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Photo-crosslinked glucose-sensitive hydrogels based on methacrylate modified dextran–concanavalin A and PEG dimethacrylate

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ABSTRACT

A series of glucose-sensitive hydrogels based on glycidyl methacrylate modified dextran (Dex-G), ethylene glycol acrylate methacrylate modified concanavalin A (Con A–E) and poly (ethylene glycol) dimethacrylate (PEGDMA) were synthesized by photopolymerization. The Dex-G precursor was prepared through ring-opening reaction, while Con A–E was obtained from Michael addition reaction. FT-IR was used to characterize the structures of pre-polymers and hydrogels. The degree of substitution (DS) of Dex-G was confirmed by ^{1}H NMR and the activity of modified Con A was approved by fluorescence spectroscopy. Swelling test, component loss measurement and SEM observation demonstrated that the size of hydrogels changed differently in the medium of different glucose concentrations and the glucose sensitivity was influenced by the content of component, especially PEGDMA. SEM image also displayed that hydrogels had microporous structures. All the results indicated that the hydrogels had glucose-sensitive property and good biocompatibility, which could be prospectively applied as glucose biosensor and intelligent insulin delivery carrier.

1. Introduction

Stimuli-sensitive hydrogels, also known as "smart" or "intelligent" materials, which can sense environmental changes and induce structural changes by themselves, have attracted considerable attention in the biomedical and biochemical areas (Miyata, Uragami, & Nakamae, 2002). Glucose-responsive hydrogels, which exhibit swelling changes in response to glucose concentration, are very useful for applications such as biosensing, microfluidics and bio-microelectromechanical systems, as well as implantable drug delivery systems for diabetes management applications (Kost & Langer, 2001; Qiu & Park, 2001; Robert & Nicholas, 2003). Four types of glucose-sensitive hydrogels have been intensively investigated, which are on the basis of glucose oxidase (Qi et al., 2009), concanavalin A (Che, Liu, Huang, Wang, & Xu, 2008; Kim & Park, 2001), phenylboronic acid (Jin et al., 2009; Matsumoto, Ikeda, Harada, & Kataoka, 2003) and glucose binding protein (Jason et al., 2009).

Concanavalin A (Con A), a sugar-binding lectin from jack bean, shows reversible strong affinity for non-reducing α-D-mannose, α-D-glucose, N-acetyl-D-glucosamine and polysaccharide as dextran with unmodified hydroxyl groups at C-3, C-4 and C-6, and the competitiveness of these substrates decrease following the sequence mentioned above, which makes it useful for fabricating glucose-sensitive systems (Kim & Park, 2001). Many researches, such as Obaidat and Park (1997), Ballerstadt and Schultz (1998), and You, Lu, Li, Zhang and Li (2002), have reported Con A reacted with polymers containing terminal or pendant glucose moieties to form glucose-sensitive hydrogels. However, these hydrogels are vulnerable to component loss, especially Con A loss, which could lead to weak glucose sensitivity and undesirable biocompatibility. Therefore, it is necessary to develop efficient crosslinking hydrogels and covalently immobilize Con A to polymer matrix. Some investigations have developed Con A covalent-binding gels by using carbodiimide reaction, ring-opening reaction and Schiff base reaction (Ladmiral et al., 2006; Tanna, Joan Taylor, Sahota, & Sawicka, 2006; Tanna, Sahota, Clark, & Taylor, 2002; Tanna, Sahota, Sawicka, & Taylor, 2006; Zhang, Tang, Bowyer, Eisenthal, & Hubble, 2006). Hydrogels obtained from these approaches have been shown to resist component loss and maintained glucose sensitivity to a certain extent. But available modification of protein calls for mild synthetic condition and high reaction efficiency. More efficient way to modify Con A and crosslink hydrogel components should be investigated.

Hence, here we introduced Michael addition reaction of Con A and ethylene glycol acrylate methacrylate (EGAMA) at room temperature to covalently bind Con A efficiently, then form hydrogel matrix with glycidyl methacrylate modified dextran (Dex-G) and the crosslinker poly (ethylene glycol) dimethacrylate (PEGDMA)

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by using photopolymerization. Dextran, composed of α-D-glucose residues, is polysaccharide with favorable biocompatibility and has been used in various biomedical applications (Sarmento, Ribeiro, Veiga, & Ferreira, 2006). Glycidyl methacrylate modification of dextran has been a complimentary method to develop immobilized dextran hydrogels with the objective of biomaterial applications (Lee, Boettiger, & Composto, 2008). PEGDMA is an unsaturated linear polyether with methacrylate double bonds that can be crosslinked by photopolymerization. Cross-linked PEGDMA, which have been successfully used by several groups both in vitro and in vivo, have been shown to be biocompatible (Lin-Gibson et al., 2004; Zhou, Yang, Nie, Ren, & Cui, 2009).

In this study, hydrogels with glucose-sensitive property and improved biocompatibility on the basis of methacrylate derivatives of dextran and Con A were fabricated. We first prepared the precursors of Dex-G and Con A–E, followed by cross-linking hydrogels through UV-polymerization. FT-IR was recorded to assess the structures of pre-polymers and hydrogels. The degree of substitution (DS) of Dex-G was confirmed by ^{1}H NMR and the activity of modified Con A was approved by fluorescence spectroscopy. Swelling test, component loss measurement and SEM were used to study the glucose sensitivity, the effect of hydrogel compositions, the advantage of Con A modification, and the microstructures of different hydrogels.

