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Investigation of Salecan/poly(vinyl alcohol) hydrogels prepared by freeze/thaw method

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ABSTRACT

Salecan is a novel water-soluble extracellular-glucan produced by a new kind of salt-tolerant strain *Agrobacterium* sp. ZX09 and can be applied in food and medicine industries. In this work, Salecan (Sal) was incorporated into poly(vinyl alcohol) (PVA) to prepare novel Sal/PVA hybrid hydrogels by repeated freeze–thaw processing. Physicochemical and biological characteristics of the hydrogels were investigated to evaluate their potential as cell adhesion materials. By increasing the Salecan content in the hybrid hydrogels, their swelling capacity increased notably, while the compressive modulus decreased. Observed by SEM, Sal/PVA hydrogels had a homogeneous porous structure. The degradation rate of the hydrogels can be controlled by tailoring the composition ratio of Sal/PVA. Furthermore, cells could adhere well on the surface of Sal/PVA hydrogels. In conclusion, these results make Sal/PVA hydrogels attractive materials for biomedical applications.

1. Introduction

Hydrogels are hydrophilic three-dimensional polymeric networks that can imbibe large quantities of water without dissolution or loss of their structural integrity. Because of their characteristic properties of high water content, together with their inherent mechanical strength, hydrogels have attracted considerable attention as promising materials for biomedical applications involved in drug carriers (El-Sherbiny, 2010), tissue engineering (El-Sherbiny & Yacoub, 2013), scaffolds for cell cultures (Almany & Seliktar, 2005), wound dressings (Yang et al., 2010) and filtration/separation processes (Dragan, 2014; El-Sherbiny, Abdel-Hamid, Rashad, Ali, & Azab, 2013). Both synthetic (Almany & Seliktar, 2005; Aouada, Pan, Orts, & Mattoso, 2009; Dinu, Přádný, Drăgan, & Michálek, 2013a,b) and natural polymers (Bortolin et al., 2012; Dragan & Apopei, 2013; Yang et al., 2010; Zhang & Edgar, 2014) can be used for the production of hydrogels, achieved by methods such as physical crosslinking, chemical gelation or self-assembly. In the last few years, hydrogels based on natural polymers, especially polysaccharides have been widely utilized due to its prominent biocompatibility, bioactivity, biodegradability, hydrophilicity and low toxicity (Aouada, Moura, Lopes da Silva, Muniz, & Mattoso, 2011).

Poly(vinyl alcohol) (PVA) is a water soluble synthetic polymer of great interest because of its many desirable characteristics (Yang et al., 2011). PVA hydrogels are non-toxic, noncarcinogenic, biocompatibility, good film forming ability and processability (Sahoo, Panyam, Prabha, & Labhasetwar, 2002). Applications of PVA hydrogels in the biomedical area include wound dressing (Kim et al., 2008), artificial articular cartilages (Kobayashi & Oka, 2004) and drug delivery (Ossipov, Kootala, Yi, Yang, & Hilborn, 2013). PVA gels can be prepared via chemical or physical crosslinking. Although the chemical crosslinking methods could efficiently prevent the dissolution and enhance the mechanical properties of PVA hydrogels, the potential toxic environments, which are created from chemical crosslinking, may have harmful effects on cells. Thus, researchers are attempting to stay away from this method (Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009).

In contrast, PVA physical hydrogels (prepared by freeze–thawing process) have drawn a greater research attention. Cryotropic treatment (single or repeated cycles of freeze–thawing) of concentrated PVA aqueous solution induces crystallization and leads to the formation of a network structure in which PVA crystallites acted as junction points (Hassan & Peppas, 2000a,b). This method addresses toxicity issues because it does not result in the reagent residuals and chemical toxicity, and consequently, no toxicity agents are leaching out from the gel matrix (Kim et al., 2008). Furthermore, these physically cross-linked hydrogels, which are inter-connected by hydrogen bonding, exhibit more porous, spongy, rubbery and higher elastic properties than PVA hydrogels prepared by other methods (Hassan & Peppas, 2000a; Plieva

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et al., 2006). Nowadays these gel matrices have been widely implemented in biotechnology fields, specifically in molecules (protein, peptides) (Hassan & Peppas, 2000a) and whole cell immobilization (Lozinsky & Plieva, 1998). However, pertinent to certain biomedical applications, being conducive to cell adhesion is an essential requirement. Regretfully, PVA cryogels are intrinsically bio-inert like most synthetic hydrogels and non-adhesive to cells and proteins. An effective way to overcome this drawback is to integrate PVA hydrogel with natural polymers (such as proteins and polysaccharides) to form blending hydrogels (Liu, Vrana, Cahill, & McGuinness, 2009).

