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Thermosensitive phase behavior and drug release of in situ N-isopropylacrylamide copolymer

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ABSTRACT

In this work, a novel in situ gel based on N-isopropylacrylamide as monomer and acrylate terminated poly(L-lactic acid)-b-poly(ethylene glycol)-poly(L-lactic acid) (PLEL) as biodegradable crosslinker was studied. The prepared poly(N-isopropylacrylamide) (PNIPAM) copolymer undergoes a temperature-dependent sol–gel transition, for it is a flowing sol at ambient temperature and turns into a non-flowing gel at around physiological body temperature. The sol–gel phase transition was recorded by using the methods of test tube-inverting and differential scanning calorimetry (DSC), which depended not only on chemical composition of copolymer, but also on molecular weight of poly(ethylene glycol) (PEG) of PLEL. The in vitro release behaviors showed that ofloxacin as model drug could be released sustainedly from the PNIPAM copolymer hydrogel system. Therefore, PNIPAM copolymer hydrogel might be very useful for its application in biomedical fields such as injectable drug delivery system.

1. Introduction

The in situ gel-forming polymer has drawn much attention as a promising material for drug delivery systems in the past few years [1–3]. The aqueous solution of polymer is in a sol state at room temperature but in a gelling state at body temperature [4,5]. In particular, thermosensitive polymers with a sol–gel transition point between room temperature and body temperature are expected to be useful for injectable polymer systems in biomedical applications [6]. These systems are injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired site [7].

Poly(N-isopropylacrylamide) (PNIPAM) is a representative thermosensitive polymer. The lower critical solution temperature (LCST) of PNIPAM in water is about 32 °C near the temperature of the human body and can be easily manipulated by copolymerizing with other hydrophilic or hydrophobic comonomers [8,9]. In situ gelling of aqueous PNIPAM copolymers has been studied as a candidate material for injectable drug delivery systems. However, wider application of PNIPAM hydrogels in many biomedical fields has been greatly restricted because these polymers are nonbiocompatible [10].

Considering biomedical applications, biocompatible and biodegradable linkages introduced into PNIPAM backbone are an important issue [11,12]. Currently, the most extensively studied biocompatible and biodegradable polymers are poly(lactic acid) (PLA) and their derivatives. PLA has been approved by the U.S. Food and Drug Administration (FDA) in biomedical fields. One of the greatest advantages is their degradability by simple hydrolysis of the ester backbone in aqueous environment. Polymeric micelles usually use poly(ethylene glycol) (PEG) as the hydrophilic segment. PEG possesses good water solubility and it has been approved by the FDA [13–15].

In present study, we first developed a polymerizable macromonomer of PLA–PEG–PLA terminated with diacrylate, which was biocompatible and biodegradable. Then, the macromonomer was used to prepare PNIPAM hydrogels as a biodegradable crosslinking agent and the bioaffinity of conventional PNIPAM hydrogel was improved. The high molecular weight PNIPAM are not biodegradable or soluble in vivo, but low molecular weight PNIPAM are non degradable but soluble and can be excreted. Therefore, a copolymer PNIPAM–PLLA–PEG–PLLA–PNIPAM would demonstrate thermoresponsive properties and it could be resorbable and biocompatible, but probably not fully biodegradable. A series of bioaffinity thermosensitive polymers were prepared in the presence of various contents and components of crosslinker. The temperature-sensitive properties and phase transitions of the microgel dispersions were studied in this work. PNIPAM copolymer hydrogel might be very useful for injectable drug delivery system. Therefore, ofloxacin was chosen as a model hydrophilic drug which was entrapped into the hydrogel to study the drug vitro release behavior.

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2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAM) monomer (Acros Organics America) was recrystallized from a 65:35(v/v) mixture of hexane and benzene and dried in vacuum. Poly(ethylene glycol) (PEG) (Belgium) with molecular weight of 800, 4000, 6000 was purchased. PEG was dried in vacuum for 4 h and DL-lactide was purified twice by recrystallization from ethyl acetate prior to use. Sodium pyrosulfite (SPS, Sinopharm Chemical Reagent Co., Ltd) as an accelerator was used as received. Ammonium persulfate (APS, Sinopharm Chemical Reagent Co., Ltd) as an initiator was further purified by recrystallization. All other chemicals used were of reagent grade and used without further purification.

2.2. Synthesis of biodegradable crosslinking agents macromonomer

A series of biodegradable crosslinking agents, diacrylate of polyethylene glycol and polylactides (PLA–PEG–PLA) were synthesized according to Scheme 1. The typical acrylate terminated PLA–PEG–PLA copolymer was prepared as follows: [16–18] calculated amount of LA and PEG were first introduced into a dried 100 ml three-necked flask equipped with a magnetic stirrer under a nitrogen atmosphere. Stannous octoate ($Sn(Oct)_2$) as catalyst was added when the solid melted. The reaction system was kept at 150 °C for 6 h. The resultant copolymer was cooled to room temperature. The just-obtained copolymer was dissolved in AR-grade dichlormethane (DCM), precipitated with anhydrous ether, filtered, and vacuum dried at 40 °C to get the copolymer (PLE) of LA and PEG. Then, acryloyl chloride (AcCl, 6 mol per PLE) dissolved in DCM was slowly dropped into PLE and triethylamine (TEA, a mol per AcCl mol). The reaction mixture was stirred for 12 h at 0 °C and room temperature respectively. After removal of precipitate of triethylamine hydrochloric acid by microfiltering, the solution was precipitated by cooled diethyl ether and filtered. The precipitate was dried for 24 h in vacuum at room temperature to obtain the biodegradable crosslinking agents (PLEL).

