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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

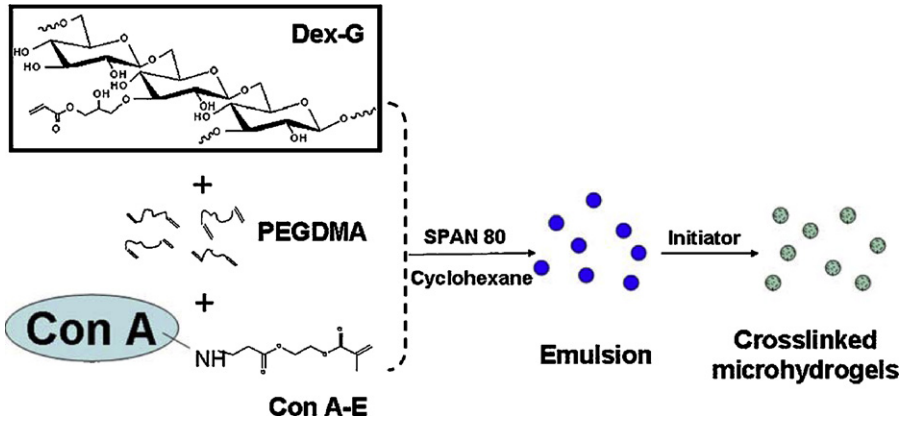
The structure of Dex-G with different DS was investigated by ^{1}H nuclear magnetic resonance (NMR) with a Bruker AV600 unity spectrometer operated at 600 MHz, with D_2O as solvent and tetramethylsilane (TMS) as the internal standard. The DS of different Dex-G were calculated according to the peaks in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



Scheme 1. A schematic presentation of the fabrication process of the blank microhydrogels.

2.6. Fluorescence microscope

Fluorescence images were measured by using an inverted microscope Olympus IX81 fitted with fluorescence microscope techniques. The samples were excited by UV light (345–385 nm).

2.7. DLS measurements

The dynamic light scattering (DLS) measurements were performed by using Brookhaven Instruments ZetaPALS/90plus. The samples were prepared in ethanol solution, filtered through a Chromafil filter (10 μm) and measured at room temperature.

2.8. In vitro release study

The weighed amount (40 mg) of the microhydrogels (DS of Dex-G was 15) with a theoretical loading amount of insulin 0.27 mg was placed in a centrifuge tube. Insulin release from insulin-loaded microhydrogels was analyzed by incubating these microhydrogels at 37 °C (±0.5 °C) in PBS (pH 7.4) while shaking (100 rpm) as a function of time and a stepwise change in glucose concentration (0, 4 and 10 mg/mL). Insulin release in response to step changes in glucose concentrations (0 and 10 mg/mL) in several alternated cycles was also tested. At appropriate time intervals, after centrifuging at 10,000 rpm for 1 min, the insulin concentration in the supernatant was measured by a Hitachi F-4500 fluorescence spectrophotometer at an excitation wavelength of 276 nm and an emission wavelength of 305 nm. The release study was continued after replacement with an equal volume of fresh solution to maintain a constant volume. The release data were expressed as mean ± standard deviation (SD) based on three independent measurements.

The release behaviors of insulin from different microhydrogels obtained from Dex-G with different DS were determined by incubating the microhydrogels (40 mg) at 37 °C (±0.5 °C) under shaking (100 rpm) in PBS solutions with glucose concentration of 0 and 10 mg/mL. The effect of DS of Dex-G on the release behavior was investigated.

2.9. Activity evaluation of released insulin

The activity of the released insulin was determined by analysis of the structure stability of released insulin by using fluorescence emission spectrum at an excitation wavelength of 276 nm and emission wavelength of 305 nm. The resulting spectrum was compared to standard insulin. The standard insulin solution was prepared in PBS (pH 7.4) to a final concentration of 0.01 mg/mL.

