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Biodegradable in situ gel-forming controlled drug delivery system based on thermosensitive PCL–PEG–PCL hydrogel. Part 2: Sol–gel–sol transition and drug delivery behavior

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Abstract

In this work, a biodegradable and injectable in situ gel-forming controlled drug delivery system based on thermosensitive poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCEC) hydrogel was studied. The prepared PCEC hydrogel undergoes temperature-dependent sol–gel–sol transition, which is a flowing sol at ambient temperature and turns into a non-flowing gel at around physiological body temperature. Furthermore, the sol–gel phase transition mechanism was investigated using ^{13}C-nuclear magnetic resonance imaging and a laser diffraction particle size analyzer. The in vitro release behaviors of several model drugs, including a hydrophilic small-molecule drug, a hydrophobic small-molecule drug and a macromolecular protein drug, from PCEC hydrogel were also investigated in detail. The results showed that the model drugs could be released from the PCEC hydrogel system over a sustained period. In addition, an anaesthesia assay was conducted using the tail flick latency (TFL) test to evaluate the in vivo controlled drug delivery effect of the PCEC hydrogel system. In the TFL assay, a lidocaine-loaded PCEC hydrogel produced significantly longer-lasting local anaesthetic effects compared with lidocaine aqueous solution at the same dose. Therefore, PCEC hydrogel is promising for use as an injectable local drug delivery system.

Keywords: Biodegradable; Thermosensitive hydrogel; Sol–gel–sol transition; Controlled release; Anaesthesia assay

1. Introduction

Local drug delivery has attracted much attention recently. Compared with systemic delivery, local drug delivery could enhance local drug concentration, prolong local drug residence time and reduce systemic adverse drug reactions. Local drug delivery systems (LDDSs) are intended to deliver drugs at a predetermined locus for predefined periods, which might overcome the shortcomings of conventional drug formulations by reducing systemic adverse drug reactions and improving the therapeutic effects and life quality of the patients [1,2]. Thus, a suitable local controlled drug delivery system is extremely important for local delivery. An optimal LDDS would maintain the drug concentration at an effective level over a long period and would be biodegradable, biocompatible and nontoxic.

Recently, smart polymers with specific responses to various environmental stimuli, including temperature, pH and electric field, have been extensively investigated [3–8]. In particular, thermosensitive hydrogels, consisting of hydrophobic and hydrophilic blocks, have attracted increasing attention for their biomedical applications, e.g. as a drug

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delivery system, for cell encapsulation and for tissue engineering [9–16]. An injectable thermosensitive hydrogel with a lower sol–gel transition at around physiological temperature enables a pharmaceutical formulation to be easily mixed in the sol state at low temperature, which will then form a gel when injected by syringe at the target location, to work as a sustained delivery depot of the drug.

Pluronic F127, a commercial copolymer whose aqueous solution can form a thermosensitive hydrogel, has been widely used as an emulsifier, wetting agent and solubilizer [17–19]. However, the critical micelle concentration of Pluronic F127 is very high due its high polyethylene oxide (PEO) content (70%). Pluronic F127 forms a fast-eroding gel and cannot persist longer than a few hours in vivo. Furthermore, Pluronic F127 has been found to induce the toxic enhancement of plasma cholesterol and triglycerol because it is non-biodegradable and can accumulate in the body [20,21]. Thus, the wider application of Pluronic F127 in many biomedical fields has been greatly restricted. In our pervious work, we synthesized a biodegradable Pluronic F127 analog, poly(ethylene glycol)–poly(ε-caprolactone)–poly(ethylene glycol) (PEG–PCL–PEG, PECE) copolymer, and the formed hydrogel could persist for at least 2 weeks in vivo [22].