2.3. Synthesis of thermoresponsive and biodegradable copolymers

Various amounts of PLEL and NIPAM (1.0 g) were dissolved in 30 ml of distilled water in a 100 ml round bottomed flask. Then, the solution was bubbled more than 30 min with nitrogen under slow stirring to remove the oxygen in the solution. When the reaction temperature was reached, the solution of APS and SPS as a pair of redox initiators dissolved in 2 ml of H_2O was injected, respectively. The polymerization was carried out at 60 °C for 7 h with continuous N_2 bubbling. Finally, the obtained PNIPAM was purified by dialysis (Solarbio dialysis membrane, MWCO 12000) against pure water frequently, which lasted for 5 days.

2.4. Characterization

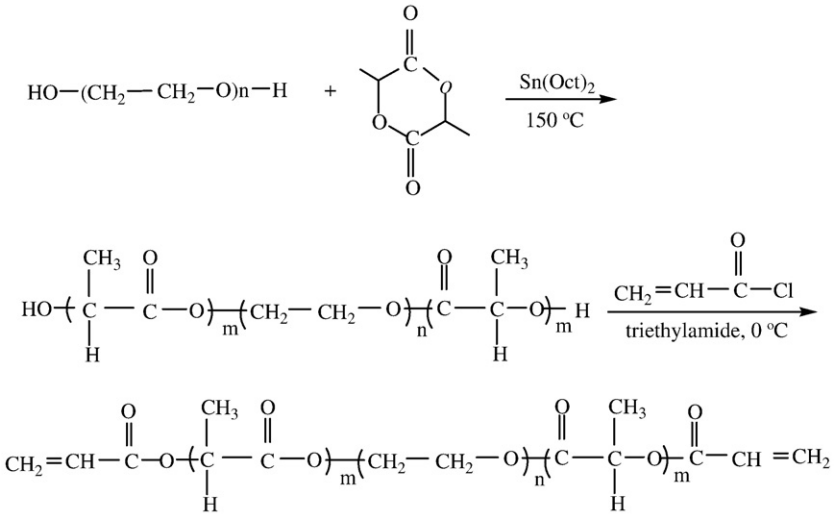
The structure of PLEL and the copolymers were confirmed by Fourier transform infrared spectroscopy (FT-IR spectra, AVATAR370, NICOLET). For FT-IR analysis, the PLEL was dissolved in chloroform and cast on KBr plates [19]. The copolymer sample was measured in pellet form and diluted with KBr powder.

Thermal analysis was performed with a differential scanning calorimeter (Diamond, DSC, Perkin-Elmer). 20 mg of polymer solution sample after purification was hermetically sealed in an aluminum pan. Heating scans were recorded in the range of 20–100 °C at a scan rate of 1 °C min^{-1}. Deionized water was used as a blank reference.

The Mw of the PNIPAM–PLLA–PEG–PLLA–PNIPAM was measured by Gel Permeation Chromatography (GPC, SEC/GPC-RI-MALLS, WYATT USA). The measurement conditions of GPC methods as follows: The eluent is 0.1 mol/L NaCl solution(the solvent is high-purity water), the temperature is 25 °C, the flow is 1.0 ml/min, and the columns are used as Shodex OHpak SB (Guard column), SB-801HQ, and SB805HQ.

The gelation and gel dissolution were determined by a test tube-inverting method with temperature increments of 1 °C per step. Polymer solutions (1 ml) at various polymer components in glass vials were immersed in a thermostate water bath at a constant designated temperature for 1 h to further equilibrate. The gelation temperature was characterized visually when the polymer solution did not flow within 2 min by inverting the vials [20,21]. Generally, after the sample has equilibrated for 1 h, one can easily distinguish the states of transparent sol, opaque sol, opaque gel, and syneresis.

Ofloxacin, a water-soluble antibacterial agent, was used as a model drug in this study. The drug-loading and in vitro release experiments were preformed in a method similar to that described elsewhere [22–24]. Ofloxacin was added to microgel dispersion at 0.1 mg/ml concentrations to evaluate the release rate of the model drugs in vitro. All preparations were clear solutions. The drug-loaded microgel dispersion was incubated at 37 °C to form gel within a few seconds, and 3.0 ml of release medium (phosphate buffer saline: PBS, pH 7.4) at 37 °C was added to the preformed gel. At certain



Scheme 1. Synthesis of biodegradable crosslinking agents.