



A novel thermo-responsive hydrogel based on salean and poly(N-isopropylacrylamide): Synthesis and characterization



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ABSTRACT

Salecan is a novel microbial polysaccharide produced by *Agrobacterium* sp. ZX09. The salt-tolerant strain was isolated from a soil sample in our laboratory and the 16S rDNA sequence was deposited in the GenBank database under the accession number GU810841. Salecan is suitable to fabricate hydrogel for biomedical applications due to the excellent hydrophilicity and biocompatibility. Here, salean has been introduced into poly(N-isopropylacrylamide) (PNIPAm) network to form novel thermo-sensitive semi-interpenetrating polymer networks (semi-IPNs). The structure of salean/PNIPAm semi-IPNs was confirmed by Fourier transformation infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Thermogravimetric analysis (TGA) proved the stability of the semi-IPNs. Rheological and compressive tests revealed an elastic solid-like behavior and good mechanical properties of the hydrogels. Swelling behavior test showed the hydrogels possessed high water content at room temperature. An excellent thermo-sensitive property of fast response rates to temperature had been demonstrated as well. In vitro degradation measurements ensured the semi-IPNs were degradable. Cytotoxicity and cell adhesion study suggested the synthesized salean/PNIPAm hydrogels were non-toxic and biocompatibility. The results indicated the novel thermo-responsive hydrogels could be a suitable candidate for biomedical applications.

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1. Introduction

Hydrogels are hydrophilic polymer of three-dimensional networks able to swell in aqueous solutions without losing integrity of their structure [1–3]. They can be cast into practically any shape, size, or form [4] for different applications such as controlled drug release devices [5–7], tissue engineering scaffolds [8] and biosensors [9]. In recent years, stimuli-responsive polymers have been vigorously investigated due to their attractive properties: volume or phase transitions in response to slight environmental changes including temperature [10], pH [11], salt concentration [12] and magnetism [13]. Particularly, thermo-sensitive hydrogels have been in the spotlight for a long period for its easy temperature control and feasibility both in vitro and in vivo [14].

Poly(N-isopropylacrylamide) (PNIPAm), undergoes a phase transition at 32 °C, is extensively studied as a stimuli-responsive polymer [15,16]. Crosslinked PNIPAm has a dramatically swelling

behavior that absorbs water to a swollen state below its lower critical solution temperature (LCST, 32 °C) and shrinks with a volume decrease above LCST [17]. This phenomenon is governed by the balance of hydrophilicity (amide) and hydrophobicity (isopropyl) of PNIPAm side groups which make the amphiphilic nature [18]. The main mechanism of the thermally induced water release above its LCST is the increasing intra- and inter-molecular hydrophobic interactions between the isopropyl groups [19]. Owing to this unique property, PNIPAm hydrogels has been applied in many fields including drug release [11], molecular separation [20], cell harvest [21], etc. However, the slow response rates and poor mechanical property of PNIPAm hydrogels have been considered as the main factors that hinder their applications [22,23]. In the past decades, several strategies have been proposed to improve the response rates and mechanical property [24–26]. An effective way is to introduce hydrophilic polymers into PNIPAm networks to form semi-IPNs [27].

In general, semi-IPNs are synthesized by simultaneously polymerizing and crosslinking monomers in the presence of linear polymer chains, yielding a hydrogel with linear chains physically entangled within a network [28]. Since no chemical bonding happens between the two components, each polymer is independent

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and retains its own properties [29]. Semi-IPNs technique has been developed as a convenient route to prepare multi-functioned polymer materials [30]. Generally, semi-IPNs based on natural polymer chains and synthetic polymers may possess unique characteristics of good biocompatibility and mechanical strength that make them fascinating in the biomedical field [31,32].

Salecan, produced by *Agrobacterium* sp. ZX09, is a novel water soluble extracellular β -glucan (Cas. No. 1439905-58-4). The structure of salecan is a linear (1 \rightarrow 3)- β -D-glucan consisted of β -1-3-linked glucopyranosyls with a small quantity of α -1-3-linked [33]. As a novel polysaccharide, salecan has been utilized in the fields of both food and medical in our laboratory [34–38]. With a high density of hydroxyl groups, the large molecular weighted (2×10^6 Da) salecan could be further modified for multifarious applications. Recently, salecan was successfully used in the preparation of novel hydrogels with certain polymers by our group [39,40]. It was indicated that the hydrophilic salecan could be a suitable candidate for biomedical applications.

In this work, a novel thermo-responsive material based on semi-interpenetrating networks (semi-IPNs) was synthesized using free-radical polymerization, comprised of PNIPAm hydrogels with salecan chains physically entangled with the network. To the best of our knowledge, salecan/PNIPAm semi-IPNs have not been reported before. As a research for a novel material, characterizations including Fourier transformation infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermal-gravimetric analysis (TGA), scanning electron microscopy (SEM), rheological measurement and compressive tests have been performed. The water content and the phase transition temperature (T_p) of the hydrogels have been determined by swelling behavior study. In vitro degradation has been researched as well. Finally, cytotoxicity and cell adhesion property of the semi-IPNs have been studied. As novel thermo-responsive semi-IPNs, salecan/PNIPAm hydrogels have great potential in biomedical applications.

