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Glucose-responsive zwitterionic dialdehyde starch-based micelles with potential anti-phagocytic behavior for insulin delivery

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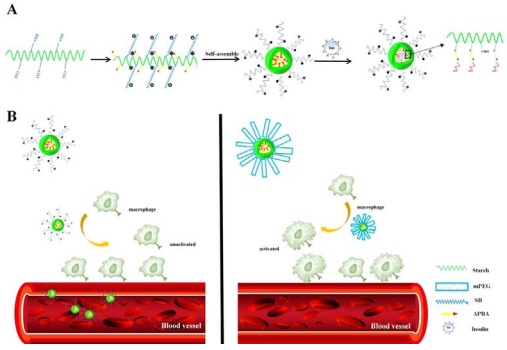
GRAPHICAL ABSTRACT



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ABSTRACT

Both blood stability and glucose responsiveness for carriers are the most important issues in insulin controlled release studies. However, few reports focus on reducing macrophage response performance for drug carriers themselves. Zwitterionic self-assembled micelles mitigated recognition by immune cell has evolved to address this limitation. In this work, novel zwitterionic dialdehyde starch-based micelles, sulfobetaine (SB) as the hydrophilic shell, and 3-aminophenylboronic acid (APBA) as glucose-responsive groups to the dialdehyde starch (DAS) backbones (SB-DAS-APBA) with glucose-responsive behavior were developed. Insulin release from the nanocarriers is sensitive to the different concentration of the glucose, rapidly at the condition of 3 mg/mL of glucose at pH 7.4. Methyl thiazolyl tetrazoliumviability (MTT) assay confirmed that zwitterion micelles were cytobiocompatible and low cytotoxic activity with A549 cells. For comparison, PEGylation micelles, monomethyl ether (mPEG) as the hydrophilic shell, and APBA as glucose-responsive groups to the DAS backbones (mPEG-DAS-APBA), were also synthesized. Confocal laser scanning microscopy (CLSM) and flow cytometry results displayed that PEGylation and zwitterion coating micelles have different recognition processes in contact with macrophages. After 4 h, doxorubicin (Dox) fluorescence of the Dox-loaded mPEG-DAS-APBA micelle groups were mostly located in nuclei. However, SB-DAS-APBA micelle groups were slightly detected in both nuclei and cytosol. In addition, the fluorescence signals of mPEG-DAS-APBA micelles showed enhanced trend than that of SB-DAS-APBA micelles in macrophage cells. These results suggest that, as drug carriers, zwitterionic self-assembled micelles SB-DAS-APBA not only possessed glucose-responsive insulin delivery property, but also reduced macrophage response performance.

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1. Introduction

Starch, which is a major natural polysaccharide, exhibits non-toxicity, compatibility, non immunogenicity and low antigenicity [1,2]. It has been widely used to constitute multifarious functional materials for controlled drug release and gene delivery [3–6]. Starch based drug carriers have loaded anti-cancer drugs and anti-inflammatory drugs, for instance, Dox, [6,7] hydroxycamptothecin (HCPT), [8] curcumin (Cur), [9] flufenamic acid, [10] prednisone [4] and indomethacin. [11] However, it is noteworthy that few starch derivatives have been reported for insulin delivery. Li et al. [3] grafted L-glutamic acid with starch, which showed pH-responsive behavior for insulin release. Akhlesh et al. [12] succeeded in making mucoadhesive nanoparticles by crosslinking starch as viable trans-nasal insulin carriers. Release rate of insulin was evaluated in the streptozotocin induced diabetes. In spite of great potential as drug carriers, starch and starch derivative polymers have been applied in inhalation, [13] ingestion [14] and injection [15] for therapeutic procedure except for reducing macrophage response performance.

Self-defense of immune cells builds along barriers by phagocytosis to get rid of foreign body, and maintains homeostasis *in vivo* [16]. The regulation of macrophage with immune-responsive function as a key tactics can avoid recognition and mitigate clearance of drug delivery nanomaterials *in vivo* [17,18]. Consequently, drug carriers can prolong circulation time and improve stability of carriers in blood. In terms of materials, one way to tackle this problem is to coat the surface of carriers with neutral hydrophilic or cell membrane biomimetic polymers to avoid excessive interaction between carriers and immune cells [19–21]. As a traditional modified surface material, nonionic poly(ethylene glycol) (PEG) has been widely used. However, it exists inevitable drawbacks of strong immune response, [22] instability in the presence of oxidative damage *in vitro* [23] and *in vivo* [24], PEGylated antibody production and accelerated blood clearance [25]. Meanwhile, PEG-chain segments with flexible and hydrophilic properties have limited cell escapes activity [26]. Thus, there is a need of developing suitable alternatives to PEGylation, and zwitterion coatings have recently emerged as promising candidates [27,28].