2. Methods

2.1. Materials

Dextran ($\bar{M}_w$ 40 kDa) was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Concanavalin A (Con A; type IV, extracted from Jack Bean, $\bar{M}_w$ 102 kDa, BR) and D-glucose anhydrouse (AR) were purchased from Yuanju Bio-Tech Co., Ltd (Shanghai, China). Acryloyl chloride was supplied by Shanghai Chemical Reagents Company (China). 2-Hydroxyethyl methacrylate (HEMA) was purchased from Beijing Chemical Reagents Company (China), distilled under reduced pressure and stored at 4 °C until use. Chloroform and triethylamine were also obtained from Beijing Chemical Reagent Company (China). 4-(N,N-dimethylamino) pyridine (DMAP, 99%) was purchased from J&K chemical, Ltd., Beijing, China. Glycidyl methacrylate (GMA) and polyethylene glycol (600) diamethacrylate (PEGDMA) were donated by Sartomer Company, Inc., USA, and used without further purification. The photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacures 2959) was offered by Ciba-Geigy Chemical Co. (Switzerland). Other reagents were all obtained from Beijing Chemical Agent Co. (Beijing, China) and used as received.

2.2. Synthesis of methacrylated dextran

Methacrylated dextran (Dex-G), was synthesized according to the literature (van Dijk-Wolthuis et al., 1995) with slight modification. Dextran (5.0 g) was dissolved in DMSO (50 mL) in a round bottom flask under nitrogen purge, followed by adding DMAP (1.0 g) and GMA (1.0 g) to get a clear solution. The reaction took place at 33 °C under stirring for 48 h, after which it was stopped by adding HCl to neutralize the DMAP. Subsequently, the solution was poured into 200 mL of ethanol to remove the unreacted reactant, filtrated to collect solid, then dialyzed against distilled water at 4 °C for 4 days. The product Dex-G was freeze-dried and stored at −20 °C before use with yield of 80%.

2.3. Synthesis of methacrylated Con A

Ethylene glycol acrylate methacrylate (EGAMA) was first prepared according to the previously reported method (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Briefly, 105 mL of triethylamine and 83.81 g of HEMA were dissolved in 250 mL of toluene in a three-necked flack equipped with stirrer, thermometer and dropping funnel, under cooling. 79.79 g of acryloyl chloride dissolved in 50 mL of toluene was dropped into the mixture within 4 h. After dropping, the mixture was allowed to stir for another 4 h and then stand overnight. The obtained solution was filtered and then the filtered liquid was extracted three times with 1 mol/L HCl, 1 mol/L $NaHCO_3$ solution and deionized water, respectively, then anhydrous Na_2SO_4 was added overnight to get rid of residual water. In the end, toluene was removed by rotary evaporation and the obtained yellow liquid was dried under vacuum at room temperature, then purified by silica gel column chromatography. The yield was up to 70%.

Methacrylated Con A (Con A–E), was then synthesized through Michael addition reaction. 0.5 g of Con A was dissolved in 200 mL phosphate buffer solution (PBS, pH 7.4). 0.15 g of EGAMA dissolved in 1 mL ethanol was then dropped into the solution and stirred for 36 h at room temperature. After that, the solution was extensively dialyzed against distilled water at 4 °C for 5 days and freeze-dried to get pure Con A–E with yield of 66%. The product was stored at −20 °C before use.

2.4. Preparation of photo-crosslinked hydrogel

The hydrogel was prepared by first mixing Dex-G with Con A–E solution (PBS, pH 7.0, 0.1 M KCl, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, 6 h before utilization to allow the reactivation of the denatured lectin by Ca^{2+} and Mn^{2+}) for 3 h, then adding PEGDMA and photoinitiator I 2959 (0.1 wt%) to get a final mixture. After mild stir and ultrasonication, the mixture was transferred into a flat mold consisting of two glass plates (25 mm × 25 mm) separated by a 2-mm thick spacer (diameter 12 mm), and then exposed to ultraviolet light (365 nm, EXFO lite, EFOS Corporation, Mississauga, Canada) at intensity of 30 mW/cm^2 for 3 min. Subsequently, the resultant hydrogels were washed with distilled water to remove the unreacted components, then freeze dried and stored at 4 °C prior to use.

The final concentration of reactant in the mixtures was about 12 wt% for all polymerized mixtures, and hydrogels with different weight ratio of Dex-G, Con A–E and PEGDMA were synthesized here, which was listed in Table 1. The hydrogel using unmodified

Table 1
Weight ratio of reactant and glucose-sensitive swelling behaviors of hydrogels.

Sample code	Dex-G/Con A–E (weight ratio)	PEGDMA/(Dex-G + Con A–E) (weight ratio)	ESR[b] (in glucose 0 mg/mL)	ESR[b] (in glucose 4 mg/mL)	T_{ESR} (min)	RI
Hydrogel-1	10/3	1/10	4.22	3.64	3	−0.58
Hydrogel-2	10/10	1/10	4.56	3.83	2	−0.73
Hydrogel-3	10/1	2.5/10	3.51	3.56	15	0.05
Hydrogel-4	10/5	2.5/10	4.58	4.79	20	0.21
Hydrogel-5	10/10	2.5/10	4.36	4.67	25	0.31
Control sample[a]	10/10	2.5/10	4.23	4.58	7	0.35

[a] Control sample used unmodified Con A instead of Con A–E.
[b] ESR of hydrogel-1, hydrogel-2 and control sample were maximum swelling ratio, since these three could not achieve equilibrium owing to component loss.