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NANOMEDICINE

Novel self-assembled core–shell nanoparticles based on crystalline amorphous moieties of aliphatic copolyesters for efficient controlled drug release

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ABSTRACT

Poly(propylene succinate-co-caprolactone) copolymers [P(PSu-co-CL)] with different ε-caprolactone (ε-CL) to propylene succcinate (PSu) monomer ratios were synthesized using ring opening polymerization. These polymers consisted of crystalline poly(ε-caprolactone) (PCL) and amorphous poly(propylene succinate) (PPSu) moieties, as shown by WAXD. In vitro biocompatibility studies showed that these copolyesters are biocompatible. Drug-loaded nanoparticles, using tibolone as a model drug, were prepared by the solvent evaporation method. Nanoparticle size ranged between 150 and 190 nm and decreased with increasing propylene succinate (PSu) ratio in the copolymers. Nanoparticle yield, encapsulation efficiency, and drug loading increased with increasing PSu ratio. Scanning Electron Microscopy (SEM) revealed that the prepared nanoparticles had a spherical shape and Transmission Electron Microscopy (TEM) showed that they were self-assembled in core–shell structures. Amorphous PPSu and crystalline PCL comprised the core and shell, respectively. The drug is mainly located into the amorphous core in the form of nanocrystals. Drug release studies showed that complete release of the drug from the nanoparticles occurs over a period of 50 h. The release rate is greatly influenced by the copolymer composition, nanoparticle size, and encapsulation efficiency. Among the main advantages of the nanoparticles produced in this study is the absence of burst effect during drug release.

1. Introduction

During the past few decades there has been an increasing interest in the development of biodegradable nanoparticles for effective drug, peptide, protein and DNA delivery [1]. Incorporation of the drug into a particulate carrier can protect the active substance against degradation in vivo and in vitro, improve therapeutic effect, prolong biological activity, control drug release rate, and decrease administration frequency [2,3]. One of the major problems associated with the use of nanoparticles as polymeric drug carriers is their rapid elimination from the blood stream through phagocytosis after intravenous administration and recognition by the macrophages of the mononuclear phagocyte system [4]. In order to maintain the required level of the active substance in the blood stream for longer time periods, long-circulating polymer nanoparticles may be designed and used [5–7]. Currently, one of the most used techniques to prepare such nanoparticles is PEGylation [8–16], in which a large number of polymer structures can be used as the hydrophobic segment [17]. Poly(ε-caprolactone) (PCL), owing to its biodegradable/biocompatible characteristics and advantage of being high permeable to drugs, has attracted attention and became an important candidate for drug delivery applications [18]. However, the application of PCL as drug delivery system reveals certain drawbacks, such as slow rate of biodegradation in human tissue due to the polymer's high degree of hydrophobicity and crystallinity [19], and very slow drug release rates. For example, the release of the whole amount of a drug encapsulated in PCL often requires weeks or months. In order to overcome these problems it is essential to modify the polymer properties in a way that meets the requirements of the drug release application. The most common and relatively easy way to enhance the properties of PCL is by means of copolymerization or blending with other polymers. In the present study, improvement of the drug release properties of PCL is attempted through the synthesis of PCL copolymers with poly (propylene succinate) (PPSu).

Poly(propylene succinate), a relatively new biodegradable polyester that is fast biodegradable [20] and biocompatible [21], can be produced from monomers derived from renewable sources using environmentally friendly methods and a variety of microorganisms [22]. PPSu is a very soft material with a low melting point ($T_m = 44$ °C) and glass transition temperature ($T_g = -36$ °C) and exhibits high biodegradation rate due to its lower degree of crystallinity [23].

In the present study, copolymers of ε-CL with propylene succinate (PSu) were synthesized, characterized, and used as candidate polymers towards the development of effective nanoparticle drug delivery systems. Our main objective is to prepare nanoparticles without the appearance of burst effects that can release the drug over a relatively short period of time, typically less than 2 days.

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Tibolone was used as a model hydrophobic drug to be encapsulated into the polymeric nanoparticles. Tibolone, [(7a,17a)-17-hydroxy-19-nor-17-pregn-5(10)-en-20-yn-3-one], is a synthetic steroid known to have combined oestrogenic, progestogenic and androgenic characteristics and is structurally related to the progestogens norathindrone and norethynodrel. It is a known tissue specific and effective agent that can be used in hormone replacement therapy (HRT) in (post)menopausal woman, for the treatment of menopausal and postmenopausal disorders, including climacteric complaints, vasomotor symptoms, osteoporosis and vaginal atrophy [24–27].

2. Experimental

2.1. Materials

ε-Caprolactone (ε-CL) (99% Sigma-Aldrich) was dried over CaH_2 and purified by distillation under reduced pressure prior to use. Succinic acid (purum 99+%), 1,3-Propanediol (1,3-PD) (purity: >99.6%) and Tetrabutyl Titanate (TBT), used as catalyst, were purchased from Aldrich Chemical Co. Polyphosphoric acid (PPA) used as heat stabilizer was supplied from Fluka. Sodium cholate was obtained from Sigma. Crystalline tibolone (TIBO) with an Assay of 99.59% was supplied from Zhejiang Xianju Junye Pharmaceutical Co. LTD.

