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A poly(ether-ester) copolymer for the preparation of nanocarriers with improved degradation and drug delivery kinetics

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ABSTRACT

This paper reports the synthesis and the physicochemical, functional and biological characterisations of nanocarriers made of a novel di-block biodegradable poly(ether-ester) copolymer. This material presents tunable, fast biodegradation rates, but its products are less acidic than those of other biosorbable polymers like PLGA, thus presenting a better biocompatibility profile and the possibility to carry pH-sensitive payloads. A method for the production of monodisperse and spherical nanoparticles is proposed; drug delivery kinetics and blood protein adsorption were measured to evaluate the functional properties of these nanoparticles as drug carriers. The copolymer was labelled with a fluorescent dye for internalisation tests, and rhodamine B was used as a model cargo to study transport and release inside cultured cells. Biological tests demonstrated good cytocompatibility, significant cell internalisation and the possibility to vehiculate non-cell penetrating moieties into endothelial cells. Taken together, these results support the potential use of this nanoparticulate system for systemic administration of drugs.

1. Introduction

The efficacy of systemically administered therapeutic agents is sometimes limited by rapid clearance, unspecific distribution outside the target organ or tissue, and poor penetration of biological barriers. For all these reasons, the use of multifunctional carriers to protect the active molecules, to transport the drug through cell membranes and biological barriers, and to target specific cell types and tissues is often advocated as a strategy to deliver pharmacological therapies mere effectively. Nanocarriers can improve biodistribution [1] of therapeutic agents, resulting in an increase of therapeutic index and reduction of side effects [2]. In addition, they may be used for the preparation of multifunctional systems [3,4] for targeted drug delivery [5,6], or for the co-administration of multiple drugs [7–9]. Other important advantages include their ability to improve bioavailability of poorly water-soluble drugs [10,11] and to protect short-lived therapeutic agents, like peptides and oligonucleotides, from enzymatic reduction. Lastly, nanocarriers can be used for the delivery of drugs to organs and tissues that are not easily accessible by conventional therapeutics, like the central nervous system (CNS) that is protected by the blood–brain barrier (BBB) [12,13]. A promising approach to the development of nanoparticles for drug delivery is the use of biodegradable polymers. Indeed, these polymers are broken up to small molecules in the physiological environment and can be readily bioresorbed. Biodegradable polymers like poly(lactide-*co*-glycolide) (PLGA) and poly(ε-caprolactone) (PCL) have been already approved by the Food and Drug Administration for the preparation of biomedical devices, and have been employed to synthesise nanoparticles for drug delivery applications [14,15]. PLGA is the most common biodegradable polymer for the preparation of nanocarriers with potential applications in the treatment of CNS disease [16–19], cancer [20–24], tuberculosis [25,26]. However, fast degrading polyesters like PLGA may saturate the clearance capacity of the tissue, and the release of degradation products can lead to the acidification of the surrounding microenvironments [27] causing instability of pH-sensitive cargos, inflammatory responses and, in some cases, cytotoxicity. Slowly degrading materials like PCL, on the other hand, do not present this problem, but their degradation kinetics are too slow for practical applications to drug delivery. In order to make degradation kinetics more compliant with those requested for a drug carrier, PCL can be chemically modified to increase hydrophilicity and chain softness, with the aim to obtain faster clearance of the polymeric component. Several examples of block copolymers of PCL with polyethylene glycol [28–31], α-tocopheryl polyethylene glycol succinate [32], poly(N-isopropylacrylamide) [33], poly(N-vinylpyrrolidone) [34], xyloglucan [35], or grafted with oligomeric chains [36] to obtain micellar nanoparticles, are reported in the literature; some efforts were also made to modify the polyester PCL block by introducing a second monomer

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such as glycolide [37] or other monomers [38]. These literature reports highlighted significant variations of hydrophilicity, drug delivery profiling and, in some cases, increased bio- and cytocompatibility with respect to PCL.

Recently we have reported the synthesis and the characterisation of the copolymer metoxy polyethyleneglycol-*block*-poly(ε-caprolactone-*co*-4-hydroxyvalerolactone) (mPEG-*block*-P(CL-*co*-4HV)), a novel material with properties that fill a gap in the available range of biodegradable polymers [39]. Indeed, this copolymer showed biodegradation rates comparable with PLGA, but its degradation products were less acidic than those of PLGA, thus resulting in an improved safety profile and compatibility with a wider range of cargoes, including pH-sensitive moieties and peptides.

Here, we report the assessment of our novel copolymer for the preparation of biodegradable nanocarriers for drug delivery. We describe methods for the preparation of monodisperse nanoparticles, marked with a fluorescent label, and loaded with rhodamine using a surfactant-free procedure. These nanocarriers were characterised to evaluate their degradation kinetics, protein adsorption and cytotoxicity. Finally, internalisation and delivery of a model cargo were demonstrated in an endothelial cell line. Taken together, these tests confirm the potential of this material for nanomedicine applications, and support further development of these biodegradable nanoparticles as potential drug delivery systems.

