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Thermosensitive porphyrin-incorporated hydrogel with four-arm PEG–PCL copolymer: Preparation, characterization and fluorescence imaging in vivo

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ABSTRACT

A biodegradable thermosensitive hydrogel based on four-arm PEG–PCL copolymer was prepared with porphyrin as a fluorescence tag. Its structure and composition were characterized by FTIR, ^{1}H NMR and GPC. Sol–gel–sol transition was evaluated by the test tube-inverting method and rheological analysis. The optical properties of hydrogel were investigated by UV–vis and fluorescence spectroscopy in vitro and by fluorescence imaging system in vivo. The results show that the thermosensitive hydrogel possesses dual function of fluorescence and injectability in vivo with good biocompatibility. Consequently it can be potentially applied in biomedical field as a visible implant for in situ monitoring.

1. Introduction

Noninvasive monitoring in vivo could benefit the design of implantable biomaterials, since the clinical implants could not been evaluated objectively and in situ by in vitro or ex vivo method. With the development of medical imaging in vivo, they play an important role in the monitoring of implants except of general diagnosis of disease. Ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) have been applied for tracking or monitoring of implants in vivo [1–3]. Although these medical imaging modalities have made some progresses in the investigation of implants in situ, fluorescence imaging as a novel imaging modality would have a wide prospect due to its low cost, high sensitivity, low-energy radiation and non-invasion [4,5]. Nowadays, fluorescence imaging has been expanded to the tracking of biomaterials from the diagnosis of disease [6–10]. Artzi et al. investigated the erosion of biodegradable hydrogels using fluorescein and Texas red as labels in vivo. Material erosion can be calculated from the decay in total material fluorescence signal with non-invasive imaging [8]. Cunha-Reis et al. monitored the degradation of chitosan for tissue engineering with tetramethylrhodamine isothiocyanate labeling and identified the dispersion pathway of the chitosan membrane degradation products in vivo [9]. Moller et al. synthesized Lucifer yellow tagged hydrogels and tracked the in vivo process by fluorescence imaging [10]. Versatile fluorescent biomaterials have been developed with significant efforts owing to their promising application in biomedical fields [11]. To enhance the sensitivity and ensure the safety of fluorescence biomaterials, novel fluorescence tags with high emission efficiency and good biocompatibility need to be further developed.

Porphyrin compounds have been paid considerable attention in biological, photo-physical, and catalytic fields due to their special optical properties from extended π-conjugated electronic macrocycle structure [12–14]. Especially in biomedical fields, porphyrins and their derivatives have been widely developed as new-generation photosensitizers, fluorescence imaging probes or oxygen carriers [15–17], because they have large visible and near infrared absorption, molar extinction constant, acceptable fluorescent quantum yields and excellent biocompatibility. Fluorescence imaging with porphyrin is one of our research interests. A porphyrin-based near-infrared fluorescence probe with simple approach was investigated for in vivo imaging in nude mice [18]. We also reported a glucose conjugate porphyrin dimmer for bioimaging [19]. Recently, porphyrin conjugated polymers attract most attention due to their precise structure, good stability, biodegradability, low immunogenicity and so on. Zhang et.al investigated a micelle base on star-shaped amphiphilic copolymer with porphyrin core for bioimaging and drug delivery [20]. Lovell et al. reported a porphyrin-cross-linked hydrogel for fluorescence-guided monitoring and surgical resection [21]. All these confirmed good biocompatibility and feasible fluorescence efficiency of porphyrin for in vivo imaging.

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Hydrogel is a hydrophilic polymer network retaining a large amount of water with a semi-solid morphology. It is widely applied in soft tissue engineering, drug delivery, surgical implant and so on [22–24]. Thermosensitive hydrogel can be easily injected to the surgical site as a flowable liquid solution and immediately turn into a standing gel after administration. Consequently it attracts considerable interest for its potential applications in the injectable controlled-delivery system and implant [25,26]. Poly(ethylene glycol)(PEG) and poly(ε-caprolactone)(PCL) have been widely exploited as the constitutive units of thermosensitive hydrogel because they are both well-known FDA-approved biodegradable materials [27,28]. The thermosensitive hydrogels based on PEG and PCL have made great progress in drug delivery, tissue repair and surgical implant [29,30]. In order to track the status of hydrogel in vivo, the combination of hydrogel and fluorescent imaging will pave an important way.

Herein a biodegradable thermosensitive porphyrin hydrogel based on four-arm PEG–PCL copolymer (POR–PEG–PCL) was prepared with porphyrin as a fluorescence tag. The structure of copolymer and the sol–gel–sol transition was evaluated. The fluorescence hydrogel was monitored by in vivo imaging system with mice as models. It possesses dual function of fluorescence and injectability, so it can be a potential biomedical implant for in situ monitoring in vivo.

2. Experimental section

2.1. Materials

Poly(ethylene glycol) (PEG,M_n = 1000, Merck) was vacuum-dried at 60 °C for 12 h before use. ε-Caprolactone(ε-CL,Aladin) was purified by vacuum distillation. Stannous octoate ($Sn(Oct)_2$,Aladin) and other reagents were all analytical reagent (AR) grade.