2.10. MTT assay

Mouse fibroblast cell line (L929) was cultured on a 96-well tissue culture plates (100 μL/well; Cellstar) at 1×10^5 cells/mL in DMEM (Dullecco's modified Eagle's medium; Sigma–Aldrich, USA) containing 10% fetal bovine serum (FBS, Sigma–Aldrich, USA), and then incubated at 37 °C in 5% CO_2 for 24 h. Cytotoxicity of the Dex-G/Con A-E microhydrogels was evaluated using MTT assay by an indirect extract method (ISO 10993-5) (Chen et al., 2007; Dev et al., 2010). Microhydrogels (20 mg) were incubated with 1 mL DMEM with agitation for 5 days for extraction. The extract was collected and was used for the cytotoxicity assay. For reference purposes, cells were seeded to medium containing 0.64% phenol (positive control) and a fresh culture medium (negative control) under the same seeding conditions, respectively. The presence of cytotoxic leachates in the extract was verified by MTT assay after incubating the cells with the extract for 24, 48 and 72 h. In the assay, fresh media containing 10% of MTT replaced the medium and the plate was incubated at 37 °C in CO_2 incubator for 4 h. After that, the unreacted dye was removed by aspiration. The produced formazan crystals were dissolved in DMSO (100 μL/well). The absorbance of the solution was measured in a microplate reader (Model 680, Bio-Rad) at a wavelength of 490 nm.

The cell viability (%) relative to negative control cells cultured was calculated from $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ and $[A]_{control}$ are the absorbance values of the wells (with the extract) and control wells (without the extract), respectively. For each sample, the final absorbance was the average value measured from six wells in parallel.

3. Results and discussion

3.1. Calculation of DS of Dex-G by ^1H NMR

Dex-G was achieved by ring-opening reaction of glycidyl methacrylate with dextran, and the structure was confirmed by FT-IR and ^1H NMR in our previous study (Yin et al., 2010). The ^1H NMR spectra of Dex-G were shown in Fig. 1A, and Fig. 1A(a)–(c) represented three kinds of Dex-G with different DS. In all these three spectra, the signal from the proton at the anomeric carbon of the α-1, 6 linkages (H^a, at 4.9 ppm) was well separated from the multiplet peaks from 3.3 to 4.0 ppm. The low-intensity signal at 5.3 ppm was assigned to the proton at the anomeric carbon of the α-1, 3 linkages ($H^{a'}$). From the ratio of the integrals of 5.3 and 4.9 ppm, on the average 4.0% of α-1, 3 linkages were calculated. The typical peaks from the methacryloyl group were observed at 1.95 ppm (methyl protons, H^b) as well as at 5.75 and 6.2 ppm (protons at the double bond, H^c), having an integral ratio of 3:2 as expected.

