Carbohydrate Polymers 105 (2014) 135–144

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and characterization of a novel semi-IPN hydrogel based on Salecan and poly(N,N-dimethylacrylamide-co-2-hydroxyethyl methacrylate)

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ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form
22 November 2013
Accepted 18 January 2014
Available online 4 February 2014

Keywords:
Salecan
Poly(DMAA-co-HEMA)
Semi-IPN hydrogels
Porous structure
Robust materials

ABSTRACT

Salecan is a novel water-soluble, high molecular mass extracellular β-glucan produced by *Agrobacterium* sp. ZX09. Salecan has excellent physicochemical and biological properties, making it very suitable for hydrogel preparation. In this study, a series of novel semi-interpenetrating polymer network (semi-IPN) hydrogels containing Salecan and poly(N,N-dimethylacrylamide-co-2-hydroxyethylmethacrylate) (poly(DMAA-co-HEMA)) were synthesized by radical polymerization and semi-IPN technology. Structure and morphology of the hydrogels were characterized by FTIR, XRD, TGA and SEM. The semi-IPNs had a well-interconnected porous structure with tunable pore size ranging from 6 to 41 μm. Swelling capability of the hydrogels was improved by introducing the hydrophilic Salecan. Rheological results indicated that the incorporation of poly(DMAA-co-HEMA) into hydrogels enhanced the storage modulus. Compression tests revealed that these semi-IPNs were robust materials with compressive modulus between 13.3 and 90.5 kPa, the addition of Salecan increased the fracture strain from 71.1% to 88.8%. Degradation and cytotoxicity tests demonstrated that semi-IPNs were degradable and non-toxic.

1. Introduction

Hydrogel are hydrophilic three-dimensional polymeric networks that are able to absorb and retain a large quantity of water within their structure. Because of the characteristic properties of water in the hydrogels, they have been utilized in a wide range of applications, such as controlled drug delivery (Dumitriu, Oprea, & Vasile, 2009; El-Sherbiny, 2010), tissue engineering (Ma et al., 2010; Tang et al., 2010), column packing materials for chromatography (Gölgelioğlu, Bayraktar, Celebi, Uğuzdoğan, & Tuncel, 2012), wastewater treatment (Aouada, Pan, Orts, & Mattoso, 2009; El-Sherbiny, Abdel-Hamid, Rashad, Ali, & Azab, 2013) and agriculture (Bortolin et al., 2012). Hydrogels can be obtained by crosslinking of both natural and fully synthetic hydrophilic polymers. In general, hydrogels prepared from natural polymers such as polysaccharides offer great advantages, including excellent biocompatibility, biodegradability and low toxicity (Kulkarni, Mangond, Mutalik, & Sa, 2011; Shalviri, Liu, Abdekhodaie, & Wu, 2010). However, many of these polysaccharide-based hydrogels possess poor mechanical properties (Huang, Onyeri, Siewe, Moshfeghian, & Madihally, 2005). They are naturally brittle and cannot withstand the forces imposed in vivo, which restrict their widespread use in various biomedical applications. By contrast, hydrogels prepared from synthetic polymers usually present favorable mechanical properties, good processibility, easily tunable molecular weight and chemical compositions (Geng, Mo, Fan, Yin, & Fang, 2012; Yu & Ding, 2008). Unfortunately, most of them require relatively long response times for external environment change due to slow diffusion of water. Besides that, they may lack informational structure for biological response (Gil & Hudson, 2004; Tan & Marra, 2010). The fusion of polysaccharide and synthetic polymers in the form of (semi-) interpenetrating polymer network (IPN) hydrogels may offer to address these drawbacks and combine the most useful characters of both systems (Aouada, de Moura, da Silva, Muniz, & Mattoso, 2011; Dumitriu, Mitchell, & Vasile, 2011a,b; El-Sherbiny, Salama, & Sarhan, 2011).

Semi-IPN hydrogels are usually made by diffusing the linear polymer chains into a preformed polymer network, two polymers are independent of each other while being physically interlocked (Myung et al., 2008). In this way, the mechanical stability of the obtained hydrogels could be improved due to physical entanglements and network interactions (Bao, Yang, Mao, Mou, & Tang, 2008; Myung et al., 2008). Furthermore, semi-IPN hydrogels have interconnected porous network structures, which lead hydrogels to have much larger special surface areas (Zhao, Sun, Ling, & Zhou, 2009).

Salecan is a novel water-soluble extracellular β-glucan (Cas.No.1439905-58-4) produced by a new strain, *Agrobacterium* sp. ZX09. This strain was isolated from a soil sample from ocean coast of Shandong province (China) by our laboratory, its 16S rDNA

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http://dx.doi.org/10.1016/j.carbpol.2014.01.051

sequence was deposited in the GenBank database under the accession number GU810841 (Xiu et al., 2010). The large scale production of Salecan is convenient, low cost and reproducible. Salecan is a linear (1→3)-β-D-glucan comprising β-1-3-linked glucopyranosyls with a small number of α-1-3-linked which was reported by our laboratory in 2010 (Xiu et al., 2010). As a novel microbial polysaccharides, Salecan has excellent biological activities including antioxidation and non-toxicity (edible safety). Our previous studies had shown that Salecan can be utilized in both the food and medical fields (Xiu et al., 2011; Xiu, Zhou, Zhu, Wang, & Zhang, 2011; Chen et al., 2011, 2012; Zhang et al., 2013; Zhou et al., 2013). Salecan also has unique physico-chemical properties. The molecular weight of Salecan is 2×10^6 Da and its solution has high viscosity, which represents good mechanical properties (Xiu et al., 2011; Xiu, Zhou, et al., 2011). In addition, Salecan contains a high density of hydroxyl groups, which can be modified to provide greater flexibility in the preparation of hydrogels. More importantly, the high solubility of Salecan allows large amounts of functional groups to be incorporated onto the polysaccharide chains without compromising its solubility in water. These features make Salecan a promising candidate for fabricating hydrogels used in different biotechnological applications.