Kunming mice (seven weeks old, 20–25 g) were used. All the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, China. Animals had free access to food and water.

2.2. Synthesis of POR–PEG–PCL copolymer

POR–PEG–PCL copolymer was synthesized by ring-opening copolymerization of ε-CL initiated by porphyrin-conjugated PEG using stannous octoate as catalyst according to Fig. 1. PEG 1000 (4 g) was reacted with 5,10,15,20-tetra(4-carboxyphenyl)porphyrin (20 mg) in DMF (20 mL) at room temperature for 24 h under the catalysis of EDC and DMAP. The reacted solution was washed with water, precipitated with cool ether and dried under vacuum to give the crude porphyrin-conjugated PEG. Without further purification, the crude porphyrin-conjugated PEG, ε-CL (4 g) and $Sn(Oct)_2$ (0.1 g) were polymerized in a polymerization tube under a vacuum at 120 °C for 24 h. The mixture was dissolved with dichloromethane, then precipitated with cold petroleum ether, filtrated and dried to provide the POR–PEG–PCL copolymer.

2.3. Characterization of POR–PEG–PCL copolymer

Infrared spectra were recorded on a Nicolet 2000 instrument from 4000 to 400 nm^{-1}. The copolymer samples were cast on KBr plates for infrared spectra analysis. ^1H NMR spectra were recorded on a Varian Mercury instrument at 300 MHz using $CDCl_3$ as solvent and TMS as an internal reference. Gel Permeation Chromatography (GPC) of Agilent 110 HPLC was used to determine molecular weight and polydispersity of the copolymers. The samples were dissolved in DMF at a concentration of 2 mg/mL. The molecular weights of samples were calculated from PMMA standard samples with narrow molecular weight distribution. Differential scanning calorimetry (DSC) of Thermo Fisher from USA was used to analyze the thermal properties of the copolymers at a temperature range from 0 to 80 °C under a nitrogen atmosphere at a heating and cooling rate of 5 °C min^{-1}.

2.4. Sol–gel–sol phase transition behavior

Sol–gel–sol phase transition behaviors of POR–PEG–PCL copolymers were observed using the test tube-inverting method in a 4 mL tube at a concentration of 40% with a heating rate of 1 °C/min from 10 °C to

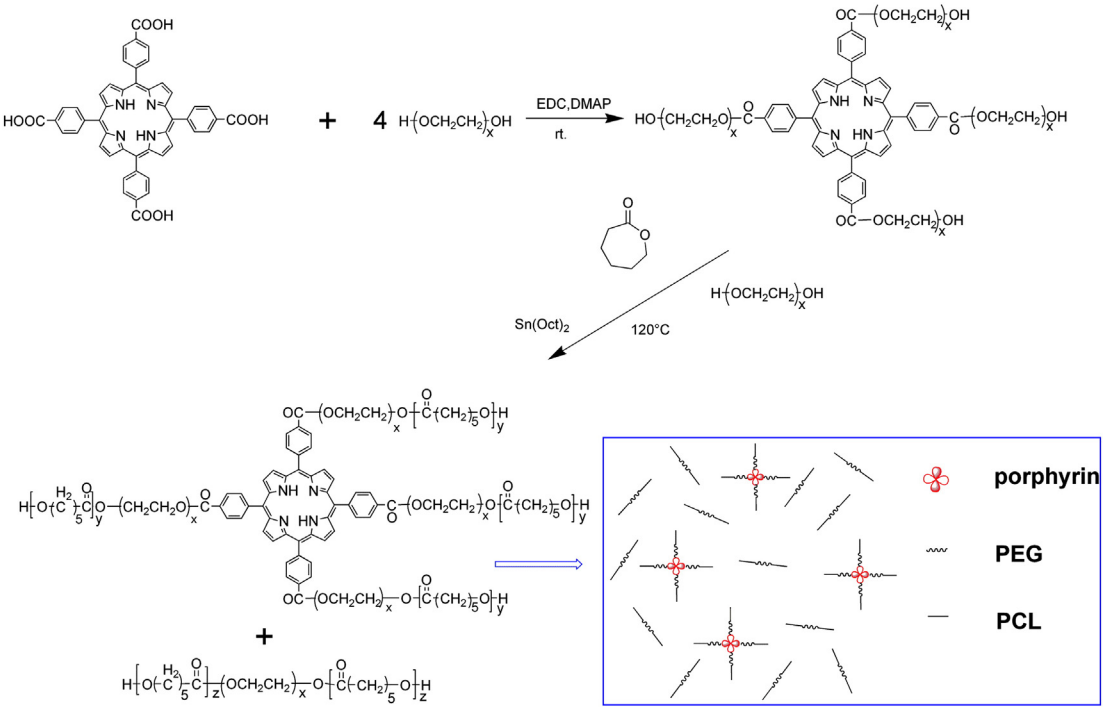

Fig. 1. Synthetic route of porphyrin-incorporated hydrogel.