely investigated polymers, it is abundant, renewable, inexpensive and available [10,11]. But native starch is not suitable for controlled drug release due to its physical and chemical properties [12,13]. To further meet and improve its properties and extend the application of starch in the food and biomedical areas, all kinds of physical and chemical modifications including blending [14] and chemical modification [15,16], such as oxidation [17], crosslinking [18,19] and hydroxypropylation [20], have been considered by many researches. In particular, the great interest has been focused on the crosslinked starch nanoparticles with disulfide-functionalized linkages.

The reduction-sensitive polymers containing disulfide bonds have paid great attention as the fascinating biomaterials and could facilitate controlled release of encapsulated drug from crosslinked starch nanoparticles. However, the disulfide linkages are an important and typical character among the reduction-sensitive polymers [21]. The fact has been found that the disulfide linkage could be cleaved to the thiols in the presence of reducing agents, such as tributylphosphine, tris(2-carboxyethyl)phosphine, dithiothreitol (DTT) and glutathione tripeptide (GSH) [22–25] due to the thiol–disulfide exchange reaction [26]. According to the research report [27], the intracellular environment of GSH is ~10 mM, which is significantly higher than the concentration in the extracellular environment (~2 μM). And the GSH concentration in some tumor cells has been reported, which was the several times higher than that in the normal cells [28]. Disulfide-functionalized crosslinker, *N*,*N*-bisacryloylcystamine (BAC), has been developed and utilized widely in the preparation of reduction-sensitive polymers for drug-carrier application [29–32].

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In this study, BAC with the disulfide linkage was chosen to modify soluble starch as crosslinker, a novel type of reduction-sensitive starch nanoparticles was fabricated by free radical reaction of hydroxyl groups of starch to ethylene groups of BAC *via* the reversed-phase microemulsion method. To obtain the optimal conditions, a series of experiments were designed under different conditions with the starch amount, O/W ratio and surfactant amount. Subsequently, the degradable behavior and cell viability assays were investigated for exploring biocompatibility as biomaterical carriers. *In vitro* cytotoxicity of BAC crosslinked starch nanoparticles was evaluated using the methyl thiazolyl tetrazolium (MTT) method with human HeLa cancer cells. The results showed that starch nanoparticles were non-cytotoxic at low concentrations and suitable as a drug carrier. The BAC crosslinked starch nanoparticles could be degraded into oligomers with thiol groups in the presence of DTT. 5-Aminosalicylic acid (5-ASA) was loaded into the starch nanoparticles as a model drug. Based on the reduction-sensitive behavior, the drug-release was investigated from 5-ASA-loaded crosslinked starch nanoparticles with or without DTT, showing a sustained release. To serve as a control, *N*,*N*-methylenebisacrylamine (MBA) was chosen as crosslinker without the disulfide linkage, a reduction-insensitive MBA crosslinked starch nanoparticles with an analogous structure but without disulfide bonds were also prepared in this work. Meanwhile, the degradability and *in vitro* release properties of BAC crosslinked starch nanoparticles were compared with that of MBA crosslinked starch nanoparticles. Therefore, the reduction-sensitive starch nanoparticles have a great potential for controlled drug release.

2. Experimental

2.1. Materials and methods

Soluble starch was purchased from Zhejiang Linghu Chemical Reagent Factory, chemical purity grade. Sorbitan monooleate (Span 80), ammonium persulfate (APS) and liquid paraffin were purchased from Fine Chemical Industry Institute of Tianjin Guangfu, chemical purity grade. Acryloyl chloride (Acros Organic, 97%) was distilled under vacuum. Cystamine dihydrochloride was purchased from Darui Fine Chemicals Company, Ltd. Dithiothreitol (DTT) was from Sangon Biotech (Shanghai) Co., Ltd. *N*,*N*-Methylenebisacrylamine (MBA, chemical grade, Tianjin Bodi Chemical Engineering Company) was recrystallized from ethanol prior to use. The other reagents were of analytical grade and used without further purification.

