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Heparin-mimetic polyurethane hydrogels with anticoagulant, tunable mechanical property and controllable drug releasing behavior

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ABSTRACT

In the present study, novel heparin-mimetic polyurethane hydrogels were prepared by introducing chemical crosslinked sulfated konjac glucomannan (SKGM). Scanning electron microscopy (SEM) results indicated that the introduction of SKGM and the increase of the molecular weight of diol segments could enlarge the pore sizes of the hydrogels. The swelling behavior corresponded with the SEM results, and the hydrogels could absorb more water after the modification. The modification also led to an improvement in the mechanical property. Meanwhile, the SKGM and the modified polyurethane hydrogels showed excellent hemocompatibility. The thromboplastin time of SKGM could reach up to 182.9 s. Gentamycin sulfate (GS) was used as a model drug to be loaded into the hydrogels, and the loading amount was increased ca. 50% after the introduction of SKGM, thus resulting in high bactericidal efficiency. The results indicated that the introduction of SKGM and the alternation in the diol's molecular weight bestowed polyurethane hydrogels with promising properties of integrated blood-compatibility, mechanical properties and drug loading-releasing behavior. Therefore, the heparin-mimetic multifunctional polyurethane hydrogels have great potential to be used in biomedical applications.

1. Introduction

Polyurethanes (PUs) are materials composed of alternated hard and soft blocks, the microphase separation of which may enhance their thermal and mechanical properties [1]. The hard segment is mainly comprised of diisocyanates and chain extenders while the soft one is generally comprised of polyester, polyether, or polycarbonate diol [2]. The synthesis routine of PU materials is flexible and the properties of PU can be well-controlled under the adjustment of the structure of monomers and other reactants. Because of their versatile chemistry, millions of materials have been fabricated based on PUs [3]. In modern life, PUs have demonstrated their brilliance in biomedical devices [4], due to their mechanical properties and biocompatibility [5]. Recently, they were studied as materials for the immobilization of cells [6], vascular grafts [7] and etc [8]. When constructing polyurethanes, poly(ethylene glycol) (PEG) is the commonly used diol because it can increase hydrophilicity, reduce protein adsorption [9] and prevent the adhesion of endothelial cell (EC) [10]. Meanwhile, PEGs with different molecular weights can provide the synthesized PUs with viable properties.

Urethane polymers have been widely studied as foams [11], coatings [12], adhesives [13], elastomers [14], fibers [15] and etc. Among them, PU hydrogels are in vast applications in biomedical fields [16,17]. Typically, they can be made into soft contact lens [18], soft tissues substitute [19] and wound dressing [20]. Compared to other hydrogels, the PU hydrogels have remarkable strength and elasticity [21], pore capacity [22] and biodegradability [23]. In addition, these properties can be substantially enhanced or even tunable after modification [24]. Meanwhile, the difference in the design of polyurethane segments can bring new properties like the responses to different stimuli [25,26] and bacteria repulsion [27]. However, few researches have reported the modification of polyurethane hydrogels in their functions as blood-contacting materials.

Blood-contacting materials are one kind of biomedical materials widely used in clinical field, which require good hemocompatibil-

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ity, including anticoagulant property [28]. Heparin, a commonly used injectable anticoagulant reagent in clinical practice, plays the anticoagulant role by interacting with the factors XIa, IXa, Xa and IIa (thrombin), especially Xa in the blood clotting cascade. These factors may bind to the heparin polymer at a site proximal to the pentasaccharde because of the strong electrostatic interactions [29]. Recently, the hydrogels based on heparin or modified heparin have been increasingly investigated. However, heparin is a relatively high-cost bio-macromolecule, which may restrict the scalable production of heparinized hydrogels. Konjac glucomannan (KGM) is a kind of polysaccharides extracted from natural plants [30,31]. KGM and its derivatives are highly biocompatible [32]. They possess a similar glycan structure as heparin. Compared with heparin, whose main source is animal, the polysaccharide from plants will largely reduce the cost if it could be modified to mimic the structure of heparin. Researchers have taken efforts to introduce sulfate groups into KGM [33–35]. After sulfation, the anticoagulant property of KGM was improved and it could bring anti-HIV property to the molecules, and the effect was almost as high as that of the AIDS drug [33].

In this study, a heparin-mimetic compound was synthesized by introducing sodium sulfate (-OSO_3Na) into KGM using sulfamic acid. The sulfated konjac glucommannan (SKGM) was introduced into polyurethane hydrogels with different diol segments. The anticoagulant property, swelling ability, mechanical performance and drug loading behavior were investigated. Gentamycin sulfate (GS), an antimicrobial drug, was chosen as the model drug for the drug loading and releasing experiments. The antibacterial activities of the drug-loaded polyurethane hydrogels were also determined.

