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Preparation of the chitosan/poly(glutamic acid)/alginate polyelectrolyte complexing hydrogel and study on its drug releasing property

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ABSTRACT

In the current study, a novel semi-dissolution/acidification/sol-gel transition (SD-A-SGT) method was explored for the preparation of polyelectrolyte complexing (PEC) composite hydrogels with natural polymers only. A chitosan (CS) powder was uniformly dispersed in a solution of poly(glutamic acid) (PGA) and alginate (SA) to form a semi-dissolved slurry mixture that was then exposed to an gaseous acidic atmosphere. CS was gradually dissolved and interacted with PGA and SA to form a CS/PGA/SA PEC composite hydrogel with a homogeneous structure. The SD-A-SGT procedure was able to overcome the shortcomings of direct mixing method via the PEC interaction. The effects of the hydrogel composition on its structure and properties were investigated by FTIR, XRD, rheology study, XPS, SEM, and swelling kinetics. The drug delivery performance of the CS/PGA/SA hydrogel was explored using piroxicam (PXC) as a model drug. PXC was *in situ* embedded in the hydrogel by the SD-A-SGT method. The hydrogel exhibited pH responsive drug release behaviors that were affected by the hydrogel composition. In all, the SD-A-SGT method for preparing PEC composite hydrogels has a great application potential in constructing the CS based hydrogels as medical materials.

1. Introduction

Hydrogels are high molecular weight 3D structures made of hydrophilic polymers connected to each other by physical or chemical cross-links (Kim et al., 2017). They are capable of holding water or biological fluids without being dissolved. Due to their high water contents, numerous studies have been conducted on the applications of hydrogels in different fields, such as tissue engineering materials, regenerative medicines, and drug delivery systems (Chen, Liu, Zeng, & Liu, 2017). As a drug delivery system, hydrogels have exhibited lesser side effects, improved drug utilization, drug targeting to specific sites, and lower cost etc. (Hu et al., 2017).

Recently, hydrogels of natural polymers, such as chitosan, alginate, cellulose, poly(glutamic acid), and so on, have received increasing interests in many fields, especially using as biomedical materials (Zou, Zhao, & Ye, 2015), due to their good biocompatibility and biodegradability. However, most of natural polymer hydrogels are prepared by chemical crosslinking or graft copolymerization (Spagnol et al., 2012). The introduction of chemical structures may bring cytotoxicity to the hydrogels, and/or affect the biodegradability of the natural polymers, which is unfavorable for their application as biomedical materials.

The PEC interaction between polyanions and polycations is an effective, safe and green method for hydrogel construction (Li, Zhang, Sun, & Wong, 2016). In recent years, many hydrogels, especially physical hydrogels of complete natural polymers, which can highlight the excellent properties of natural polymers, have been prepared via PEC interaction (Kang, Park, Lee, & Son, 2007; Ji, Kuo, Wu, Yang, & Lee, 2012; Tsao et al., 2010). Among all of natural polymers, chitosan (CS) is the only natural basic polysaccharide that can combine with H^+ (Paini et al., 2015). CS can be used as the polycationic skeleton to prepare natural polymer-based PEC hydrogels. It is known that the marine polymer, alginate (SA), is an excellent biocompatible polyanion (Traffano-Schiffo, Cavo, Castro-Giraldez, Fito, & Santagapita, 2017) and poly(glutamic acid) (PGA) is highly hydrophilic polyanion (Yan et al., 2017). Therefore, it can be anticipated that the CS/PGA/SA composite hydrogel can be prepared via the PEC interaction, where SA can promote the strength and structure stability of the hydrogel, and PGA can promote the hydrophilicity and swelling property of the hydrogel. The polyampholyte structure of CS/PGA/SA composite hydrogel might possess distinctive pH sensitive swelling properties, and would have

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application potentials in drug delivery, tissue engineering, and wound dressing, etc.

However, CS is only soluble in acidic solutions, which confines the preparation conditions of PEC hydrogels. In addition, the cationic CS can rapidly interact with polyanions due to the strong electrostatic interactions, which cause precipitations during the preparation process or form hydrogels with irregular shapes, and thus influences the homogeneity, stability, and swelling property of the prepared PEC hydrogels. For example, Zhao et al. (2009) reported the turbidities and precipitations of the reaction solution for the preparation of polyelectrolyte complexes of CS and sodium carboxymethyl cellulose. Therefore, exploring novel preparation methods of full natural polymer-based PEC hydrogels with homogeneous and stable structures is needed.

In the present work, a novel SD-A-SGT method was proposed for preparing the CS based PEC hydrogels with homogeneous structures and excellent performances. A CS powder was uniformly dispersed in the polyanion solution of PGA and SA to form a semi-dissolved slurry mixture that was then exposed to an acetic acid gaseous atmosphere. The CS was gradually dissolved and reacted with the polyanion to form a visible sol-gel transition, eventually, the CS/PGA/SA polyelectrolyte complexing hydrogel of natural polymers only was prepared. This process can overcome the shortcomings of the direct mixing of polyelectrolytes with opposite charges. The tunable structure and properties, especially swelling properties, of the hydrogel were characterized. Its potential application as a pH sensitive drug delivery system was explored.

