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Glucose-responsive insulin delivery microhydrogels from methacrylated dextran/concanavalin A: Preparation and in vitro release study

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ABSTRACT

Glucose-responsive systems are very important for self-regulated insulin delivery. The aim of the present study was to evaluate the potential of insulin loaded microhydrogels fabricated from methacrylate derivatives of dextran (Dex-G) and concanavalin A (Con A-E) as a insulin delivery system releasing insulin in response to different glucose levels. Insulin-loaded microhydrogels were prepared through a reversed-phase emulsion crosslinking method. The morphology and size of obtained microhydrogels were characterized by SEM, fluorescence microscope and dynamic light scattering, which showed that these microhydrogels were formed with sphere-like shape and diameters less than 5 μm. In vitro release of insulin from these microhydrogels and release kinetics were studied. The results indicated that insulin release was reversible in response to different glucose concentrations and the released insulin was shown to remain active since the tertiary structure was not destroyed. The degree of substitution (DS) of dextran methacrylate derivatives had effects on the release rate and surface burst release of the microhydrogels and high DS of Dex-G (DS 32) restricted the glucose sensitivity of the microhydrogels. The MTT assay from L929 cell line indicated that these microhydrogels possessed noncytotoxicity. The results suggested that these microhydrogels might be suitable for self-regulated insulin delivery and find potential applications in biomedical fields.

1. Introduction

Self-regulated insulin delivery systems are significant for the treatment of insulin-dependent diabetes mellitus to control the blood glucose level, in which insulin can be released in response to glucose concentrations in the blood (Steil, Panteleon, & Rebrin, 2004). Glucose-responsive carriers which can exhibit swelling changes in response to different glucose concentrations are very useful for the development of self-regulated insulin delivery systems (Kim, Kim, Jeon, Kwon, & Park, 2009; Kost & Langer, 2001; Miyata, Uragami, & Nakamae, 2002; Motornov, Roiter, Tokarev, & Minko, 2010; Qiu & Park, 2001; Roy, Cambre, & Sumerlin, 2010). Four types of glucose-sensitive systems have been intensively investigated, which are based on of glucose oxidase, concanavalin A (Con A), phenylboronic acid and glucose binding protein (Che, Liu, Huang, Wang, & Xu, 2008; Ding, Guan, Zhang, & Zhu, 2009; Gordijo, Shuhendler, & Wu, 2010; Jason et al., 2009; Jin et al., 2009; Qi et al., 2009).

Concanavalin A, a saccharide-binding lectin from jack bean, shows reversible strong affinity for non-reducing α-D-mannose, α-D-glucose, N-acetyl-D-glucosamine, polysaccharide and glycopolymers with unmodified hydroxyl groups at C-3, C-4 and C-6 in pyranose ring. The reactivity of Con A with non-reducing α-D-mannose and α-D-glucose is stronger than that of other ring forms (Brownlee & Cerami, 1979; Gerald et al., 1972). The specific saccharide-binding property of Con A makes it capable of causing affinity gelation of polysaccharide or glucose moieties containing polymer. Free glucose can interact with the specific binding sites of Con A-polymer complex leading to the dissociation of the complex thus forming glucose-sensitive systems (Kim & Park, 2001). Obaidat and Park (1997), Ballerstadt and Schultz (1998), and You, Lu, Li, Zhang, and Li (2002) have used dextran and synthetic polymers containing terminal or pendant glucose moieties to react with Con A, thus forming glucose-responsive hydrogels.

However, this system is vulnerable to component loss, especially Con A loss, which could lead to weak glucose sensitivity and undesirable biocompatibility. Therefore, it is necessary to develop an efficiently crosslinked network and covalently immobilize Con

Abbreviations: Con A, concanavalin A; EGAMA, ethylene glycol acrylate methacrylate; Con A-E, ethylene glycol acrylate methacrylate modified Con A; GMA, glycidyl methacrylate; Dex-G, glycidyl methacrylate modified dextran; PBS, phosphate buffer solution; PEGDMA, polyethylene glycol diamethacrylate; TMS, tetramethylsilane.

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A to the polymer matrix. Some researches have developed Con A covalent-binding hydrogels by using carbodiimide reaction, ring-opening reaction and Schiff base reaction (Tanna, Sahota, Sawicka, & Taylor, 2006; Tanna, Taylor, Sahota, & Sawicka, 2006; Taylor, Tanna, Sahota, & Voermans, 2006; Zhang, Tang, Bowyer, Eisenthal, & Hubble, 2006) which restricted the loss of Con A and improved the glucose-responsive properties to a certain extent. Hence, in our previous study, we synthesized methacrylate derivatives of dextran (Dex-G) and concanavalin A (Con A-E) through the ring-opening reaction of dextran and glycidyl methacrylate (GMA) and Michael addition reaction of concanavalin A and ethylene glycol acrylate methacrylate (EGAMA), respectively, thus provided an available method to obtain an efficiently crosslinked network which covalently immobilized Con A to the polymer matrix (Yin, Wang, Han, & Nie, 2010). The obtained Con A-E from the previous mild synthetic condition and high reaction efficiency was proved to stay active and keep the property of reversible binding to saccharide.