2. Experimental

2.1. Material

The 2×10^6 Da salecan (CAS: 1439905-58-4) were made by Center for Molecular Metabolism, Nanjing University of Science & Technology. N-isopropylacrylamide (NIPAm) (Aladdin Reagent Co., Ltd., Shanghai, China) was recrystallized twice from n-hexane. N,N'-methylenebis(acrylamide) (BIS), ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Aladdin and used as received. MTT cell proliferation and cytotoxicity detection kit was obtained from Nanjing KeyGen Biotech Co., Ltd., China.

2.2. Preparation of salecan–PNIPAm hydrogels

The thermo-responsive hydrogels were synthesized by free-radical polymerization using ammonium persulfate (APS) as initiator and N,N,N',N'-tetramethylethylenediamine (TEMED) as accelerator, respectively. N,N'-methylenebis(acrylamide) (BIS) was selected to be the crosslinking agent in the semi-IPN system. The polymerization process used in this work was similar to that reported in our latest literature [39]. Briefly, NIPAm monomer (10% (w/v) based on water) and BIS solution were mixed with salecan solution (2%, w/v) according to the desired ratios. The mixture was stirred at 0 °C under a nitrogen atmosphere to get a uniform solution. After that, TEMED and APS were added to initiate the reaction. The solution was thoroughly stirred, immediately poured into a glass mold and covered with a glass plate. The solution was allowed to polymerize at 25 °C for 1 day. The gels were carefully removed

from the mold and immersed in deionized water for a week, during which time the unreacted monomers were eliminated by changing the water repeatedly. The swollen hydrogels were freeze-dried at -70 °C to completely remove water. The feed ratios are listed in Table 1.

2.3. Characterization

2.3.1. Stability of salecan in semi-IPN hydrogels

Stability of salecan in the semi-IPNs was monitored by measuring the concentration of salecan in washing solution of the gels. In this work, phenol-sulfuric acid method was employed [41]. Briefly, 1 ml 6% (w/w) phenol aqueous solution was added to 2 ml washing solution. Followed 5 ml concentrated sulfuric acid was added dropwise. The mixture was placed on a shaker for 10 min to get a uniform solution. The amount of salecan released in the medium was calculated using a standard curve ($y = 10.993x + 0.0785$, $R^2 = 0.9992$) from its absorbance at 490 nm of UV spectrograph. All the samples were performed in duplicate.

2.3.2. FTIR measurement

Fourier transform infrared (FTIR) spectra were obtained through a Nicolet iS10 FTIR instrument (Thermo Fisher Scientific, USA). Attenuated total reflectance (ATR) was applied to the lyophilized hydrogels. Each spectrum was obtained by an average of 32 repeated scans and 4 cm^{-1} scan resolution. Background measurements were performed and subtracted from the readings.

2.3.3. X-ray diffraction (XRD) analysis

The X-ray diffraction patterns of the lyophilized salecan/PNIPAm hydrogels were obtained using a XRD instrument (DMAX-2200) with Cu K α radiation ($\lambda = 0.154$ nm). Data were collected from $2\theta = 10$ – 60° at a voltage of 30 kV and 20 mA.

2.3.4. Morphology

Scanning electron microscopy (SEM) (JEOLJSM-6380LV) was used to observe the surface morphology and internal cross-sections of the semi-IPNs. The freeze-dried hydrogels were sputter-coated with gold for 10 min to enhance conductive before observation. SEM images were acquired at operating voltage of 30 kV.

2.3.5. Thermal stability

Thermogravimetric analyses (TGA) of salecan, PNIPAm and salecan/PNIPAm hydrogels was investigated by a TA instrument (Model Q600 thermal gravimetric analyzer). The samples were heated from 25 °C to 600 °C under a nitrogen atmosphere at a heating rate of 10 °C/min.

2.3.6. Rheological test

Rheological experiments were performed with a MCR 101 rheometer (Anton Paar) using a parallel-plate (diameter 50 mm). First, dynamic strain sweep was carried out to confirm the linear viscoelasticity region. The storage modulus G' and loss modulus G'' was assessed by dynamic frequency sweep measurement (0.01% strain) at 25 °C as a function of frequency. The normal force loaded onto each hydrogel film was automatically controlled at 1.0 N.

2.3.7. Mechanical properties

The mechanical properties of the hydrogels were characterized by unconfined, uniaxial compression tests performed on an Instron 4464 mechanical tester equipped with a 500 N load cell at room temperature. The cylindrical hydrogel samples ($\varnothing 22$ mm \times 10 mm) were compressed at a rate of 2 mm/min until cracking. The compressive modulus was determined by the initial linear slope of the compressive curve in the strain range of 20–30%. The fracture strain

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