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Synthesis, characterization and evaluation of in vitro toxicity in hepatocytes of linear polyesters with varied aromatic and aliphatic co-monomers

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ABSTRACT

Polyesters are extensively used in drug delivery because of their controllable biodegradation properties and perceived favorable cytocompatibility. However, new ester-based materials are continually being sought which can be produced from readily accessible monomers, which can be tuned for drug encapsulation and which retain good cellular compatibilities. In this study, 5 polyesters of similar molar mass were synthesized by reacting 1,10-decanediol with different ratios of succinic acid/phenylsuccinic acid and the effect of the phenyl side-chain group addition on polymer properties relevant to drug delivery was investigated. A polymer with a 70/30 ratio of succinic acid and phenylsuccinic acid was selected based on its ability to encapsulate a model dye in nanoparticle (NP) formulations, and was found to be slowly degradable in phosphate buffered saline (PBS) but more rapidly degraded in the presence of a lipase. The compatibility of NP formulations of this polymer either with or without a Pluronic F68 stabilizing coating was assessed in vitro using the C3A hepatocyte cell line. Cell viability was assessed, at NP concentrations ranging from 4.68–300 μg mL^{-1} 24 h post-exposure, using the Alamar Blue, CDFA and Neutral Red assays. C3A cells internalized both coated and uncoated polyester NPs to a similar extent, with uptake observed to increase over time (10–1440 min). Although cell viability was >80% at the concentrations tested, in all assays, it was found that a Pluronic F68 coated poly (decanediol-phenylsuccinate-*co*-succinate) stimulated significant DNA damage driven by an oxidant mechanism, whereas the non-coated polyester analogue and the Pluronic F68 alone had no effect. The results obtained suggest that new polyesters can be synthesized with desirable properties from the materials perspective but formulation with additional excipients requires careful evaluation for drug delivery applications.

1. Introduction

In recent years, there has been a renewed interest in polyesters as drug delivery devices due to their favorable biocompatibility and controllable biodegradation profiles [1–4]. Accordingly, polyester materials are regarded as a material of choice for biomedical applications including drug delivery [5–9], as diagnostic agents [10,11] and for tissue engineering [12–16]. For these applications, polyesters with a range of physicochemical, biomechanical and biological properties are needed, along with appropriate biocompatibility, biodegradability and storage stability [17]. A balance of these properties can be obtained through incorporation of suitable constituent monomers into the polyester backbone.

For use as therapeutic agents, polyesters need to have strong associative interactions with active ingredients. An ability to release the drug at a suitable rate for therapy is important to reduce frequency of treatment [18]. Accordingly, there have been many variations made on the compositions and co-monomers used for polyesters, with a view to obtaining the most favorable controlled drug incorporation and release properties. More recently, there has been a strong economic and ecological drive for polymer precursors to be derived from sustainable building blocks, and for polymers to be produced with minimal use

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of solvents [19]. The synthesis of polyesters for drug delivery has been very thoroughly explored, with many variations around the use of poly(lactides), poly(caprolactone) and poly(carbonates) [3,20,21]. However, these polyesters by themselves are not easy to formulate into nanoparticles that are stable for storage in solution or for intravenous injection. Accordingly, methods are needed to enhance the colloidal properties of polyester nanoparticles, and the most simple of these, and thus the most appealing from an industrial perspective, is the surface adsorption to the nanoparticles of stabilizing layers. The concept of sterically shielding hydrophobic polyester surfaces by attaching hydrophilic polymers is well-established, with an extensive literature on adsorbed amphiphilic block co-polymers [22–27]. These types of stabilized nanoparticles have long been known to evade uptake by the mononuclear phagocyte system (MPS) [28], and so for practical drug delivery purposes there is a strong rationale to develop polyesters which are easy to synthesize from renewable resources, and which can be easily formulated into drug-loaded nanoparticles.

Here we report the synthesis of linear polyesters from the readily available monomers succinic acid, phenyl succinic acid and 1, 10-decanediol, with progressive variation with respect to the phenyl side chain content from the polyester backbone. The polymers were made using scandium (III) triflate as a catalyst under solvent-free conditions [29–33] and evaluated for drug-loading via nanoprecipitation in the presence of coumarin-6 dye. The polymer exhibiting the highest dye loading was tested for colloidal and hydrolytic stability when formulated into nanoparticles (NPs). In addition, the toxicity of polyester nanoparticles, with and without a sterically-stabilizing adsorbed Pluronic F68 block co-polymer layer. The toxicity of these NPs was assessed in vitro, using the human C3A hepatocyte cell line. Hepatocytes were selected as the liver is the primary site of nanoparticle accumulation following exposure via different routes (e.g. intratracheal instillation, ingestion, intravenous injection), and existing evidence suggests that C3A cells respond similarly to primary human hepatocytes [34–37]. The internalization of coated and uncoated polymer nanoparticles by hepatocytes was assessed over time, and their effects on cell viability evaluated using three assays; Alamar Blue, Neutral Red, and 5-CFDA-AM [5-carboxyfluorescein diacetate, acetoxymethyl ester] CFDA-AM. The ability of nanoparticles to stimulate cytokine production (IL-8) and cause genotoxicity (DNA damage) was assessed in order to evaluate sub-lethal impacts on cell function. These data showed that while the new selected polyesters were rapidly internalized with or without the Pluronic F68 coating, there were differences in DNA damage induced by the NP formulations which were a consequence of the combination of the coating and 'core' NP, and not a function of the individual components alone.

