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Novel thermosensitive hydrogels based on methoxy polyethylene glycol-co-poly(lactic acid-co-aromatic anhydride) for cefazolin delivery

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Abstract

Thermosensitive micelles composed of a copolymer of methoxy polyethylene glycol (mPEG), polylactic acid (PLA), and 1,6-bis (p-carboxyphenoxy) hexane (CPH), namely methoxy polyethylene glycol-co-polylactic acid-co-aromatic anhydride (mPEG-PLCPHA), were fabricated for application as a promising hydrophilic drug carrier. The copolymer can self-assemble into micelles in PBS by hydrophobic interaction. The diameters of these micelles increased as the environmental temperature increased. An increase in viscosity with sol-to-gel transition occurred as temperature increased from room temperature to body temperature. During the *in vitro* degradation process, hydrogels demonstrated a more stable degradation rate. Both *in vitro* and *in vivo* cytotoxicity results showed that the materials had excellent biocompatibility due to less acidic products formation. *In vitro* cefazolin release profiles showed a stable release for 30 days. The hydrogel encapsulated cefazolin exhibited a good antibacterial effect. Based on these results, mPEG-PLCPHA can serve as an injectable depot gel for drug delivery.

From the Clinical Editor: In this study, thermosensitive hydrogel encapsulated cefazolin was found to exhibit good antibacterial effects with sustained levels for up to 30 days, enabling the development of an injectable depot gel for long-term drug delivery.
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Key words: Antibiotics; Biodegradable; Drug delivery; Hydrogel; Micelle; Thermosensitive

Cefazolin sodium has been used to treat bone and joint infections.[1] However, since the release of cefazolin sodium occurs very quickly,[2] a requirement for sustained-release should be met by special dosage form. To this purpose, a novel thermosensitive hydrogel was used as a sustained-release carrier.

Thermosensitive amphiphilic copolymers can self-assemble into micelles or hydrogels in aqueous environment at different temperature, depending upon their concentration.[3] Thermosensitive hydrogel is called a 'smart material', because it can respond to environmental changes. Specifically, it is a sol at room temperature but forms a gel at body temperature, above certain concentrations. It has been widely used in biomedical applications, such as an *in situ* injectable and also for stable carriers for both hydrophilic and hydrophobic drugs.[4]

Several amphiphilic biodegradable copolymers have used. For example, aliphatic polyesters, such as poly (ε-caprolactone) (PCL),[5] poly (D,L-lactide) (PLA),[6] poly (glycolide) (PGA),[7] and their copolymer, poly (lactide-co-glycolide) (PLGA),[8] were used as hydrophobic segments, and polyethers (such as polyethylene glycol (PEG)) as hydrophilic segments. The thermosensitivity of these micelles can be modulated by altering the concentration of their aqueous solution or the composition of the copolymers. Their thermoresponsive and biodegradable properties make them potential candidates for cell therapy, drug delivery and tissue engineering via *in situ* injection.[9] However, certain drawbacks prevent their wider application, such as low cell adhesion and local acidity.[10] Acidic environment due to the formation of acids from the degradation of these materials is deleterious to bioactive proteins or cells encapsulated and may cause an *in vivo* non-bacterial inflammation in the surrounding tissue.

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The micelles and hydrogels formed by mPEG-PLA have shown to give initial burst release.[11] In contrast, mPEG-aromatic polyanhydride micelles have a more stable release profile.[12–14] However, mPEG-aromatic polyanhydride is less thermoresponsive. Polyanhydrides have been approved by the United States Food and Drug Administration for clinical use, with demonstrated degradation, metabolism, and excretion in humans.[15] The aromatic polyanhydride acids, in particular, exhibit moderate pH microenvironments, superior protein stabilization, and better mechanical properties and stability, along with longer release and degradation times.[16,17]

A novel biodegradable copolymer was synthesized by polymerization of aromatic polyanhydrides, methoxy polyethylene glycol (mPEG) and polyesters. Our aim was to manufacture a new type of thermosensitive methoxy polyethylene glycol-co-polylactic acid-co-aromatic anhydride (mPEG-PLCPHA) copolymer for stable drug release. The thermosensitive, mechanical, biodegradable and biocompatible properties of this copolymer were evaluated. We also validated mPEG-PLCPHA copolymer as an antibiotic carrier by bacterial inhibition assay of the encapsulated drug.