Salecan is a new extracellular water-soluble microbial polysaccharide (Cas. No. 1439905-58-4) produced by strain of *Agrobacterium* sp. ZX09. This novel strain was isolated from soil samples collected from ocean coast of Shandong province, PR China by our laboratory in 2009 and its 16S rDNA sequence was deposited in the GenBank database with accession No. GU*810841 (Xiu et al., 2010). Large scale production of Salecan is low cost, convenient and easy processing. Salecan is a linear (1 → 3)-β-D-glucan comprising β-1-3-linked glucopyranosyls with a small number of α-1-3-linked, and is composed of the following repeating unit: → 3)-β-D-Glcp-(1 → 3-[β-D-Glcp-(1 → 3)-β-D-Glcp-(1 → 3)]3-α-D-Glcp-(1 → 3)-α-D-Glcp-(1 → (see Fig. S1 in Supporting information) (Xiu et al., 2010). This unique linkage pattern, was reported by our laboratory in 2010, endows Salecan with prominent biological activities including anti-oxidation and non-toxicity (edible safety) (Chen et al., 2011; Xiu et al., 2011a; Xiu, Zhou, Zhu, Wang, & Zhang, 2011b). Salecan can be utilized in food fields as a new source of thickening additive (Chen et al., 2012; Xiu et al., 2011a,b) and medical fields for preventing and treating constipation *CN Pat.*, 102058616A, 2011. Furthermore, Salecan also has distinctive physicochemical properties: (i) Salecan ($M_w = 2 \times 10^6$ Da) solutions are highly viscous even at low polymer concentrations (Xiu et al., 2011a,b); (ii) Salecan possesses a large amount of pendant hydroxyl functional groups making it prone to chemical modification (Meng, Matson, & Edgar, 2014). These features make it an excellent candidate for the preparation of hydrogel. In the past, our group has already studied the properties of Salecan-based hydrogels (Hu et al., 2014a,b). Hu et al. (2014b) found that cells could not grow on the surface of the pure PAAm (polyacrylamide) hydrogel, but it could adhere well on the surface of the Salecan/PAAm semi-IPN hydrogels due to the addition of Salecan (Hu et al., 2014b). These results remind us that the incorporation of Salecan into the PVA cryogels may enhance the cell adhesion.

In this work, we report the synthesis of Sal/PVA blended cryogels by the freeze-thaw technique. The novelty of the present study is to evaluate the cell adhesion ability of bio-inert PVA cryogels with the use of Salecan for the first time. Furthermore, their physicochemical properties, such as gel fraction, swelling behavior, thermal stability, morphology and mechanical strength were also investigated. Due to its biomimetic composition and a green fabrication procedure, the novel Sal/PVA hybrid hydrogels have great potential in biomedical engineering.

2. Experimental

2.1. Materials

PVA (average $M_w = 125{,}000$ g/mol, degree of polymerization ~2800, degree of hydrolysis 98.0–98.8 mol%) was obtained from Sigma–Aldrich Chemie GmbH, Riedstr., Germany. Salecan (average $M_w = 2000{,}000$ g/mol, Xiu et al., 2010) was made by Center for Molecular Metabolism, Nanjing University of Science & Technology. 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide and penicillin/streptomycin, all purchased from Nanjing KeyGen Biotech Co., Ltd (China), were used as received.

Table 1
The preparation and the compositions of Sal/PVA solutions.

Ingredient	Designation					
	Pure PVA	S10V90	S20V80	S30V70	S40V60	S50V50
Salecan (2%,w/v) (mL)	0	10	20	30	40	50
PVA (10%,w/v) (mL)	100	90	80	70	60	50

2.2. Fabrication of Sal/PVA hybrid hydrogels

Sal/PVA hydrogels were obtained by freezing–thawing (F–T) cycle (Peppas & Stauffer, 1991). The preparation scheme is presented in Fig. 1. First, 10.0 g PVA was added to 100 mL deionized water and stirred continuously at 90 °C for 2 h to form a homogeneous solution of 10% (w/v). Salecan solution (2%, w/v) was prepared by dispersing the required amount of Salecan in deionized water under moderate stirring at 60 °C for 2 h. The PVA and Salecan solutions were cooled to room temperature prior to assessment. Then, a volume of Salecan solution was mixed with the PVA solution at five ratios, as listed in Table 1. When the volume of Salecan solution was higher than 50% (v/v), the mixture was too diluted that cannot form a hydrogel. The blends stirred vigorously with mechanical stirrer at room temperature for 2 h. The homogeneous Sal/PVA solution was sonicated in an ultrasonic bath at room temperature for 0.5 h to get rid of air bubbles. Finally, the mixtures were poured in petri dishes, followed by freezing at −20 °C for 18 h and thawing at 25 °C for 6 h, for three consecutive cycles. All samples were left in de-ionized water for 72 h to extract leachable sol fraction from polymer matrix for further characterizations and the influence of Salecan concentration on the gel fraction is shown in Fig. S2 (Supporting information).

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the cryogels were recorded on an FTIR spectrometer (Nicolet IS-10) working in attenuated total reflectance mode, within a spectral range of 400–4000 cm^{-1}.

2.4. X-ray diffraction measurements (XRD)

Wide-angle XRD patterns of the dried hydrogels were measured using an X-ray generator (PW 1720, Philips) operated at a voltage of 30 kV and current of 20 mA with Cu$K\alpha$ radiation ($\lambda = 0.154$ nm) in the 2θ range of 5–60°. The relative degree of crystallinity was calculated according to the method described in the literature (Costa-Júnior, Barbosa-Stancioli, Mansur, Vasconcelos, & Mansur, 2009).

2.5. Differential scanning calorimetry (DSC)

DSC experiments were performed with a differential scanning calorimetry (DSC, Mettler Toledo 823/e) under nitrogen purge gas and at a heating rate of 10 °C/min in the temperature range of 25–300 °C. The T_g was taken at the midpoint of the baseline shift (Fathi, Atyabi, Imani, & Alinejad, 2011). The degree of crystallinity (%) was calculated as:

$$\text{degree of crystallinity}\,(\%) = \left(\frac{\Delta H}{\Delta H_0}\right) \times 100$$

where ΔH was determined by integrating the area under the melting peak of the PVA based hydrogel over the range 200–250 °C, ΔH_0 was the thermodynamic enthalpy required to melting a 100% crystalline PVA (138.6 J/g) (Peppas & Hansen, 1982).