2. Experimental section

2.1. Materials

Succinic acid (ACS reagent, ≥99.0%), phenylsuccinic acid (98%), 1,10-decanediol (98%), scandium (III) triflate (99%), coumarin-6 (98%), Pluronic F-68, deuterated chloroform ($CDCl_3$), 1 mM sodium pyruvate, 1% non-essential amino acids, phosphate buffer saline solution, CFDA-AM, Neutral Red, acetic acid, 95% ethanol, ammonium chloride, Triton-X100, DAPI, H_2O_2, HBSS, low melting point agarose, agarose, lysis buffer base, dimethyl sulfoxide, HEPES, potassium chloride, ethylenediaminetetraacetic acid [EDTA], Bovine Serum Albumin, sodium hydroxide, Tris base and GelRed were purchased from Sigma-Aldrich. All solvents were purchased from Fischer Scientific UK.

Cell culture reagents including MEM medium, foetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, phenol red free MEM medium, 10 × trypsin (2.5%) and 0.4% v/v Trypan Blue were purchased from Gibco, Invitrogen. The human hepatocyte cell line C3A was purchased from ATCC, Manassas, VA, USA.

2.2. Measurements

Nuclear magnetic resonance (NMR) spectra of ^{1}H NMR and ^{13}C NMR were recorded at 400 MHz (^{1}H) and 101 MHz (^{13}C) using a Bruker DPX400 Ultrashield spectrometer and deuterated chloroform ($CDCl_3$) as the solvent. Spectral analysis was performed using MestRENova 6.0.2 software copyright© 2009 Mestrelab Research S.L. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances ($CDCl_3$: δ_H 7.26, δ_C 77.16).

Fourier transform infrared (FTIR) spectroscopy was performed on solid or liquid samples using a Cary 630 FTIR spectrophotometer equipped with a single bounce diamond ATR. MicroLab software was used for data analysis.

A Polymer Laboratories GPC 50 instrument was used to determine M_n (number-average molecular weight), M_w (weight average molecular weight) and Đ (polydispersity index, M_w / M_n). The instrument was fitted with a Polymer Laboratories PLgel guard column (50 × 7.5 mm, 8 μm) followed by a pair of PLgel Mixed-D columns (300 × 7.5 mm, 8 μm) and a refractive index detector. The flow rate of HPLC grade $CHCl_3$ at 30 °C was 1 mL min^{-1}. The column calibration was achieved using narrow molar mass distribution polystyrene standards. Polymer Laboratories Cirrus 3.0 software was used for data analysis.

The thermal properties of the polymers i.e. T_m (melting temperature) and T_g (glass transition temperature) were probed using a TA-Q2000 DSC (TA Instruments) under a nitrogen atmosphere. Typically, the samples (5–10 mg) were exposed to two cooling-heating cycles from −90 to 150 °C at a rate of 10 °C min^{-1}.

The particle sizes (z-average diameter) of nanoparticles were measured in HPLC water determined by dynamic light scattering (DLS) using a NanoZS instrument (Malvern, UK) at 25 °C using 633 nm (4 mW) wavelength laser. The scattered light was detected at an angle of 173° and analysis was performed using zetasizer software version 7.03. The zeta potentials of the NPs were determined in HEPES 10 mM buffer (pH 7.4). The size and surface zeta potentials of NPs were also measured in complete MEM cell culture medium (MEM medium supplemented with 10% heat-inactivated foetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, 1 mM sodium pyruvate and 1% non-essential amino acids) containing 125 μg mL^{-1} of NPs.

A Tecnai G2 (FEI, Oregon, USA) microscope was utilized for transmission electron microscopy (TEM). One drop of polymer suspension in HPLC grade water (typically 25–50 μg mL^{-1}) was dropped onto a copper grid and allowed to dry in air. The sample was put on a copper grid and allowed to air dry. The imaging was performed without staining.

A Zeiss LSM880 confocal microscope was used for imaging uptake of labelled polymers and nanoparticles, and the Zeiss Zen program was used for data analysis.

2.3. Methods

2.3.1. Synthesis of polyesters

Poly(decamethylene succinate) [PDeMS], poly(decamethylene phenylsuccinate) [PDsMPS] and their copolymers poly(decamethylene succinate-*co*-phenylsuccinate) [PDeMS-*co*-PS] were synthesized by a solvent-free melt polycondensation method (Scheme 1). The diacid and diol ratio was maintained at 1:1 in all polycondensation reactions. A scandium (III) triflate: diol ratio of 5 × 10^{-4} (0.05 mol%) was used in all the reactions. Polymer names, abbreviations and codes along with feed compositions are given in Table 1.

2.3.1.1. Poly(decamethylene succinate) [PDeMS] polymer (P01). The polymer was synthesized by mixing succinic acid (0.61 g, 5.2 mmol, 1 eq) and 1, 10-decanediol (0.90 g, 5.2 mmol, 1 eq) in a reaction vessel at 80 °C for 30 min under stirring for melting and mixing of the monomers.