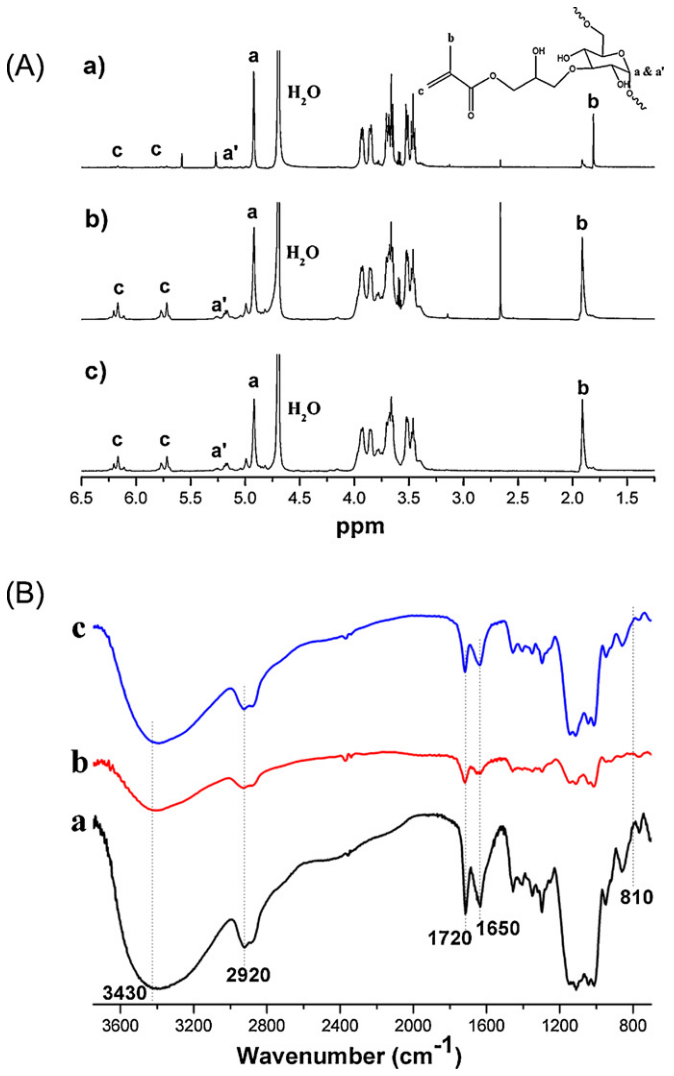


Fig. 1. ^{1}H NMR analysis of Dex-G structure with D_2O as solvent: (a)–(c) represent Dex-G with different DS (A); FT-IR spectra of the obtained microhydrogels from Dex-G with DS 15 (a), 25 (b) and 32 (c) (B).

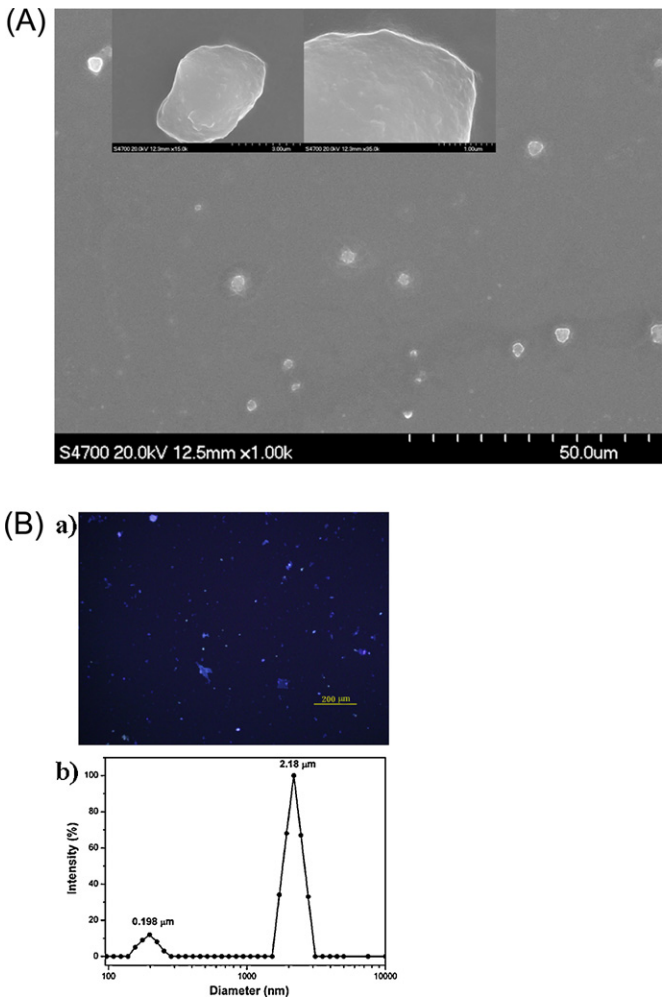


Fig. 2. Morphology of microhydrogels observed by SEM with lower and higher magnification after freeze-drying (A); fluorescence microscopy image of insulin-loaded microhydrogels (a) and particle size distribution from DLS analysis (b) (B).

According to the assignment of the ^{1}H NMR spectra, the DS of Dex-G was calculated as 100X/Y (Van Dijk-Wolthuis et al., 1995), in which X was the average peak area of the protons at the double bond, and Y was the integral of the anomeric proton at 4.9 ppm with addition of 4.0% of α-1, 3 linkages. The DS of (a), (b) and (c) were 15, 25 and 32 (the number of substituent per 100 rings), respectively.

3.2. FT-IR spectra of microhydrogels

The insulin-loaded microhydrogels were prepared through reversed-phase emulsion crosslinking method initiated by $(NH_4)_2S_2O_8$ and Na_2SO_3 in cyclohexane stabilized by Span 80. The obtained microhydrogels were analyzed by FT-IR (Fig. 1B). The spectra a, b and c represented microhydrogels prepared using Dex-G with DS of 15, 25 and 32, respectively. From the spectra a to c, we could see that there were no novel peaks and no significant peak shifts occurred in these microhydrogels. Typical characteristic peaks of the reactants, Dex-G, Con A-E, and PEGDMA, could be observed, such as 3430 cm^{-1} (O—H and N—H stretch), 2920 cm^{-1} (C—H stretch), 1720 cm^{-1} (C=O stretch of ester groups), 1650 cm^{-1} (C=O stretch of amide groups). But the peak originating from the methacrylate double bond (810 cm^{-1}) disappeared after polymerization, indicating that the microhydrogels were successfully cross-linked.