In this study, we prepared a kind of biodegradable and injectable poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL–PEG–PCL, PCEC) hydrogel that undergoes sol–gel–sol transition, which is different from the gel–sol transition behavior of PCEC hydrogel reported previously by our laboratory (due to the difference in the PEG/PCL ratio and the total molecular weight) [23]. The aqueous solution of the present PCEC triblock copolymer is a free-flowing sol at ambient temperature and becomes a gel at body temperature. The structure–property relationship of the sol–gel–sol transition, its in vitro degradation behavior and its in vitro drug release behavior are also investigated in this paper. Furthermore, the in vivo controlled drug delivery effect was evaluated by an antinociceptive assay.

Compared with the PECE triblock copolymers previously reported by our group, the PCEC copolymers have several advantages: first, PCEC copolymer can be synthesized in one step without using any coupling agent; secondly, PCEC hydrogel has a wider gel window; and thirdly, PCEC hydrogel can persist for a longer period in vivo (about 3 weeks). Both PCEC and PECE hydrogels can play important roles in the biodegradable in situ gel-forming controlled drug delivery system because they show sol–gel–sol transition, are compatible and non-toxicity, can persist for at least 2 weeks in vivo and can release drugs over an extended period. We could thus choose to use PCEC or PECE hydrogel according to the practical needs of different applications.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) (PEG, $M_n = 600$, 1000 and 1500, Fluka, USA), poly(ethylene glycol) methyl ether (MPEG, $M_n = 550$, Aldrich, USA), ε-caprolactone (ε-CL, Alfa Aesar, USA), hexamethylene diisocyanate (HMDI, Aldrich, USA), stannous octoate ($Sn(Oct)_2$, Sigma, USA), bovine serum albumin (BSA, BR, BoAo Co. Ltd., China), VB_{12} (Sigma, USA) and lidocaine (99%, ZheDaChem, China) were used without further purification. Honokiol were isolated and purified in our laboratory. All the materials used in this article were analytic reagent (AR) grade and used as received.

Sprague–Dawley (SD) rats, weighing 250 ± 20 g, were used for the tail flick latency (TFL) assay. The animals were purchased from the Laboratory Animal Center of Sichuan University and housed at a controlled temperature of 20–22 °C and a relative humidity of 50–60%, with 12 h light–dark cycles. Free access to food and water was allowed. All the animals were quarantined for a week before treatment. All animal care and experimental procedures were conducted according to Institutional Animal Care and Use guidelines.

2.2. Synthesis and purification of PCEC copolymers

PCEC triblock copolymers were prepared by ring-opening copolymerization of ε-CL initiated by PEG using $Sn(Oct)_2$ as a catalyst, as reported previously [23]. Briefly, calculated amounts of ε-CL and PEG were introduced into a dry glass ampoule under a nitrogen atmosphere, and a specific amount of $Sn(Oct)_2$ was added into the reaction vessel under mild agitation. The reaction system was kept at 130 °C for 6 h. After the mixture was degassed under vacuum for another 1 h, the resultant copolymer was cooled to room temperature. The just-obtained PCEC copolymer was first dissolved in AR-grade dichloromethane, then reprecipitated from the filtrate using AR-grade excess cold petroleum ether, after which the mixture was filtered and vacuum dried to a constant weight. The purified PCEC copolymers were kept in desiccators for further use. In this paper, the copolymers were denoted as PCL–PEG–PCL (X–Y–X) where X and Y represented the number average molecular weight (M_n) of PCL and PEG block, respectively.

For comparison, PECE copolymer was synthesized by ring-opening copolymerization of ε-CL initiated by an MPEG and HMDI coupling step, which was reported previously by our group [11,22].

2.3. Characterization of PCEC copolymers and hydrogel

2.3.1. Nuclear magnetic resonance analysis (^{1}H-NMR and ^{13}C-NMR)

^{1}H-NMR spectra (in $CDCl_3$) were recorded on a Varian 400 spectrometer (Varian, USA) at 400 MHz to characterize the chemical composition and molecular weight of the copolymer.

^{13}C-NMR spectra (in $CDCl_3$ and D_2O) were recorded on a Varian 400 spectrometer (Varian, USA) at 200 MHz.

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