2. Experimental

2.1. Materials

CS with a deacetylation degree of 90.97% (determined by potentiometric titration method as shown in supplementary document) and an average molecular weight of 230 kDa was purchased from Zhejiang Aoxing Biotechnology Co., Ltd., China. Sodium alginate was obtained from Aladdin Industrial Co., Ltd (Shanghai, China). According to the supplier, the M_w and G/M ratio of it were 120 kDa and 35/65, respectively. Poly(glutamic acid) with molecular weight of 2000 kDa, tested by GPC method, was purchased from Shandong Freda Biotechnology Co., Ltd., China. Piroxicam (PXC) of HPLC grade with purity ≥98% was purchased from Shanghai Aladdin Reagent Company, China. Other regents were all analytical grades and obtained from Beijing Tongguang Fine Chemicals Company, China.

2.2. Preparation of CS/PGA/SA composite hydrogels

Certain amounts of PGA and SA were dissolved in water, followed by the addition of a certain amount of CS and stirring. The total concentration of PGA, SA and CS in solution was kept at 5%. The slurry solution of CS in the PGA/SA solution was poured into a petri dish, put in a sealed larger vessel containing a petri dish filled with acetic acid and kept at room temperature for 18 h until the slurry solution was transformed into a hydrogel. The hydrogel was quickly washed with distilled water to remove acetic acid residues, and sealed before use. The mass ratio between PGA and SA, and that between polycation to polyanion (m_{CS}:(m_{PGA} + m_{SA})) were varied to study the effects of hydrogel composition on its properties. The m_{CS}:(m_{PGA} + m_{SA}) was fixed at 1:1 for the studies on the effects of m_{PGA}:m_{SA} on the structure and properties of the hydrogel. Similarly, m_{PGA}:m_{SA} was kept at 3:2 for the studies on the effects of m_{CS}:(m_{PGA} + m_{SA}) on the structure and properties of the hydrogel.

The lyophilized hydrogels were prepared as the following process for the structural characterization. Hydrogels were frozen in the freezer at −20 °C for 12 h, and then freeze-dried in a FD-1E lyophilizer (Beijing Detianyou Technology Development Co., Ltd, China) at −60 °C under the pressure of 5 Pa.

The PXC loaded CS/PGA/SA hydrogel was prepared using the same procedure, except that 1% PXC was dispersed along with CS in the PGA/SA solution. The slurry solution of CS in the PGA/SA solution containing PXC was stirred, poured into a petri dish, put in a larger vessel containing a petri dish filled with acetic acid, and kept at room temperature for 18 h until the slurry solution was transformed into hydrogel. The hydrogel was quickly washed with distilled water to remove acetic acid residues, and sealed before use.

2.3. Characterizations

The FTIR spectra were measured on a Nicolet 380 FTIR spectrometer (Thermo Fisher Scientific, USA) in the range of 500–4000 cm^{-1} at a resolution of 1 cm^{-1} with 16 scans. The lyophilized hydrogels were used and the test specimens were prepared by the KBr-disk method.

Wide angle X-ray diffraction (XRD) of lyophilized hydrogels was recorded on a Rigaku D/Max-1200 type X-ray diffractometer (Rigaku Co., Japan) with Cu Kα characteristic radiation (wavelength λ = 0.15 nm) at a voltage of 40 kV and a current of 40 mA. The scanning rate was 5°/min and the scanning scope of 2θ was from 5° to 50°.

X-ray Photoelectron Spectroscopy (XPS) analyses of the lyophilized hydrogels were conducted on a PHI QUANTERA-II instrument (Ulvac-PHI Inc., Japan) equipped with a monochromatized Al KRX-ray source operated at 25 W and 15 kV. High-resolution spectra were collected at a 26.00 eV pass energy using a step size of 0.025 eV. The XPS results were further fitted in a nonlinear least squares curve fitting program (XPS-peak-41 software, Version 4.0).

To observe the surface morphology, the lyophilized hydrogels were mounted on a metal stub with a conductive tape and sputter-coated with a thin gold layer. The morphologies of the hydrogels were imaged under a scanning electron microscopy (SEM, Hitachi S-4800, Japan) at an acceleration voltage of 5 kV.

The rheological properties of the composite hydrogels with different composition were determined by measuring their storage modulus (G') and loss modulus (G") using an Anton Paar instrument (Physica MCR 301, Germany) equipped with a parallel-plate geometry (25 mm diameter). The measurements of G' and G" as a function of angular frequency at strain = 0.5% were carried out at 25 °C in the sweeping angular frequency from 0.1 to 100 rad s^{-1}.

The swelling behavior of the lyophilized hydrogels was investigated using the tea-bag method (Chen, Liu, Tan, & Jiang, 2009) and then the swelling kinetics of the hydrogels was simulated using the Voigt model. The detailed process was described in the supplementary document.

The accumulative release amount of PXC through the CS/PGA/SA composite hydrogel in different media was measured as the methods described in the supplementary document.

3. Results and discussion

3.1. Formation mechanism of CS/PGA/SA composite hydrogels

The CS/PGA/SA composite hydrogel was formed via the PEC interaction between the polycation and polyanion. In general, polyanions and polycations tend to flocculate if they are directly mixed due to the strong PEC interactions, forming hydrogels with poor homogeneity and stability. In contrast, for the SD-A-SGT method, the unprotonated chitosan was uniformly dispersed in the polyanion solution, gradually protonated in an acid atmosphere, and interacted with the polyanion to gradually form a hydrogel, which ensured a homogeneous and stable structure of the prepared hydrogel. The SD-A-SGT method is not influenced by the disadvantages of chitosan which can only be dissolved in acid solutions. On the contrary, the acid soluble property of chitosan facilitated the hydrogel formation process. Fig. 1 shows the formation mechanism of the CS/PGA/SA PEC hydrogel via SD-A-SGT method.