2. Materials and methods

2.1. Materials

N, N-dimethylformamide (DMF) (99.0 wt.%) and ethanol (99.0 wt.%) were derived from Chengdu Kelong Inc. (China). Sulfamic acid, heparin sodium salt (Hep), polyethylene glycol (M_W = 1000 Da, PEG1000; M_W = 2000 Da, PEG2000), diethylene glycol (DEG), 2,2′-dimethylol propionic acid (DMPA), 1,6-diisocyanatohexane (HDI), dibutyltin dilaurate (DBTDL), triethanolamine (TEA) and gentamycin sulfate (GS) were purchased from Aladdin reagent Co. Ltd. (China). Dialysis membranes (M_W = 3500 Da) and KGM ($M_W \approx$ 200,000 to 2000,000, 99.0 wt.%) were obtained from authentic Chinese producers. De-ionized (D.I.) water was used in the experiment as solvent in aqueous solution.

2.2. Synthesis of SKGM

KGM and sulfamic acid were dissolved in DMF, and maintained at 80 °C for 4 h. After cooling to room temperature, the mixture was filtered and the solution was added into alcohol (three times of the solution volume) dropwise. After the second filtration, the precipitation was immersed into a solution consisting of NaOH (4 wt.%), D.I. water (10 wt.%) and alcohol (86 wt.%), and then the whole system was stirred at room temperature for 24 h, followed by the third filtration. The pellet was redissolved in D.I. water and dialyzed against water for three days (refreshing the water every 4 h, and changing the water into alcohol as outer atmosphere if the dialysis membranes were full of water). Then, the resulting product was obtained after freezing-dried.

53.9 g and 36.0 g sulfamic acid were reacted with 10 g KGM to produce SKGMs with two different degrees of substitution, respectively. The SKGM with a high degree of substitution was named as HSSK, and the lower one was named as LSSK.

2.3. Preparation of hydrogels

The hydrogels were prepared by using SKGM, HDI and diols. Before the preparation, the water in the reagents was eliminated to prevent side reaction with HDI. SKGM was firstly dissolved in DMF in a glass mold (φ 60 × 15 mm height); after the addition of HDI, diol, TEA and DBTDL (details are shown in Table 1), the solution was degassed and kept at 60 °C for 24 h. After the completion of the reaction, the resulting hydrogel was removed from the glass mold and immersed in D.I. water at ambient temperature for 7 days. The water was refreshed four times a day to guarantee the removal of the unreacted monomer, soluble polymer and catalyst. Finally, the hydrogel was washed with phosphate buffered saline (PBS pH 7.4) and lyophilized.

2.4. Characterization of SKGM

Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet-560 spectrophotometer (Nicol, US) between 4000 and 500 cm^{-1} (the resolution was 2 cm^{-1}). ^{13}C NMR data were obtained with a Bruker spectrometer (600 MHz; Bruker, Germany).

To determine the content of carbon (C), sulfur (S), hydrogen (H), and nitrogen (N), elemental analysis was performed by a Carlo Erba 1106 elemental analyzer (Italy) with carrier gas (He, at a flow rate of 100 mL/min) at a combustion temperature of 1000 °C with the solid samples. According to the results of elemental analysis, the DS (degree of substitution) was calculated as below:

$$DS = \frac{S\%}{32} \Big/ \frac{C\%}{12 \times 6} \quad (1)$$

where S% and C% stand for the percentage of sulfur and the percentage of carbon in total mass, respectively.

2.5. Characterization of hydrogels

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were obtained on a Nicolet-560 spectrophotometer between 4000 and 700 cm^{-1} with a resolution of 2 cm^{-1}.

The cross-sectional morphology of the hydrogels was observed by scanning electron microscopy (SEM, Hitachi, Japan). To prepare SEM samples, the hydrated hydrogels were sectioned after freezing. Then, the samples were attached to a support and coated with a gold layer. All the SEM images were taken at an accelerating voltage of 5 kV.

The swelling ratios of the hydrogels were measured by a gravimetric method [36,37]. The lyophilized hydrogels were cut into pieces, weighed and immersed in PBS (pH 7.4) at room temperature. The weights of the hydrogels after swelling at different time intervals were directly obtained after gently removing the excess water by filter papers. At least three repetitive measurements were

Table 1
Synthetic conditions for the hydrogels.[a]

Samples	SKGM (g)	HDI: Diols (g)	TEA (g)	DBTDL
B-PEG2000	0	0.93: 5.00	0.14	2 drops(ca. 0.08 g)
S-PEG2000	0.3	0.93: 5.00	0.14	2 drops
B-PEG1000	0	0.93: 2.50	0.14	2 drops
S-PEG1000	0.3	0.93: 2.50	0.14	2 drops
B-DEG	0	0.93: 0.27	0.14	2 drops
S-DEG	0.3	0.93: 0.27	0.14	2 drops
B-DMPA	0	0.93: 0.34	0.14	2 drops
S-DMPA	0.3	0.93: 0.34	0.14	2 drops

[a] The amount of DMF was 50 wt.% in the whole reaction.