2.2. Synthesis of polyesters

2.2.1. Synthesis of PPSu and polycaprolactone (PCL)

Synthesis of aliphatic polyester PPSu was performed following the two-stage melt polycondensation method (esterification and polycondensation) in a glass batch reactor [28]. The bulk polymerization of ε-caprolactone in order to synthesize neat PCL, was carried out in 250 cm^3 round-bottomed flask equipped with a mechanical stirrer and a vacuum apparatus as described elsewhere [29].

2.2.2. Synthesis of P(PSu-co-CL) copolymers

P(PSu-co-CL) copolymers with various molar ratios, such as 2.5/97.5, 5/95, 10/90, and 75/25, were synthesized according to the procedures described by Seretoudi et al. [29]. Briefly, purified PPSu was added into the same apparatus used for PCL synthesis and the proper amounts of ε-CL monomer were added as well as TBT (1×10^{-4} mol TBT/mol ε-CL). Polymerization took place at 180 °C under nitrogen flow and a stirring rate of 500 rpm while the reaction was completed after 2 h. Unreacted monomer was removed through distillation by applying a high vacuum (≈5 Pa) over a time period of 15 min. Polymerization was stopped by rapid cooling to room temperature.

2.3. Polymer characterization

Intrinsic viscosity [η] measurements on the isolated polymers were performed using an Ubbelohde viscometer cap. Oc at 25 °C in chloroform at a solution concentration of 1 wt.%.

Molecular weight determinations were performed by gel permeation chromatography (GPC) method using a Waters 150C GPC equipped with differential refractometer as detector and three ultrastyragel (103, 104, 105 Å) columns in series. Tetrahydrofuran (THF) was used as the eluent (1 ml/min) and the measurements were performed at 35 °C. Calibration was performed using polystyrene standards with a narrow molecular weight distribution.

^{1}H NMR and ^{13}C NMR spectra of polyesters were obtained with a Bruker spectrometer operating at a frequency of 400 MHz for protons. Deuterated chloroform ($CDCl_3$) was used as solvent in order to prepare solutions of 5% w/v. The number of scans was 10 and the sweep width was 6 kHz.

A Setaram DSC141 differential scanning calorimeter (DSC), calibrated with indium and zinc standards, was used, for the identification of the thermal properties of the copolyesters. A sample of about 10 mg was used for each test, placed in an aluminium pan and heated from −100 °C up to 100 °C at a heating rate of 20 °C/min. The sample remained at that temperature for 5 min in order to erase any thermal history. After that it was cooled down to −100 °C at a cooling rate of −150 °C/min and scanned again (second heating) up to 100 °C using 20 °C/min as heating rate. The glass transition temperature (T_g), the melting temperature (T_m) and crystallization temperature (T_c) were recorded.

Wide Angle X-Ray Diffractrometry (WAXD) was used for the identification of the crystal (structure and changes) of the polymers and also of the drug used in case of nanoparticle samples. WAXD study was performed over the range 2θ from 5 to 50 °C, using a Philips PW 1710 diffractometer with Bragg–Brentano geometry (θ, 2θ) and Ni-filtered CuKa radiation.

2.4. Biocompatibility study of the prepared polyesters

2.4.1. Cell culture

Human umbilical vein endothelial cells (HUVEC) were grown routinely in RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS), 15 mg ECGS, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin and 2.5 μg/ml amphotericin B. The cultures were maintained at 37 °C, 5% CO_2 and 100% humidity.

2.4.2. In vitro biocompatibility study

The biocompatibility of P(PSu-co-CL) was evaluated by measuring the viability of HUVEC cells in the presence of different polymer concentrations and comparing results with those obtained from poly (lactic acid) (PLA). Cell viability was determined by means of the MTT assay. HUVEC cells were seeded in 24-well plates at a density of 30,000 cells per well in 500 μl cell culture medium. Twenty-four hours after plating, different amounts of P(PSu-co-CL) nanoparticles (suspended in water) were added in the wells. After 24 h of incubation at 37 °C, 50 μl of MTT solution (5 mg/ml in PBS pH 7.4) was added into each well and plates were incubated at 37 °C for 2 h. The medium was withdrawn and 200 μl acidified isopropanol (0.33 ml HCl in 100 ml isopropanol) was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read by a microplate reader (Biorad, Hercules, CA, USA) at 490 nm wavelength. The experiments were performed in triplicates. Biocompatibility of polymers was expressed as % cell viability, calculated as the ratio between the number of cells treated with the nanoparticles and that of non-treated cells (control).

2.5. Preparation of P(PSu-co-CL) nanoparticles loaded with tibolone

P(PSu-co-CL) copolymer nanoparticles were prepared by o/w solvent evaporation method. Copolymer (50 mg) and tibolone (5 mg) were dissolved in 2 ml of dichloromethane. The solution was transferred to an aqueous solution of sodium cholate ($V=6$ ml, $C=12$ mV) and it was probe-sonicated for 1 min [30]. The o/w emulsion formed was gently stirred until the evaporation of the organic solvent was complete. Nanoparticles were purified by centrifugation (9500 rpm for 20 min). The samples were reconstituted with deionized water. Polymer aggregates were removed by filtering the suspension through a 1.2 μm pore size microfilter.

2.6. Characterization of drug-loaded nanoparticles

2.6.1. SEM measurements

The morphology of the prepared nanoparticles was examined with a Scanning Electron Microscope (JEOL, JMS-840). The samples were coated with carbon black to avoid charging under the electron beam. Operating conditions were: accelerating voltage 20 kV, probe current 45 nA, and counting time 60 s.