2.2. Synthesis of N,N-bisacryloylcystamine (BAC)

The *N*,*N*-bisacryloylcystamine (BAC) was synthesized according to the literature [26] by the acylation of two amine groups of cystamine dihydrochloride as shown in Scheme 1. Cystamine dihydrochloride (10 mmol) was dissolved in a mixture of 15 mL of 3.5 M NaOH and 10 mL of chloroform. This solution was heated to 50 °C, and 5 mL of chloroform containing 20 mmol of acryloyl chloride was added dropwise under constant stirring over 15 min meanwhile the reaction temperature was maintained at 50 °C. After separating the phases when it is still warm, the aqueous phase was discarded. The remaining organic phase was cooled down to room temperature, and the product precipitated directly from the solution. The white crystal product was recovered by filtration and recrystallized from chloroform to give a yield 50%.

2.3. Synthesis of crosslinked starch nanoparticles

Starch nanoparticles with disulfide crosslinkers were synthesized *via* free radical reaction of hydroxy groups of starch to ethylene groups of BAC (Scheme 2).

0.5 g soluble starch was dissolved in 10 mL distilled water, and then put into boiling water bath for 10 min to form transparent solution. Finally, starch solution was ultrasonicated for 30 min as the water phase. The preparation procedures of oil phase were as follows: 0.5 g Span 80 was dissolved into 100 mL liquid paraffin and stirred in the thermostatic water container at 55 °C until emulsifier dissolved enough. The transparent water phase was added dropwise to the oil phase and stirred under mechanical agitation of 1000 rpm for 20 min. Then a certain amount of BAC was added. A certain amount of APS and $NaHSO_3$ as the initiators were added 20 min later. The final starch nanoparticles were formed with mechanical agitation for 4 h at 55 °C. The product was centrifuged and purified with distilled water and ethanol separately for several times, and then was vacuum-dried. Finally, the dried starch nanoparticles were kept in a desiccator for the analysis. When MBA was used as the controlled crosslinker without disulfide linkage for comparison with BAC, the preparation procedure of MBA crosslinked starch nanoparticles was similar (Fig. S1).

2.4. Fourier transform infrared (FTIR) spectroscopy analysis

FTIR absorption spectra of the samples were taken on a Fourier transform infrared (FTIR) spectrometer (Nicolet 670 FTIR, USA) over the region from 4000 to 400 cm^{-1}. The starch nanoparticles samples were collected using the KBr powder method prior to analysis, and pellets were dried in the vacuum before measurement.

2.5. Transmission electron microscopy (TEM) analysis

The morphology and size of the starch nanoparticles were characterized by a JEM-1200 EX/S (Hitachi, Japan) transmission electron microscope (TEM). The starch nanoparticles were dispersed in water or anhydrous alcohol with the concentration of 1 mg/mL prior to measurement in an ultrasonic bath (KQ-400KDE, KunShan Ultrasonic instrument Co., Ltd., Jiangsu, China) at 100 W for 15 min. To protect the solution from the heat build-up during sonication, the pulse function was used (pulse on, 5.0 s; pulse off, 2.0 s). Finally, the starch nanoparticles were deposited on a copper grid covered with a perforated carbon film. Observation was performed at 200 kV.

2.6. Size measurement of starch nanoparticles

Average sizes of the starch nanoparticles were determined by dynamic light scattering (DLS) at the scattering angle of 90° in anhydrous alcohol using 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation) at room temperature. According to the previous report [33], about 10 mg starch nanoparticles were dispersed in 10 mL anhydrous alcohol with the concentration of 1 mg/mL at 25 °C. Samples were ultrasonicated for 15 min to avoid the aggregation between particles during measurement. The particle size was determined at the maximum of the peak obtained in the CONTIN histogram. Further, the experimental data were given monomodal histogram analyzed by the cumulants method, giving access to some information on the average diffusion coefficient of the system. The final particle diameter was calculated from a mean of at least three measurements.