3.3. Morphology and size of microhydrogels

The shape, morphology and size of microhydrogels obtained from Dex-G with DS 15 as typical examples, were analyzed by SEM, fluorescence microscope and DLS. The SEM image of freeze-dried microhydrogels was presented in Fig. 2A. The microhydrogels showed a sphere-like shape, a slightly rough surface morphology free of cracks. Fig. 2B(a) was the fluorescence image of insulin-loaded microhydrogels after freeze drying, indicating that the microhydrogels could be relative evenly dispersed with a spot of aggregation in the medium of PBS (pH 7.4). Since the observed fluorescence emission from the fluorescence image could be attributed to loaded insulin, it could be found that insulin was almost homogeneously distributed in the microhydrogels. The size distribution of freeze-dried microhydrogels in ethanol was shown in Fig. 2B(b). It could be seen from DLS data that the microhydrogels diameters had two peaks at 0.198 and 2.18 μm with a mean value of about 1.7 μm, which was nearly consistent with the SEM and fluorescence images.

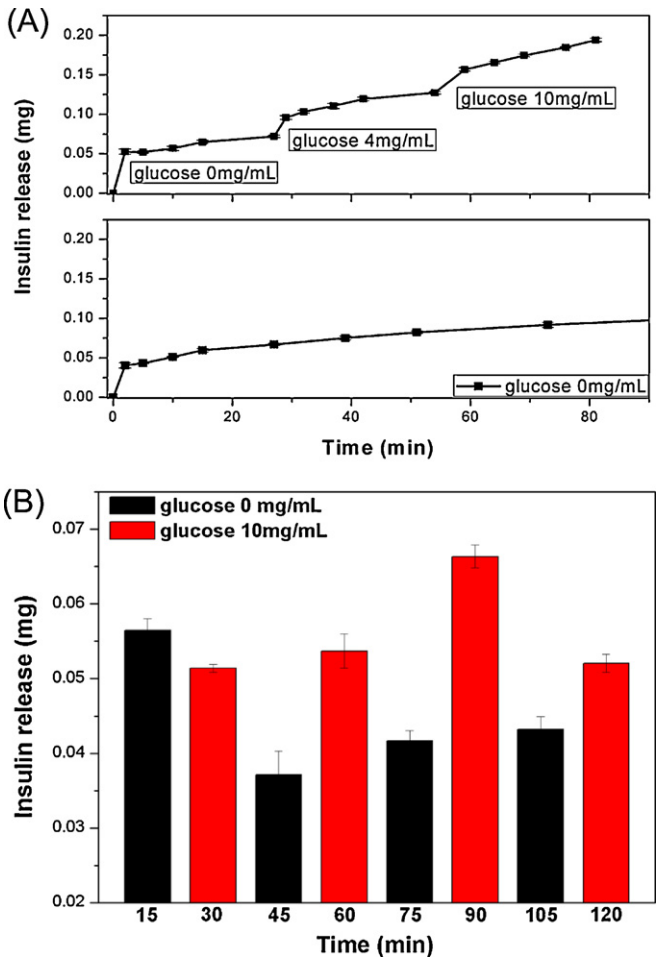


Fig. 3. Insulin release in response to change of stepwise glucose concentration (upper) and in the medium without glucose (lower) (A); insulin release in response to an abrupt change in glucose concentration in four alternated cycles (B).

