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PHEA-graft-polybutylmethacrylate copolymer microparticles for delivery of hydrophobic drugs

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ABSTRACT

Polymeric microparticles encapsulating two model hydrophobic drugs, beclomethasone dipropionate (BDP) and flutamide (FLU) were prepared by using the high pressure homogenization-solvent evaporation method starting from a oil-in-water emulsion.

For the preparation of polymeric microparticles a α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) graft copolymer with comb like structure was properly synthesized via grafting from atom transfer radical polymerization (ATRP) technique, by using two subsequent synthetic steps. In the first step a polymeric multifunctional macroinitiator was obtained by the conjugation of a proper number of 2-bromoisobutyryl bromide (BIB) residues to the PHEA side chains, obtaining the PHEA-BIB copolymer. PHEA-BIB copolymer was then used as macroinitiator for the polymerization via ATRP of the hydrophobic monomer such as butyl methacrylate (BMA) to obtain the α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide-co-(N-2-ethylen-isobutyrate)-graft-poly(butyl methacrylate) copolymer (PHEA-IB-p(BMA)). Spherical microparticles with 1–3 microns diameter were prepared. Microparticles loaded with BDP or FLU were also prepared. In vitro mucoadhesion and enzymatic degradation studies evidenced bioadhesive properties and biodegradability of prepared microparticles, while release studies showed a different release profiles for the two loaded drugs: BDP was totally released from nanoparticles until 24 h in pulmonary mimicking conditions; differently a slower FLU release rate was observed in gastro-intestinal mimicking conditions. The in vitro cytotoxicity activity was assessed using 16HBE and Caco-2 cell lines. Results showed that exposure of both cell lines to BDP-loaded microparticles do not inhibited the cell growth; on the contrary FLU-loaded microparticles inhibited the cell growth, in particular of the Caco-2 cancer cell line, in a concentration- and time-dependent manner. Finally, uptake studies demonstrated that BDP-loaded microparticles and FLU-loaded microparticles effectively increased uptake of loaded drugs in a time-dependent manner, respectively on 16HBE and Caco-2 cell lines.

1. Introduction

Polymeric micro- and nanoparticles are being increasingly investigated for producing drug delivery systems able to give sustained drug release and targeting delivery. The advantages of using micro- or nanoparticulate drug delivery systems include: the possibility to maintain drug concentration in patient's blood and/or tissues at an active level for an extended time and a biodistribution and permeation through biological barriers and cellular uptake strictly dependent to the micron and submicron dimensions. These properties can be translated into an increased bioavailability of the encapsulated drug. In many cases, microencapsulation remains the most important formulation strategy for many bioactive substances, in particular for hydrophobic drugs (Anton et al., 2012; Wischke and Schwendeman, 2008; Purvis et al., 2006; Siepmann and Siepmann, 2006).

The principal requirement for fabricating controlled-release drug delivery systems is the availability of an appropriate material, which must be absolutely harmless to the organism and possess the necessary physical–chemical, mechanical and biomedical properties, including degradability in biological media; thus, the selection of the ideal polymer for microencapsulation is not immediate. While a wide variety of polymeric particulate carriers have been devised (Kumar, 2000) to protect active molecules from inactivation by the host and to control drug release in body fluids, a special attention should be paid to the biodegradability of polymers in order to prevent local or chronic toxicity that could be encountered after administration of non-biodegradable polymers

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(Wischke and Schwendeman, 2008). On another hand, the physico-chemical characteristics of the polymer used for microparticles production may also influence drug bioavailability. At this regard, for example, uptake of nanoparticles prepared from hydrophobic polymers seems to be higher than that obtained by particles with more hydrophilic surfaces, while more hydrophilic particles may be rapidly eliminated (Jung et al., 2000).

Microspheres and microparticles have been manufactured by various techniques, including solvent evaporation and phase separation (Wischke and Schwendeman, 2008), using non-solvent addition. One of the most simple and commonly employed method, is the high pressure homogenization-solvent evaporation method starting from a oil-in-water emulsion, that showed a good encapsulation rate of water insoluble compounds. For this reason, it was employed in the present study for producing polymeric microparticles encapsulating two model hydrophobic drugs, beclomethasone dipropionate and flutamide.

Beclomethasone dipropionate (BDP) is a synthetic chlorinated glucocorticoid diester, highly hydrophobic and thus, poorly soluble in water (49 μg/mL at 25 °C) (DrugBank, 2008). It is commonly used by inhalation in the treatment of asthma, and its therapeutic regimen in human generally recommends 3–4 doses of ≤200 μg daily, which indicates the relatively short local duration of action after administration.

Differently, flutamide (FLU) is a nonsteroidal antiandrogen. It exerts its antiandrogenic action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen in target tissues or both. Being prostatic carcinoma an androgen-sensitive tumor, flutamide is primarily indicated in the treatment of this pathology. Like BPD, FLU is a poorly soluble in water drug (9.5 μg/mL at 25 °C) (DrugBank, 2008) and it has a serum half-life of about 6 h. Consequently the original dosing schedule for this medication is established at minimum 2 times daily (Murphy et al., 2004).

For the preparation of polymeric microparticles a α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) graft copolymer (PHEA-IB-p(BMA)) was properly synthesized via grafting from ATRP technique (Cavallaro et al., 2009), characterized and subsequently used to obtain microparticles by the high pressure homogenization-solvent evaporation technique.