In this study, in order to investigate the injectable administration of glucose-responsive systems for the requirement of self-regulated delivery, we fabricated glucose-responsive microhydrogels based on Dex-G and Con A-E through reversed-phase emulsion crosslinking method (Scheme 1). Dextran, composed of α-D-glucose residues, is polysaccharide with favorable biocompatibility which shows strong specific affinity to Con A, and has been used in various biomedical applications (Pescosolido et al., 2011; Sarmento, Ribeiro, Veiga, Ferreira, & Neufeld, 2007). Glycidyl methacrylate modification of dextran has been a complimentary method to develop immobilized dextran hydrogels with the objective of biomaterial applications (Chen et al., 2007; Lee, Boettiger, & Composto, 2008). Here, microhydrogels obtained from Dex-G with different substitution degrees (DS) were fabricated and characterized by FT-IR, SEM, fluorescence microscope and dynamic light scattering. In vitro insulin release in response to different glucose concentrations and the effect of DS of Dex-G on the release behavior of microhydrogels were investigated in detail. Release kinetics in different release mediums were studied by using an exponential model. The release rate and influence of surface burst were studied by using this exponential model. The activity of released insulin and the in vitro cytotoxicity of microhydrogles were also measured. These microhydrogels are very attractive in terms of self-regulated insulin delivery, as well as in other applications such as actuators and separation systems with sensitivity to glucose.

2. Experimental methods

2.1. Materials

Dextran ($\overline{M}_w$ 40 kDa) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Concanavalin A (Con A; type IV, extracted from Jack Bean, $\overline{M}_w$ 102 kDa, BR) and D-glucose anhydrous (AR) were purchased from Yuanju Bio-Tech Co., Ltd. (Shanghai, China). Insulin (bovine pancreas, >27USP U/mg, Sigma I 5500) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was supplied by Sigma (USA). Polyethylene glycol (600) diamethacrylate (PEGDMA) were donated by Sartomer Company, Inc., USA, and used without further purification. Cyclohexane, Span 80, ammonium persulfate ($(NH_4)_2S_2O_8$), sodium sulfite (Na_2SO_3) and other reagents were all obtained from Beijing Chemical Agent Co. (Beijing, China). Mouse fibroblast cells (L929) were obtained from Department of Microbiology, Peking University Health Science Center.

Glycidyl methacrylate modified dextran (Dex-G) and ethylene glycol acrylate methacrylate modified concanavalin A (Con A-E) were synthesized and characterized in our previous study (Yin et al., 2010). Dex-G was synthesized according to the literature (Van Dijk-Wolthuis et al., 1995) with slight modification. Briefly, dextran and GMA were dissolved in DMSO and the reaction was catalyzed by N,N-dimethylamino pyridine and took place at 33 °C under nitrogen purge for 48 h. Con A-E was prepared through Michael addition reaction in phosphate buffer solution (PBS, pH 7.4) at room temperature, with EGAMA dissolved in a small amount of ethanol to form homogeneous solution.

2.2. Preparation of insulin-loaded microhydrogels

Insulin-loaded microhydrogels were prepared through reversed-phase emulsion crosslinking method according to the previous report with a slight modification (Karewicz et al., 2010). First, 0.1 g Dex-G was dissolved in 1 mL insulin solution (PBS, pH 7.4, 0.6 mg/mL); then the aqueous solution was mixed with 1 mL Con A-E solution (PBS, pH 7.0, 10 mg/mL, 0.1 M KCl, 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$, 6 h before utilization to allow the reactivation of the denatured protein by Ca^{2+} and Mn^{2+}) for 3 h; at last the cross-linker PEGDMA (1.25 wt%) was added to get a final clear solution.

The round-bottom flask (100 mL) was charged with 45 mL of cyclohexane to which 0.7 g of Span 80, an emulsion stabilizing agent, was added and the mixture was stirred for 10 min at about 750 rpm to assure complete dissolution of the stabilizer. Then the Dex-G/Con A-E/insulin/PEGDMA mixture was added dropwise to that solution and stirred at the same speed for another 20 min to obtain the milk-white emulsion. Subsequently, about 0.6 mL of $(NH_4)_2S_2O_8$ and Na_2SO_3 solution was added to initiate the crosslinking reaction of double bonds. The resulting mixture was stirred for 24 h to allow for microhydrogels hardening. Finally, after standing for 1 h, the mixture was allowed to remove the upper oil, followed by precipitated by isopropanol, centrifugated, and washed with water. At last, the obtained microhydrogels were freeze dried and stored at 4 °C before use. Insulin-loaded microhydrogels using different DS of Dex-G were prepared. The obtained microhydrogels had a theoretical loading content (the ratio of total amount of insulin to the collected amount of microhydrogels) of 0.54% (for DS 15), 0.51% (for DS 25) and 0.49% (for DS 32).

2.3. ^{1}H NMR spectra of Dex-G

The structure of Dex-G with different DS was investigated by ^{1}H nuclear magnetic resonance (NMR) with a Bruker AV600 unity spectrometer operated at 600 MHz, with D_2O as solvent and tetramethylsilane (TMS) as the internal standard. The DS of different Dex-G were calculated according to the peaks in ^{1}H NMR spectra.

2.4. FT-IR spectra of microhydrogels

Fourier transform infrared (FT-IR) spectra, recorded on the Nicolet 5700 instrument, were used to confirm the structure of microhydrogels obtained from Dex-G with different DS. Samples were prepared as KBr pellet and scanned against a blank KBr pellet background as wavenumber ranging from 4000 to 650 cm^{-1} with resolution of 4.0 cm^{-1}.

2.5. SEM measurements

The morphology of insulin-loaded microhydrogels after freeze-drying was visualized by a Hitachi S-4700 field-emission scanning electron microscope (SEM) at an accelerating voltage of 10 kV. Prior to the observation, specimen was fixed on stubs with sputter coated with gold.