3.4. In vitro release study

Insulin release from microhydrogels (DS of Dex-G was 15) in response to a stepwise change in glucose concentration (0, 4, 10 mg/mL) was revealed in Fig. 3A. With the increase of glucose concentration, the cumulate release amount of insulin increased and had a dramatic change at each moment of replacing glucose concentration. Besides, compared to the release profile in glucose 0 mg/mL alone, the cumulate release amount of insulin in response to the stepwise change in glucose level was much higher, owing to the disconnection between the sugar-binding site of Con A-E and Dex-G, indicating the microhydrogels could effectively respond to different glucose levels. Fig. 3B showed insulin release in response to step changes in glucose concentrations (0 and 10 mg/mL) in four alternated cycles. The microhydrogels responded quickly to changes in glucose concentration. Except for the first 15 min, resulting from the burst release of surface, the amount of released insulin increased with increasing glucose concentration from 0 to 10 mg/mL and decreased when glucose was reduced to 0 mg/mL, owing to the reversible affinity of Con A-E and Dex-G, which indicated the reproducibility of the microhydrogels.

Fig. 4 displayed the comparison of insulin release profiles in the medium of glucose 0 and 10 mg/mL with different DS of Dex-G: (a) 15, (b) 25, (c) 32. Increasing of the DS of Dex-G allowed more permanent covalent cross-links, making it more difficult for the polymer chains to move along each other, which could prevent the loss of active components, but inversely reduced the specific binding sites of Dex-G to Con A-E, thus decreased the glucose sensitivity of the microhydrogels. When the DS of Dex-G was 32, there was no obvious difference between insulin release profiles in the medium of glucose 0 and 10 mg/mL, on account of the specific affinity of Con A-E and Dex-G became weak and predominant force of insulin release was water-absorbing swelling of microhydrogels.

3.5. Release kinetics studies

Release kinetics of insulin from the microhydrogels with different DS of Dex-G in release mediums of different glucose concentrations (0 and 10 mg/mL) were studied. To investigate the release rate, the release data (the percentage of the cumulative release amount shown in Fig. 4 over the theoretical total amount of encapsulated) were fitted to an exponential model as the following equation (Zhang et al., 2009):

$$y = Ae^{-Bx} \quad (1)$$

where y is the insulin retention in the microhydrogels, A and B are two constants, and x is the time. The values of parameters were listed in Table 1. According to the parameter r^2, the release data fitted well to the exponential model when the surface effect was excluded, and the release behavior of microhydrogels obtained from Dex-G with DS 25 was most significantly influenced by the surface burst release among all the samples. According to the characters of exponential function, the larger the constant B was, the faster the release rate was. From Table 1, it could be found that the constant B of microhydrogels obtained from Dex-G with DS 25 was the smallest, indicating the slowest release rate from the microhydrogels obtained from Dex-G with DS 25 owing to the most tightly combined physical and chemical crosslinkage.

3.6. Activity of released insulin

Fluorescence spectrophotometer was used to evaluate the conformational change of tertiary structure of insulin (Amidi et al., 2008; Murali & Jayakumar, 2005). Fig. 5 showed the fluorescence spectra of released and standard insulin. The two formulations displayed similar peaks with an emission maximum at 305 nm, indicating that the tertiary structure of insulin was not distorted. It

Table 1
Effect of DS of Dex-G, medium glucose concentration, and surface burst release on parameters of the kinetic model for the release rate.

DS of Dex-G	With surface effect[a]				Without surface effect[b]			
	Glucose (0 mg/mL)		Glucose (10 mg/mL)		Glucose (0 mg/mL)		Glucose (10 mg/mL)	
	B	r^2	B	r^2	B	r^2	B	r^2
15	0.0058	0.83	0.0063	0.78	0.0055	0.97	0.0052	0.95
25	0.0027	0.47	0.0032	0.49	0.0017	0.91	0.0018	0.92
32	0.0079	0.75	0.0085	0.80	0.0065	0.93	0.0071	0.95

[a] Experimental data from 0 min are used for fitting the kinetic model.
[b] Experimental data from 2 min are used for fitting the kinetic model.

