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Facile one-pot synthesis of glucose-sensitive nanogel via thiol-ene click chemistry for self-regulated drug delivery

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ABSTRACT

A novel glucose-sensitive nanogel was conveniently prepared through one-pot thiol-ene copolymerization of pentaerythritol tetra(3-mercaptopropionate), poly(ethylene glycol) diacrylate, methoxyl poly(ethylene glycol) acrylate and *N*-acryloyl-3-aminophenylboronic acid. The formation of core–shell nanogel was verified by proton nuclear magnetic resonance, dynamic laser scattering (DLS) and transmission electron microscopy. The successful incorporation of phenylboronic acid (PBA) in the nanogel was confirmed through Fourier transform infrared spectroscopy, inductively coupled plasma mass spectrometry and fluorescence technology. Owing to the presence of PBA, the nanogel exhibited high glucose sensitivity in phosphate-buffered saline determined by DLS and fluorescence technology. The increased amount of glucose causes an increase in the hydrodrodynamic radius and a decrease in the fluorescence intensity of PBA–alizarin red S (ARS) complex in the nanogel at pH 7.4 because of the competitive substitution of ARS to form the hydrophilic PBA–glucose complex. ARS and insulin were loaded into this glucose-sensitive nanogel. In vitro release profiles revealed that the drug release from the nanogel could be triggered by the presence of glucose. The more glucose in the release medium, the more drug was released and the faster the release rate. Furthermore, in vitro methyl thiazolyl tetrazolium assay, lactate dehydrogenase assay and hemolysis test suggested that the nanogel was biocompatible. Therefore, the PBA-incorporated nanogel with high glucose-sensitivity and good biocompatibility may have great potential for self-regulated drug release.

1. Introduction

Recently, stimuli-responsive materials have attracted much interest because of their rapid response to environmental stimuli and promising applications in various fields, such as drug delivery [1–4], gene therapy [5–8], biosensors [9,10] and diagnostics [11,12]. These environmental physical and chemical stimuli include light [13], magnetic fields [14], temperature [15], pH [16,17], ionic strength [18], etc. A typical example of a stimuli-responsive system is glucose-sensitive drug delivery for the treatment of diabetes mellitus in which the patient cannot control his/her blood glucose concentration within normal levels. The aim of glucose-sensitive drug delivery is to achieve continuous and automatic insulin release in response to an elevated level of blood glucose with minimal patient intervention and improved quality of life [19].

To develop glucose-triggered insulin release systems, glucose-sensitive materials such as glucose oxidase (GOx), carbohydrate–concanavalin A (Con A) and phenylboronic acid (PBA) derivatives have been employed [20,21]. The enzymatic reactions between GOx and glucose alter the pH of the microenvironment and cause swelling or shrinking of the carriers incorporated with GOx, resulting in insulin release in response to high glucose levels [22]. However, the use of enzymes may cause some disadvantages, such as limited pH and temperature range, and possible bioactivity loss during the preparation of carriers [23]. Carbohydrate–Con A is another system used to fabricate glucose-sensitive platforms [24]. Unfortunately, the biotoxicity and instability of Con A limit its application as a glucose-responsive insulin delivery system [25].

PBA, known to reversibly form cyclic boronic esters with *cis*-diol compounds, has selective glucose-sensitivity in an aqueous milieu [26]. In aqueous solution, the PBA exists as an equilibrium between neutral trigonal-planar species and negatively charged tetrahedral boronate species. Both the hydrophobic neutral form and hydrophilic negatively charged form can form complexes with diols, but only the negatively charged tetrahedral state can form a stable

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cyclic boronic ester with *cis*-diol compounds. Glucose-bearing *cis*-diol groups are well documented to form stable glucose–PBA complexes at neutral or alkaline pH, which makes the equilibrium shift to the charged form and improves the hydrophilicity of PBA-functionalized materials [27,28]. Therefore, when PBA or its derivatives are immobilized in a nanogel, the presence of glucose can induce the nanogel swelling and subsequent release of the payload via formation of a hydrophilic glucose–PBA complex.

In this paper, a novel glucose-sensitive nanogel was synthesized by a one-pot thiol-ene click chemistry approach. The influence of the glucose on the hydrodrodynamic radius (R_h) of the nanogel was studied. Alizarin red S (ARS), a *cis*-diol-containing fluorescent dye, has been used as a probe to examine the binding behavior between *cis*-diol compound and PBA in nanogel. The binding of ARS with PBA incorporated in the nanogel could be competitively substituted by the added glucose, resulting in a significant decrease in fluorescence intensity. In addition, ARS and insulin were loaded into nanogel, and the drug-release behaviors triggered by glucose at physiological pH were studied. Moreover, the biocompatibility of the nanogel was also investigated.