Methods

Materials

The mPEG (Mn = 550 g/mole), acetic anhydride, and stannous 2-ethylhexanoate (stannous octoate) were obtained from Sigma Aldrich (St. Louis, USA). DL-lactide acid (LA) was purchased from Purac (Lincolnshire, USA). 1,6-Dibromohexane was supplied from Acros (New Jersey, USA), 1,6-diphenyl-1,3,5-hexatriene (DPH) and live and dead cell double-staining kit were purchased from Fluka (Buchs, Switzerland). The p-hydroxybenzoic acid was supplied by Lancaster (Lancashire, UK). The FITC Annexin V Apoptosis Detection Kit was purchased from BD Pharmingen™ (New Jersey, USA). All reagents were analytical grade.

Synthesis of amphiphilic diblock copolymer

Details on synthesis and characterization of amphiphilic diblock copolymer are given in Supplementary data, S1.

Thermosensitive micelle determination

Micelle size, zeta potential, and morphology

The size and electrokinetic potential of thermosensitive micelles were measured using a spectrophotometer (Nano Series ZetaSizer, Malvern; Worcestershire, UK). The micelle solution, at a concentration of 0.1 wt%, was filtered through a 0.45-mm filter membrane and then stored at 4 °C, 25 °C and 37 °C, respectively, before measurement. The particle sizes were calculated using the Stokes-Einstein equation[18] and the zeta potential was obtained using the Helmholtz-Smoluchowski equation.[19]

^{1}H-NMR spectroscopy, with D_2O as the solvent at room temperature, was used to confirm micelle formation. The micelle morphology was characterized by transmission electron microscope (TEM, Hitachi H-7500; Tokyo, Japan). Samples were prepared by placing a drop of 0.1 wt% solution on a Formvar-coated copper TEM grid at 4 °C, 25 °C, and 37 °C, respectively. The specimens included drug-free micelles and cefazolin-loaded micelles (cefazolin loading is described in *in vitro* cefazolin release).

Critical micellization concentration

The critical micellization concentration (CMC) was determined using the dye solubilization method. A 1 wt% mPEG-PLCPHA solution and cefazolin loaded mPEG-PLCPHA solution were serially diluted by a factor of 2 into 16 vials (from 1 wt% to 3.05×10^{-5} wt%). Fluorescent dye (DPH) was used at a concentration of 0.4 mM; 2 μL DPH solution was mixed with 100-μL diblock copolymer solution and then stored overnight at 4 °C, 25 °C and 37 °C, respectively. The reaction proceeded in the dark. Using an enzyme-linked immunosorbent assay (ELISA) reader set at an excitation wavelength of 360 nm and emission wavelength of 480 nm, the ratio of fluorescence intensity was plotted against the logarithm of copolymer concentrations to determine the CMC.

Thermosensitive hydrogel

Sol-gel transition

The sol-gel transition profiles were investigated using the 'tube-flipped-upside-down' method. All samples were prepared in Eppendorf tubes and incubated at 4 °C until the temperature achieved equilibrium. Different weight concentrations of mPEG-PLCPHA solution (10%, 15%, 20%, 25%, and 30%) were prepared without cefazolin and incubated at 4 °C. An identical set of solutions of mPEG-PLCPHA was also prepared to which 20 mg/mL cefazolin was added. The temperature was then raised by intervals of 2 °C and maintained for 5 min before each sampling. The Eppendorf tubes were flipped upside down for 30 s to observe any movement so as to determine the sol-gel status. The temperatures of sol-to-gel and gel-to-sol transformation were recorded to produce a phase diagram using Gaussian regression.

The mechanical properties of sol-gel transition

The mechanical properties of hydrogels, with or without cefazolin, were measured using a rheometer (AR2000 EX system, TA Instruments; New Castle, USA) with the temperature controller set from 5 °C to 40 °C at a heating rate of 2.2 °C/min. Polymeric solutions of 15 wt%, 20 wt%, 25 wt%, and 30 wt% with 20 mg/mL cefazolin were added to the instrument to analyze the rheological behavior of the sol-gel transition. The temperature of initial G' (storage modulus) higher than G" (loss modulus) was defined as the phase transition temperature. The viscosity was measured along with the heating process. The experiments were done in triplicate. The maximal values were analyzed using two-tailed Student's t test. $P < 0.05$ was designated as statistically significant.

In vitro degradation and pH value change of hydrogel

Three different concentrations of the copolymer mixture (15 wt%, 20 wt%, and 25 wt%; 0.3 mL) were injected into release bottles and incubated in a shaking bath at 37 °C. After 30 min, 1.5 mL of PBS solution (pH 7.4) was added to the formed gels. At a predetermined time (0, 3, 6, 10, 15, 20, 25, and 30 days), three samples were taken out to assess weight loss. The

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