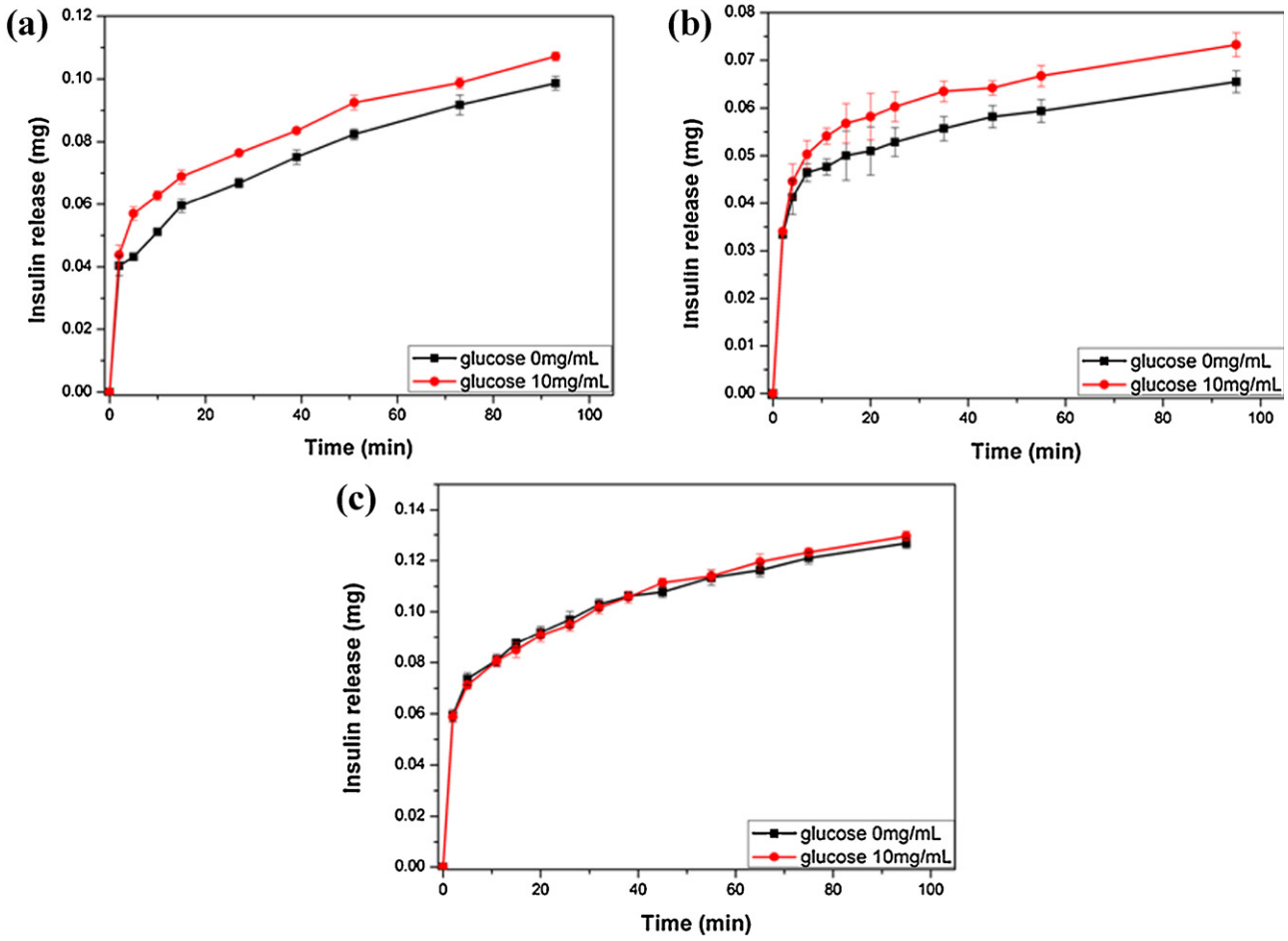


Fig. 4. Insulin release in the medium of glucose 0 and 10 mg/mL from microhydrogels formed with different DS of Dex-G: (a) 15, (b) 25 and (c) 32.

could be inferred that the insulin released from the microhydrogels stayed active.

3.7. *In vitro cytotoxicity study*

The potential applications of the Dex-G/Con A-E microhydrogels in biomedical fields were assessed by investigating the in vitro cytotoxicity using MTT assay. Mouse fibroblast cells (L929) were used as model cells. Experimental groups (microhydrogels with different DS of Dex-G) and control groups (a fresh culture medium without extract as negative control and medium containing 0.64% phenol as positive control) were studied simultaneously under the same seeding conditions. The viability of L929 culture for 24, 48 and 72 h was illustrated in Fig. 6. It could be seen that no statistically