The obtained microparticles have been evaluated in vitro in term of biodegradability, biocompatibility and mucoadhesivity properties in order to evaluate their use as drug delivery systems for pulmonary delivery of BDP and oral delivery of FLU.

2. Experimental

2.1. Materials and methods

α,β-Poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) was prepared and purified according to the previously reported procedure (Giammona et al., 1987; Mendichi et al., 2000). Spectroscopic data (FT-IR and ^{1}H NMR) were in agreement with attributed structure (Giammona et al., 1987; Mendichi et al., 2000): ^{1}H NMR (300 MHz, D_2O, 25 °C, δ): 2.82 (m, 2H, –CH–**CH_2**–CO–NH–), 3.36 (t, 2H, –NH–**CH_2**–CH_2–OH), 3.66 (t, 2H, –CH_2–**CH_2**–OH), 4.72 (m, 1H, –NH–**CH**–CO–CH_2–). PHEA average molecular weight was 48.0 kDa (Mw/Mn = 1.66) based on PEO/PEG standards, measured by size exclusion chromatography (SEC). The SEC protocol involved using two Phenogel columns from Phenomenex (104R and 103R) connected to a Water 2410 refractive index detector and using a 0.01 M LiBr DMF solution as eluent with a flow of 0.8 mL/min. The column temperature was set at 50 °C. The ^{1}H NMR spectra were recorded in D_2O (Aldrich) using a Bruker Avance II 300 spectrometer operating at 300 MHz. Centrifugations were performed using a Centra MP4R IEC centrifuge. Sample centrifugations were performed at 4 °C and 8000 rpm for 10 min. Triethylamine (TEA), SEC polyethylene glycol standards, methanol (MeOH), polyvinyl pyrrolidone (PVP) 30 kDa were purchased from Fluka (Switzerland). 2-Bromoisobutyryl bromide (BIB), butyl methacrylate, 2,2′-bipyridine (bpy, 99%), copper(I) bromide (Cu^IBr 99.999%), dimethylacetamide (DMA), dimethylformamide (DMF), beclomethasone dipropionate (BDP) and flutamide (FLU) were purchased from Aldrich and were used as received. SpectraPor dialysis tubing was purchased from Spectrum Laboratories, Inc. (Italy).

2.2. Synthesis of α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide-co-(N-2-ethylen-isobutirrate)-graft-poly(butyl methacrylate) (PHEA-IB-p(BMA)) copolymer

Derivatization of PHEA with 2-bromoisobutyryl bromide (BIB) to obtain PHEA-BIB multifunctional macroinitiator was carried out using the protocol described previously (Cavallaro et al., 2009). The product was obtained with a yield of 95 wt.%, based on the starting PHEA. The degree of derivatization (DD), determined by ^{1}H NMR spectroscopy in D_2O and calculated according to the method reported elsewhere (Cavallaro et al., 2009), was 35 mol%.

The homopolymerization of butyl methacrylate, using PHEA-BIB as the macroinitiator, was carried out according to a previously reported procedure (Cavallaro et al., 2009), by modifying some reaction parameters. Briefly, the reaction of PHEA-BIB with butyl methacrylate (being molar ratio between butyl methacrylate and BIB residue equal to 10) was carried out in a previously degassed 1:1 DMF/water (v/v) solvent mixture at 50 °C for 20 h; Cu^IBr catalyst (25.5 mg, being the molar ratio between Cu^IBr and BIB linked group equal to 1) and bpy ligand (101 mg, being the molar ratio between bpy ligand and BIB linked group equal to 4) were then added to the flask under argon. Reaction was stopped by keeping reaction mixture in contact with air oxygen until the complete oxidation of copper. The reaction mixture was added drop-wise into double distilled water and the resulting solid residue was washed twice in a 1:1 H_2O/MeOH solvent mixture in order to eliminate the great part of unreacted butyl methacrylate and other reaction impurities. The white residue, obtained after centrifugation, was suspended in double distilled water and its purification was completed through exhaustive dialysis using a SpectraPor dialysis tubing with 12,000–14,000 molecular weight cutoff. After dialysis the suspension was freeze-dried from water. Obtained PHEA-IB-p(BMA) copolymer was characterized by ^{1}H NMR and spectroscopic data were in agreement with the previous results (Cavallaro et al., 2009).

2.3. Microparticle preparation from PHEA-IB-p(BMA)

Microparticles starting from PHEA-IB-p(BMA) graft copolymer were prepared by high pressure homogenization-solvent evaporation method. An organic phase was prepared by dispersing PHEA-IB-p(BMA) graft copolymer (typical concentration: 20 mg/mL) in chloroform. This organic solution was added to 20 mL of an aqueous phase, containing PVP 1.5% (w/v) and Pluronic F68 0.25% (w/v), and a primary o/w emulsion was obtained by using an Ultra-Turrax (T 25, Janke & Kunkel Ika-Labortechnik) for 20′ at 24,000 rpm. This emulsion was broken down into nano-droplets by applying external energy (500 bars, through a homogenizer) for six cycles. In particular, homogenization was performed using an EmulsiFlex^{TM}-C5 high pressure homogenizer (Avestin Inc., Canada), equipped with Totem CCS 338 (FIAC, Italy) air compressor. Then, the extraction of the solvent was achieved by evaporation under reduced pressure. As a consequence of this extraction, microparticle hardening occurred. Obtained microparticles were purified from PVP and Pluronic F68 by dialysis for 48 h. Finally,