Zwitterionic polymers have electroneutral interface formation with positive and negative charged in both layers on the side chain, [29,30] possess phospholipid bilayer structure with highly bionic property, [31] and own high hydration. [21] Additionally, zwitterionic polymers have obvious advantages, such as excellent biocompatibility, non-specific protein-resistant, and stealth behavior of recognition by immune system. Thus, zwitterionic polymers have potential for applications in biomedicine. [32] Currently, the zwitterion-coating approaches to modulate performance of polymers are mainly used in tumor-targeting therapy for drug delivery system. A series of biomimetic hyperbranched polyether with zwitterionic shell was developed as drug carriers for protein and anti-cancer drugs [24,33,34]. Zhu et al. [32] reported newly zwitterionic amphiphiles of magnetofluorescent nanoparticles with dual imaging properties, which have potential application in tumor cell targeting. Jiang et al. [34] designed biomimetic hyperbranched polyether with zwitterionic shell (poly(carboxybetaine), PCB) as a drug carrier. Zhang et al. [35] synthesized copolymers P(MPC-*co*-PCL) with hydrophilic poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) zwitterions and degradable hydrophobic poly(ε-caprolactone) (PCL). By the evaluation of L929 and HeLa cells, polymeric micelles showed no significant cytotoxicity at 0.8 mg/mL for 24 h. *In vitro* phagocytosis by macrophages showed that polymeric micelles coated zwitterion exhibited 6-fold fluorescence intensity minimized difference from that without zwitterion. Doris et al. [36] synthesized zwitterionic diacetylene amphiphiles by photopolymerization and designed stealth micelles by self-assembly. As a tumor targeting enhanced permeability and retention effect (EPR) mediator, polydiacetylene-zwittionic (PDA-Zwitt) micelles took evaluation of the volume and margins of tumor.

In spite of several reports focus on glucose-responsive drug-carriers, most researches have spared no effort to assess the responsiveness of the material itself, ignoring pathway of polymers recognized by macrophages. Herein, two type of dialdehyde starch derivative micelles (mPEG/SB-DAS-APBA) were prepared. The endocytosis behaviors in contact with macrophage cells for PEGylation and zwitterionic micelles were compared by CLSM and flow cytometry, and illustrated in Scheme 1. Glucose responsive insulin release was determined. Cytocompatibility of micelles was examined by cell viability and hemolysis tests.

2. Materials and methods

2.1. Materials

Soluble starch (Mw 8.8 kDa) was provided from Zhejiang Linghu Chemical Reagent Factory. 1, 3-propanesultone, 1-chloro-3-dimethylaminopropane hydrochloride (CDMAP·HCl) (99%), monomethyl ether (mPEG) (Mn 1.9 kDa), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), insulin from porcine pancreas, 3-aminophenylboronic acid hydrochloride (APBA) and pyrene were purchased from Aladdin Chemistry Co., Ltd. Succinic anhydride and 4-dimethylaminopyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co., Ltd. Doxorubicin hydrochloride (Dox·HCl) and N-hydroxysuccinimide were purchased from *J & K* Reagent Company. 2-(4-amidinophenyl)-6-indolecarbamidine (DAPI) was purchased from Sangon Biotech Co., Ltd. All other chemicals were analytical reagents and used without further purification.

2.2. Synthesis and characterization of dialdehyde starch derivatives

2.2.1. Synthesis of dialdehyde starch derivatives

Two types of dialdehyde starch derivatives were synthesized. A type of sulfobetaine (SB) named as 3-dimethyl (chloropropyl) ammonium propanesulfonate (DCAPS) was chosen to prepare the zwitterionic etherifying agent. DCAPS was synthesized by conjugating 1, 3-propane sultone with 1-chloro-3-(dimethylamino) propyl according to a previous report [37]. The DCAPS modified dialdehyde starch (SB-DAS) was conveniently synthesized by conjugating DCAPS with DAS [38]. SB-DAS was synthesized using a Williams etherification reaction. Briefly, 1.43 g of dialdehyde starch was dispersed in 10 mL of NaOH (11 wt%) water solution. Then, 5 mL of DCAPS was added, and the reaction was carried out with stirring at 60 °C for 6 h. Subsequently, the reaction system was poured into cold methanol. The precipitate was washed with methanol for 3 times. Finally, the product was dried in a vacuum oven overnight. SB-DAS-APBA was synthesized as follows, SB-DAS (0.1 g) and APBA (0.1 g) were added to dimethyl sulfoxide (DMSO) with stirring at 45 °C for 12 h under a nitrogen atmosphere. The solution was purified by dialyzing against deionized water for 2 days using dialysis bags (MWCO 3.5 kDa), and then APBA-qualified SB-DAS (SB-DAS-APBA) was obtained by freeze-drying.

Similarly, the mPEGylated dialdehyde starch (mPEG-DAS) was conveniently synthesized by conjugating mPEG-COOH with DAS according to previous reports [39,40]. Firstly, DAS (0.2 g), mPEG-COOH (0.38 g), EDC HCl (0.23 g) and DMAP (0.05 g) were dissolved in DMSO. The mixture was stirred at 25 °C for 48 h. Then, the mixture was dialyzed in a dialysis bag (MWCO 10 kDa) for three days. Finally, the solution was lyophilized to obtain mPEGylated dialdehyde starch (mPEG-DAS). mPEG-DAS (0.1 g) and APBA (0.1 g) were dissolved in DMSO at 60 °C for 6 h under nitrogen atmosphere. Afterwards, the mixture was purified in a dialysis bag (MWCO 3.5 kDa) for 72 h and lyophilized to obtain APBA-functional dialdehyde starch (mPEG-DAS-APBA).

2.2.2. Characterization of dialdehyde starch derivatives

Dialdehyde starch derivatives were determined by ^{1}H NMR which was recorded on an AVANCE III, Bruker 400 MHz spectrometer (Germany) and FTIR determined by Agilent technologies carry 600