Poly(N,N-dimethylacrylamide) (PDMA) is a hydrophilic polymer, due to its remarkable properties, such as water solubility and biocompatibility, it is very useful in the biomedical applications including polymer supports for protein synthesis, two-phase catalysts and controlled drug delivery (Kondo, Nakashima, Hado, & Tsuoa, 1990; Valdebenito & Encinas, 2010). Poly(2-hydroxyethyl methacrylate) (PHEMA), an important synthetic polymer, has been successfully applied in biomedical and pharmaceutical fields such as contact lenses, wound dressings and surgical prostheses because of their high mechanical strength, stability in water and inert to normal biological processes (Peppas, Hilt, Khademhossein, & Langer, 2006; Yildiz, Isik, & Kis, 2002). Copolymerization of HEMA monomers with other monomers has been reported by previous literature (Atzet, Curtin, Trinh, Bryant, & Ratner, 2008; Tomic, Dimitrijevic, Marinkovic, Najman, & Filipovic, 2009). The obtained copolymers present excellent biocompatibility and unique physicochemical properties (Peppas et al., 2006; La Gatta, Schiraldi, Esposito, D'Agostino, & De Rosa, 2009).

In this paper, a new class of semi-IPN hydrogels composed of Salecan and poly(DMAA-co-HEMA) (PDH) were prepared by radical copolymerization of DMAA and HEMA in the presence of Salecan and crosslinking agent N,N′-methylene diacrylamide (BAAm). To our knowledge, this is the first report on the preparation and characterization of Salecan/poly(DMAA-co-HEMA) semi-IPN hydrogels (Salecan/PDH semi-IPN hydrogels). The structure and interior morphology of the hydrogels were characterized by Fourier transformation infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The equilibrium swelling ratios, as well as the water retention capacity were measured as a function of time. The mechanical properties were investigated by rheological and compressive tests. Degradation tests were carried out in phosphate buffer saline (PBS) solution. Moreover, their cytocompatibility was assessed under in vitro conditions with COS-7 cell. Particularly, the effect of Salecan/PDH ratio on these properties of the resulting hydrogels were also studied.

2. Experimental

2.1. Materials

The 2×10^6 Da Salecan were made by Center for Molecular Metabolism, Nanjing University of Science & Technology. N,N-dimethylacrylamide (DMAA), 2-Hydroxyethyl methacrylate (HEMA), N,N′-methylene diacrylamide (BAAm), Ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) were purchased from Aladdin Industrial Corporation. MTT cell proliferation and cytotoxicity detection kit was obtained from Nanjing KeyGen Biotech Co., LTD, China. The solutions of Salecan (2 wt%) were prepared by dispersing the required amount of Salecan in deionized water under slow stirring at room temperature overnight prior to assessment.

2.2. Preparation of Salecan/PDH semi-IPN hydrogels

Semi-IPN hydrogels based on Salecan and PDH were synthesized by free radical cross-linking copolymerization in aqueous solution at 25 °C. The preparation scheme is presented in Fig. 1. Briefly, 20 wt% monomer (DMAA + HEMA, DMAA/HEMA weight ratio = 1/1.5) solution containing 0.7 wt% crosslinking agent BAAm mixed with 2 wt% Salecan solution according to the desired blending ratios in 50 mL three-necked flask. Then the activator, TEMED was added and the solution was cooled to 0 °C in ice-water bath and stirred under Ar atmosphere for 1 h. After that, the initiator APS was added to the solution and stirred vigorously with mechanical stirrer for 180 s. Then 10 mL of this solution was transferred to a circular glass mold. The glass mold were sealed and placed in a thermostatted bath at 25 °C for 24 h. After polymerization, the samples were carefully removed from the mold and immersed in deionized water overnight. Then, these samples were further washed with a large excess of deionized water for 7 days, and the water was refreshed four times every day in order to remove the residual unreacted monomers and other impurities (Sarmad, Yenici, Gürkan, Keçeli, & Gürdağ, 2013). For all the cases, the washing solutions were collected and analyzed by the spectrophotometric technique to confirm the complete removal of the residual monomers (Dumitriu et al., 2011a,b). In addition, the feed composition and the samples code of the hydrogels are summarized in Table 1.

3. Characterization

3.1. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra were recorded in KBr pellets using a Nicolet IS-10 FTIR spectrometer in the region of 400–4000 cm^{-1}. Salecan, the freeze-dried PDH, SPDH2 and SPDH4 hydrogel samples were powdered, ground with KBr powder and pressed into pellets.

3.2. X-ray diffraction (XRD)

X-ray diffraction (XRD) measurements were conducted using a Philips PW 1720 X-ray generator operated at a voltage of 30 kV and 20 mA with CuKα radiation (λ = 0.154 nm) in the 2θ range of 0–60°.

3.3. Thermal-gravimetric analysis (TGA)

TGA analysis was conducted with a TA Model Q600 thermal gravimetric analyzer under a nitrogen atmosphere and at a heating rate of 10 °C/min in the temperature range of 0–600 °C.

3.4. Swelling behavior measurements

The preweighed dry hydrogels were immersed in deionized water at room temperature. At regular time intervals, the swollen hydrogels were taken out and weighed after wiping off the surface water with wet filter paper until a constant weight. The swelling ratio (SR) was determined according to the following equation:

$$SR = \frac{W_t - W_d}{W_d}$$