



Research Paper

Fabrication of Salecan/poly(AMPS-co-HMAA) semi-IPN hydrogels for cell adhesion

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ABSTRACT

Salecan is a novel water-soluble extracellular β -glucan and has excellent physicochemical and biological properties. A series of hydrogels were fabricated from Salecan and poly(2-acrylamido-2-methylpropanesulfonic acid-co-*N*-hydroxymethyl acrylamide) (poly(AMPS-co-HMAA), PAH) by semi-IPN technique. The introduction of Salecan into the PAH network endows the system with enhanced cell adhesion properties. These semi-IPN hydrogels with open interconnected pores had pH and salt-sensitive swelling behavior. The increase in the content of hydrophilic Salecan could enhance the water uptake of the hydrogels. Rheological results indicated that the incorporation of PAH into hydrogels increased the storage modulus. The degradation property of the hydrogels can be tuned by modulating the content of Salecan and BAAM. Cytotoxicity results revealed that the hydrogels were non-toxic to COS-7 cells. All these results suggested that Salecan/PAH semi-IPN hydrogels exhibited great potential for applications in soft tissue engineering.

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1. Introduction

Hydrogels are defined as cross-linked, three-dimensional network of hydrophilic polymers capable of absorbing a significant amount of water while remaining insoluble in water. Due to the unique physical, chemical, and biologic properties, hydrogels are highly popular for constructing three-dimensional scaffolds in drug delivery systems (Dragan & Cocarta, 2016; Dragan, Loghin, Cocarta, & Doroftei, 2016), tissue engineering and cell adhesion devices (Kim, Potta, Park, & Song, 2017; Tang et al., 2010). Hydrogel can be composed of either natural polymers or fully synthetic polymers (El-Sherbiny & Smyth, 2010; Owino, Arotiba, Baker, Guiseppi-Elie, & Iwuoha, 2008). Generally speaking, hydrogels obtained from natural polymers such as polysaccharides have attracted much interest because of their excellent biocompatibility, biodegradability and low toxicity (El-Sherbiny, 2009; Singh, Kaur, & Kennedy, 2015). However, many of these polysaccharide-based hydrogels are considered to be a limitation in their applications as biomaterials. For example, the primary disadvantage of hyaluronic acid-based, agarose-based and chitosan-based hydrogels is their poor mechani-

cal properties (Balakrishnan & Banerjee, 2011; Burdick & Prestwich, 2011; Huang, Onyeri, Siewe, Moshfeghian, & Madihally, 2005). The wide application of chondroitin sulfate-based hydrogel is enslaved to the high cost. While the major drawback that still remains with alginate-based hydrogel is that cells seeded within the scaffold do not directly adhere to the matrix (Balakrishnan & Banerjee, 2011). Hydrogels made of synthetic polymers always possess favorable mechanical properties, processibility, tunable and reproducible molecular parameters including composition, degradability, and conformation (Geng, Mo, Fan, Yin, & Fang, 2012; Yu & Ding, 2008). Unfortunately, these synthetic polymers like polyacrylate and polyacrylamide have several disadvantages such as poor cell-matrix interaction and lack of informational structure for positive cell biological response (Jaiswal, Koul, Dinda, Mohanty, & Jain, 2011; Tan & Marra, 2010). The toxicity and carcinogenicity of residual acrylate and acrylamide monomers are expensive and harmful to the environment. In addition, the non-biodegradability of poly(acrylic acid), poly(*N*-vinyl-2-pyrrolidone) and poly(ethylene glycol) raises great ecological and health concerns (Casadio, Brown, Chirila, Kraatz, & Baker, 2010; Peppas, Bures, Leobandung, & Ichikawa, 2000; Shi et al., 2012).

The combination of polysaccharide and synthetic polymers in the interpenetrating polymer network (IPN) style is a simple and easily feasible route to address this drawback. IPN is defined as

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a network of two polymers, at least one of which is synthesized and/or cross-linked in the presence of the other. If one polymer is linear and penetrates another cross-linked network without any other chemical bonds between them, it is termed as a semi-IPN (Dragan, 2014; Dragan & Apopei, 2013). For tissue engineering application, a satisfied hydrogel should not only provide mechanical and biochemical support during the regeneration process, but also have interconnected porous network, which ensures the enough space for cell invasion, proliferation and efficient transport of oxygen, nutrients and metabolic wastes during the cells growth (Balakrishnan & Banerjee, 2011; Drury & Mooney, 2003; Elowsson, Kirsebom, Carmignac, Mattiasson, & Durbeek, 2013). Designing polysaccharide-based hydrogels in semi-IPN style provides a convenient way to create the desirable pore structure with controllable pore size and high degree of interconnectivity due to physical entanglements of polysaccharide chains within the network of the synthetic polymers, which can endow optimal properties for cell spreading and adhesion inside hydrogels (Akpalo et al., 2011; Liu & Chan-Park, 2009).