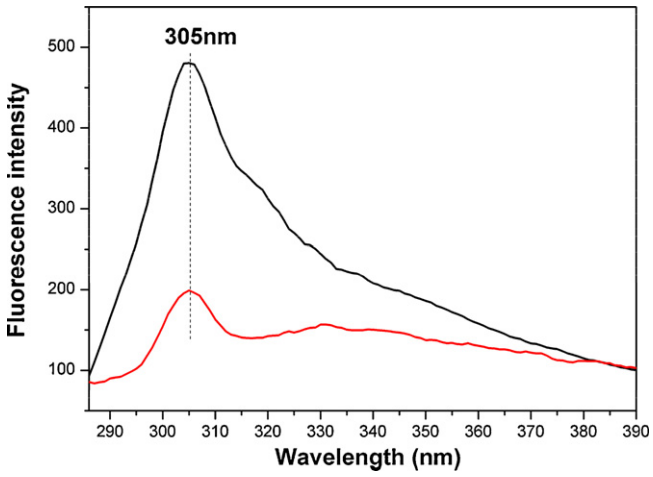


Fig. 5. Fluorescence spectra of released insulin and standard insulin.

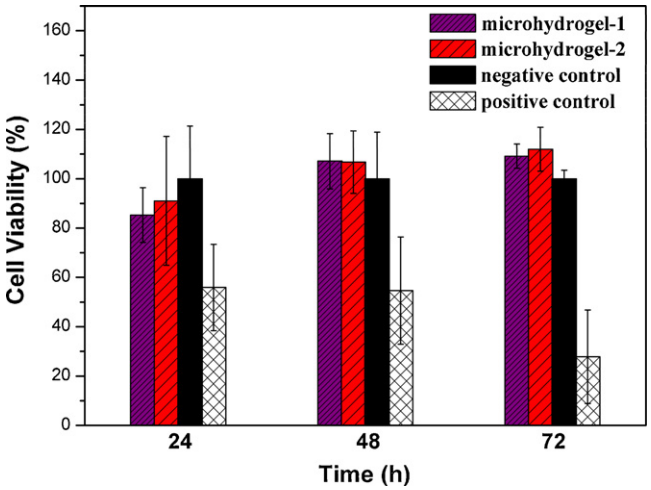


Fig. 6. In vitro cytotoxicity evaluation of methacrylated dextran/concanavalin A microhydrogels (DS of Dex-G was 15 and 25 for microhydrogel-1 and michydrogel-2, respectively) with negative and positive controls ($p < 0.05$) $^{*}p < 0.05$ when compared to the negative control of indirect cytotoxicity. The data represented mean and standard deviations of six samples.

significant differences were observed in the cell viability of L929 culture for 24 and 48 h in the presence of Dex-G/Con A-E microhydrogels extracts in comparison with negative control, although the values were lower than that of the negative control condition for 24 h. However, statistically significant differences ($p < 0.05$) were observed in the cell viability for 72 h, but the viability of cells in the presence of extracts was higher than that of the negative control, indicating there was no toxicity in the extract of microhydrogels with different DS (15 and 25) of Dex-G, which contained possible leachable and degradation products (Chen et al., 2007). The obtained results suggested that that these Dex-G/Con A-E microhydrogels were nontoxic to L929 cells and were good candidates to be used as drug carriers.

4. Conclusions

Glucose-responsive microhydrogels based on Dex-G and Con A-E have been fabricated, characterized and used for self-regulated insulin delivery. Insulin-loaded microhydrogels were prepared through reversed-phase emulsion crosslinking method. SEM, fluorescence microscope and dynamic light scattering analysis displayed that these microhydrogels were formed with sphere-like shape and diameters less than 5 μm. In vitro release study indicated that insulin release was reversibly in response to different glucose concentrations and the glucose sensitivity of microhydrogels became less significant with increase of the DS of Dex-G, extremely weak when the DS reached to 32. The release rate and surface burst release were influenced by DS of Dex-G. The released insulin was proved to remain active without destroyed the tertiary structure. In vitro cytotoxicity assessment of microhydrogels with L929 indicated that the material showed no cytotoxicity toward growth of L929 cells and had good in vitro biocompatibility. These result suggested that this microhydrogels might be a promising system for self-regulated insulin delivery as well as other applications such as actuators and separation systems with sensitivity to glucose.

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