2. Materials and methods

2.1. Synthesis of the copolymer mPEG-block-P(CL-co-4HV)

The copolymer was synthesised and purified following the procedure previously reported [39] with some few modifications. ε-Caprolactone (CL, Aldrich, Milan, Italy), γ-valerolactone (VL, Aldrich) and metoxy polyethyleneglycol (mPEG, Mn = 550 Da, Sigma-Aldrich, Milan, Italy) were stored in glass vials over molecular sieves and used as monomers and initiator, respectively, for the synthesis via ring opening polymerisation (ROP). Tin(II) 2-ethylhexanoate (Sigma) was used as catalyst without any previous treatment. CL (31.42 mmol, 3.59 g), VL (7.85 mmol, 0.79 g) and mPEG (0.33 mmol, 0.18 g) were weighted to obtain a copolymer with a theoretical molecular weight of 14 kDa and a percentage molar ratio between CL/VL of 80/20. Reactants were inserted in a round-bottom single neck reactor heated at 120 °C using a thermostatic bath. When the temperature was reached, the catalyst (0.33 mmol, 0.13 g, molar ratio between initiator and catalyst was 1:1) was added; the reactor was purged with dry N_2 for 10 min and then sealed. The reaction was maintained for 4 h at 120 °C under mild stirring, then the reactor was quenched in a water bath to stop the reaction, thus causing the solidification of the product. The copolymer mPEG-*block*-P(CL-*co*-4HV) was dissolved in dichloromethane anhydrous (CH_2Cl_2, Sigma-Aldrich) and precipitate in methanol (MeOH, Sigma, HPLC purity degree) three times to eliminate unreacted monomers and the catalyst. After purification, the polymer powder was dried for 24 h under vented hood and stored in a sealed glass vial at room temperature.

2.2. Labelling of the copolymer

The copolymer prepared as described in the previous section was marked using 5(6)-carboxyfluorescein (CF, Sigma). For this reaction, copolymer (390.0 mg), 4-(dimethylamino)pyridine (DMAP, Aldrich, 0.01 mmol, 1.8 mg) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCl, Sigma-Aldrich, 0.15 mmol, 28.7 mg) were dissolved in CH_2Cl_2 (10 ml). When the mixture was homogeneous, CF (0.30 mmol, 113.0 mg) was added to the reaction, and maintained under stirring at room temperature (25 °C) for 4 h. At the end of the reaction, the product (mPEG-*block*-P(CL-*co*-4HV)-CF) was purified by precipitation in MeOH for three times and then dried and stored at −20 °C. The yield of the conjugation reaction was evaluated by UV spectrometer. 10 mg of marked copolymer was weighted and dissolved in acetone, 1 ml of sample was introduced in a quartz cuvette and analysed. Absorption data were recorded at 25 °C in a JASCO V550 spectrophotometer (JASCO Europe, Cremello, Italy). On the basis of a calibration curve of CF solutions in acetone, the amount of CF was quantified from values of absorbance at 468 nm of the polymer solution spectrum, and the ratio between CF and polymer was evaluated. Analysis was performed for three polymer solutions.

2.3. Physicochemical characterisation

Chemical characterisation of native and labelled copolymers was carried out through FTIR ATR, ^{1}H NMR and ^{13}C NMR analyses. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) analysis was carried out to define the chain structure of the materials and to demonstrate successful conjugation with CF; FTIR spectra were recorded on a FTIR Cary 630 (Agilent Technologies, Cernusco sul Naviglio, Italy) spectrometer in the wavelength range of 4000–650 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectroscopy was carried out with a Bruker Avance 400 spectrometer, using deuterated chloroform ($CDCl_3$, Aldrich, concentration of samples was 2% w/v) as solvent. Molecular weights of the products were evaluated through Gel Permeation Chromatography (GPC), Static Light Scattering (SLS) and NMR. For GPC analysis, a Waters Alliance 2695 chromatograph, equipped with a PDA (Waters 2995) and a RI detector, and a ResiPore column 300 × 7.5 mm (Agilent Technologies, Cernusco sul Naviglio, Italy) were used; tetrahydrofuran (THF, Aldrich, Chromasolv purity degree) was used as internal mobile phase (1 ml/min), the system was kept at 30 °C; results were analysed on the basis of a calibration curve obtained by polystyrene narrow standards (Agilent Technologies, Cernusco sul Naviglio, Italy). SLS analysis was carried out using a Zetasizer ZS90 (Malvern Instruments, Malvern, UK), six sample solutions with concentration comprised between 10 mg/ml and 3 mg/ml were analysed, using toluene (Sigma-Aldrich) as standard and acetone as solvent. Analysis was performed at 30 °C and with a scattering angle of 90°; all tests were carried out in triplicate. Evaluation of the molecular weight by NMR was based on the end-groups. The ^{1}H resonance of the methoxy group was taken as reference to evaluate the average molecular weight and the molar composition of the material. Thermal characterisation was carried out through Differential Scanning Calorimetry (DSC, DSC1 Mettler Toledo, Milan, Italy). 5 ± 0.003 mg of polymer powder were weighted and inserted in Al sealed capsules. Analyses were performed applying a ramp temperature (10 °C/min) from −65 °C to 180 °C under dry N_2 flow. Thermograms of the second scan were normalised on the sample weight. The midpoint of the slope change of the heat flow plot was considered as the glass transition temperature (T_g), and the heat flow capacity change (Δc_p) associated to T_g was determined. Melting temperatures (T_m) were taken as the minimum of endothermic peaks, while crystallisation temperatures (T_c) as the maximum of the exothermic peak. Wettability of polymer surface was evaluated through static water contact angle measurements onto thin films obtained by spin-coating. 100 μl of a polymeric solution in THF (10% w/v) was deposited on a glass flat support and spin-coated at 600 rpm for 1 min, then at 1400 rpm for 2 min, finally maintained on a heated plate at 40 °C for 1 min to completely eliminate the residual solvent. Contact angles were evaluated depositing a water droplet (3 ± 0.5 μl) with a glass syringe onto the flat polymer surface, drop shape and contact angles were calculated through the Young–Laplace equation. Three polymer samples were prepared and five measurements for each sample were recorded.

2.4. Nanoparticle preparation and characterisation

Nanoparticles were obtained through the solvent displacement technique in the absence of surfactants. Briefly, 10 mg of the polymer,