Salecan, a novel water-soluble extracellular β -glucan (Cas. No.1439905-58-4), is produced by the fermentation of a salt-tolerant new strain *Agrobacterium* sp. ZX09. This strain was isolated from a soil sample from the ocean coast of Shandong and its 16S rDNA sequence was deposited in the GenBank database under the accession number GU810841 (Xiu et al., 2010). Salecan is a linear $(1 \rightarrow 3)$ - β -D-glucan comprising β -1-3-linked glucopyranosyls with a small number of α -1-3-linked which was reported in 2010 (Xiu et al., 2010). Its structure has been proven to consist of the following repeating unit: $\rightarrow 3)$ - β -D-Glcp-($1 \rightarrow 3)$ -[β -D-Glcp-($1 \rightarrow 3)$ - β -D-Glcp-($1 \rightarrow 3)$] β - α -D-Glcp-($1 \rightarrow 3)$ - α -D-Glcp-($1 \rightarrow$. As a novel microbial polysaccharide, Salecan has excellent rheological property and biological activity, as well as edible safety (Xiu, Zhan et al., 2011; Xiu, Zhou, Zhu, Wang, & Zhang, 2011). Previous studies had shown that Salecan can also be utilized in the preparation of hydrogel, the obtained products combining the advantages of Salecan with different responsive behavior have shown great potential in tissue engineering application and drug delivery system (Hu et al., 2015; Hu et al., 2017; Hu, Feng, Wei et al., 2014; Hu, Feng, Xie et al., 2014; Qi et al., 2015; Wei et al., 2015).

2-Acrylamido-2-methylpropanesulfonic acid (AMPS) is a hydrophilic vinyl monomer having nonionic and anionic groups, which has drawn more attention in the preparation of hydrogel due to its good reactivity and acidity (Durmaz & Okay, 2000). *N*-(Hydroxymethyl) acrylamide (HMAA) is one of the bifunctional, hydrophilic monomer containing a self-condensable methylol group and a reactive double bond, so that it can be homopolymerized or copolymerized (Cha, Kohman, & Kong, 2009). The copolymerization of AMPS monomer with HMAA monomer can combine the most useful characters of each polymer such as high water uptake and favorable mechanical integrity (Rastogi, Krishnamoorthi, & Ganesan, 2012). The electrostatic repulsion of ionic groups in PAMPS is expected to expand the whole polymer network, while the nonionic groups can improve the salt tolerance of the copolymer (Ozmen & Okay, 2005). Meanwhile, the introduction of PHMAA into the PAMPS network could greatly improve the mechanical strength (Chen, Liu, Liu, & Ma, 2009). In this way, poly(AMPS-co-HMAA) seems to be more appropriate for biomedical applications than the polymers used in our previous work (Hu et al., 2015; Hu et al., 2017; Hu, Feng, Wei et al., 2014; Hu, Feng, Xie et al., 2014; Qi et al., 2015; Wei et al., 2015).

In this work, a novel series of hydrogel based on Salecan and poly(AMPS-co-HMAA) (PAH) were produced by utilizing semi-IPN technique. The incorporation of Salecan into PAH network endows the material with the continuous pore structure and desirable swelling properties. The effects of Salecan and PAH content on swelling behavior, water retention, rheological property and degra-

dation were also discussed in detail. Moreover, the cytotoxic effect of the hydrogel against COS-7 cells was examined. Especially, adhesion and spreading of COS-7 cells on the hydrogel surface were observed.

2. Experimental section

2.1. Materials

Salecan was made by the Center for Molecular Metabolism, Nanjing University of Science & Technology. 2-acrylamido-2-methylpropanesulfonic acid (AMPS), *N*-hydroxymethyl acrylamide (HMAA), *N*, *N'*-methylene diacrylamide (BAAm), ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) were purchased from Aladdin Industrial Corporation (Shanghai, China). African green monkey kidney cells (COS-7 cells) were obtained from Medical School of Southeast University (Nanjing, China). An MTT cell proliferation and cytotoxicity detection kit and Live/dead viability/cytotoxicity kit were offered by Nanjing KeyGen Biotech Co., Ltd (Nanjing, China).

2.2. Hydrogel preparation

A series of Salecan/PAH semi-IPN hydrogels used in this study were synthesized by free radical polymerization at 30 °C using BAAm as cross-linker and APS/TEMED as redox-initiating pair. The feed compositions of the hydrogels are listed in Table 1.

Briefly, a calculated amount of Salecan solution (2%, w/v), 2 mL of monomer (AMPS + HMAA) solution (42.5%, w/v), 1 mL of TEMED solution (2.5%, v/v) and a calculated amount of BAAm were first mixed in a 50 mL three-neck flask, then the solution was cooled to 0 °C in ice-water bath and stirred under Ar atmosphere for 30 min. After that, 1 mL of APS solution (0.8%, w/v) was added to the solution and stirred vigorously for 30 s. The final volume of the solution was made up to 14 mL with deionized water and the resultant solution was then poured into a circular glass mold. The glass mold was sealed and placed in a thermostatted bath at 30 °C. After polymerization, the samples were carefully removed from the mold, cut into small pieces and immersed in deionized water for 1 week by changing the water four times a day in order to remove the residual unreacted monomers and other impurities. For all the cases, the washing solutions were collected and analyzed by the spectrophotometric technique to confirm the complete removal of the residual monomers and cross-linker. The result is shown in Fig. S1 of Supporting information.

3. Characterization

3.1. Swelling behavior

The swelling behavior of the hydrogels was measured in buffer solutions with different pH values at room temperature (pH 1.0, HCl/NaCl solution; pH 4.01, potassium hydrogen phthalate buffer; pH 6.86, disodium hydrogen phosphate/potassium dihydrogen phosphate buffer; pH 9.18, sodium tetraborate decahydrate buffer, the ionic strength (I) of each buffer solution was kept constant at 0.1 M by adding an appropriate amount of NaCl) and in different concentrations of KCl, CaCl₂ and AlCl₃ salt solutions. The known weights of dry hydrogel specimens were swollen to equilibrium in the media, at predetermined time intervals, the swollen hydrogels were weighed after being wiped with soft paper tissue. The water uptake of the hydrogel was calculated by the following Eq. (1):

$$\text{Wateruptake} = (W_t - W_d)/W_d \quad (1)$$

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