2. Materials and methods

2.1. Materials

D-(+)-Glucose (99%) was purchased from Alfa Aesar and used as received. Methoxyl poly(ethylene glycol) (M_n = 5000 nominal value) was purchased from Aldrich without further purification. 3-Aminophenylboronic acid (APBA), pentaerythritol tetra(3-mercaptopropionate) (QT) and acryloyl chloride were purchased from Sigma. Poly(ethylene glycol) diacrylate (PEGDA, typical M_n = 575) was purchased from Aldrich. Methoxyl poly(ethylene glycol) acrylate (mPEGA) and *N*-acryloyl-3-aminophenylboronic acid (AAPBA) were synthesized according to the literature [29,30]. All other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received.

2.2. Preparation of nanogel

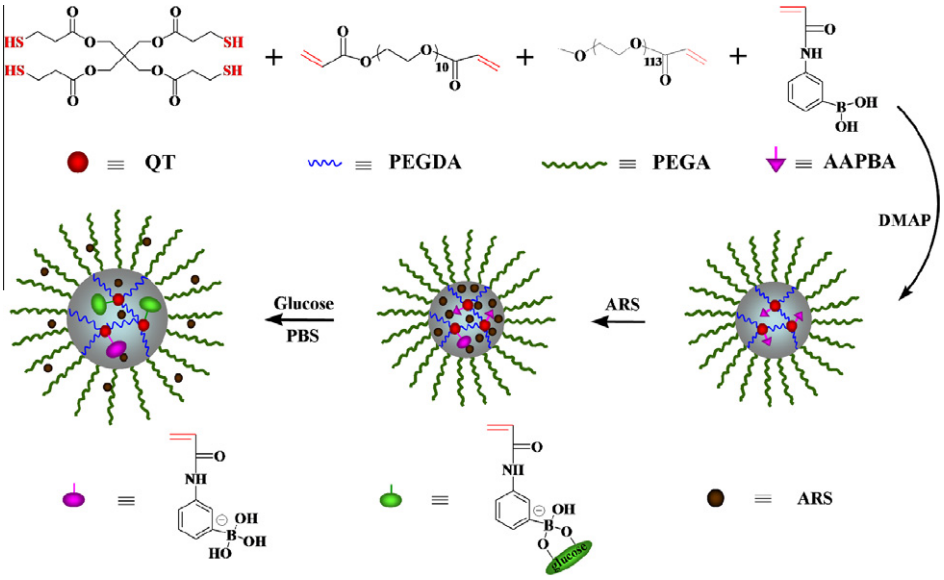
The nanogel was prepared by one-pot thiol-ene copolymerization of AAPBA with PEGDA, mPEGA and QT under the catalysis of 4-dimethylaminopyridine (DMAP) (as shown in Scheme 1). Briefly, AAPBA (0.747 g, 3.9 mmol), PEGDA (0.750 g, 1.30 mmol), mPEGA (1.629 g, 0.326 mmol) and QT (0.876 g, 1.79 mmol) were dissolved in 40.0 ml acetonitrile in a three-necked round-bottom flask. The solution was purged with nitrogen for about 30 min before DMAP (0.043 g, 0.359 mmol) was added. After further bubbling with nitrogen for 30 min, the reaction mixture was allowed to proceed for 48 h at room temperature. Then the resultant nanogel was purified by dialysis against deionized water for 3 days (MWCO 7,000 Da). The final nanogel was obtained as a white spongy solid by lyophilization (yield: 85.2%).

2.3. Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AV 400 NMR spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6) or deuterium oxide (D_2O). Fourier transform infrared (FT-IR) spectra were recorded on a Bio-Rad Win-IR instrument using the potassium bromide (KBr) method. Inductively coupled plasma mass spectrometry (ICP-MS, Xseries II, Thermoscientific, USA) was used for quantitative determination of boron in the nanogel. Transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-1011 transmission electron microscope with an accelerating voltage of 100 kV. To prepare the TEM sample, a small drop of the nanogel in phosphate-buffered saline (PBS, pH 7.4, 0.01 M) was deposited onto a 230 mesh copper grid coated with carbon, and allowed to dry at 25 °C before measurements. Dynamic laser scattering (DLS) measurements were performed on a WyattQELS instrument with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology) and 90° collecting optics. All samples were prepared in aqueous solution at a concentration of 0.50 mg ml^{-1} and measurements were carried out at 25 °C. Each sample was kept in the thermostat of the apparatus for 30 min to reach equilibrium prior to measurement.

2.4. In vitro drug loading and release

ARS was used as a model molecule to study the glucose-sensitive drug release profiles. The complexation of nanogel with ARS was performed in PBS at room temperature. Briefly, 10.0 mg



Scheme 1. Structures of the nanogel and glucose-sensitive behavior of ARS-loaded nanogel in PBS.