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Poly(glucono-δ-lactone) based nanocarriers as novel biodegradable drug delivery platforms

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ABSTRACT

Novel biocompatible and biodegradable polymers are highly desirable and crucial in drug delivery applications in order to overcome the technical challenges and problems existing in the traditional method of poly(ethylene glycol) based drug carriers. In this study, ring-opening polymerization of a carbohydrate-derived lactone, glucono-δ-lactone (GDL), generates a new highly branched polymer (PGDL) that can form stable nanoparticles through a w/o emulsification approach. The biodegradable and biocompatible particles can carry anticancer agent 5-fluorouracil (5-FU) effectively. The controlled release of 5-FU from the PGDL particles exhibits a non-Fickian mechanism without an initial burst, with an enhanced release exponent at tumoral pH. Cell viability studies by MTT assays indicate that ovarian carcinoma cells (OVCAR-3) and macrophage cells (raw 264.7) treated with PGDL (2.5 mg/ml) show no signs of toxicity in 24 h cell incubations. The PGDL particles can interact with the OVCAR-3 cell line efficiently. Therefore, the glucono-δ-lactone based particles represent an effective delivery system for cancer therapy.

1. Introduction

It is well known that the topological and steric effects of polymer colloidal particles play a major role in intravenous drug delivery, whereas the electrostatic charges in the polymers may lead to the adsorption of serum proteins (opsonization) and elimination from the body by the mononuclear phagocyte system (Owens and Peppas, 2006; Tabata and Ikada, 1988; Schwendener et al., 1984). On the other hand, low cytotoxicity, good protein-resistance and high functionalizability properties of synthetic polymers are crucial for various applications in drug delivery, polymer therapeutics, and tissue engineering (Duncan, 2003; Haag and Kratz, 2006; Andrade et al., 1996; Lee et al., 1995). These criteria have partially been met by biocompatible poly(ethylene glycol) (PEG) in stabilization of colloidal particles. Modification by PEG results in electrically neutral surfaces, minimal interaction with serum proteins, "stealth-like" properties, and hence, a prolonged circulation *in vivo* (Klibanov et al., 1990; Photos et al., 2003; Yamaoka et al., 1994). For these reasons, PEG and its derivatives have become the frequently used hydrophilic material in drug delivery research to enhance the efficacy of therapeutic agents (Harris and Chess, 2003). However, the inert CH_2CH_2O repeating unit after PEGylation leads to lack of enough sites for functionalization such as the introduction of targeting structures or dye labeling. Accumulation of PEG may cause side effects in certain *in vivo* applications, due to its low or poor biodegradability (Knop et al., 2010; Ulbricht et al., 2014). Although PEGylated drug vehicles can avoid uptake by the reticuloendothelial system, cellular internalization is also slowed down due to weak interactions between vehicles and cellular membranes. Therefore, alternative biocompatible and biodegradable polymers are highly desirable and crucial in drug delivery applications (Knop et al., 2010; Scherer et al., 2016; Ishihara et al., 1999; Statz et al., 2005).

Carbohydrate derivatives have recently received great attention in the field of drug delivery because of their satisfactory biodegradable, biocompatible, and bioadhesive properties (Liu

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et al., 2008; Spain and Cameron, 2011; Jain et al., 2012; Gu et al., 2014; Zhang et al., 2015). They are derived from a variety of natural resources with functional groups useful for numerous chemical modifications. Their participation in many biological processes signifies the importance of exploiting them as structural building blocks for bioactive carriers. Glycosylation of polymers through conjugation and modification may impart a wide range of merits, including reducing toxicity and immunogenicity (Agrawal et al., 2007; Höbel et al., 2011), improving serum stability, and promoting bioadhesion (Yin et al., 2015). Chitosan or starch possesses multiple hydrophilic functional groups amenable to interactions with biological tissues, therefore, carriers made of chitosan may have long retention time in tissues and improved absorption of loaded drugs (Makhlof et al., 2010).

In this work, we report a novel carbohydrate-derived polymer with very satisfactory biocompatibility. Glucono-δ-lactone (GDL) is a cyclic ester of D-gluconic acid, manufactured with the acid by fermentation of glucose. As a "Generally Regarded as Safe" (GRAS) ingredient listed by Food and Drug Administration of USA, GDL is widely used as a food additive and administered with some drugs in the form of gluconates. Poly(glucono-δ-lactone) (PGDL) with the multiple groups valuable for functionalization is demonstrated here to have very low cytotoxicity. The PGDL nanoparticles can be efficiently delivered to human ovarian cancer cells. Controlled release of 5-fluorouracil (5-FU) from PGDL nanoparticles shows that PGDLs are promising platforms for effective treatment of ovarian tumors.

2. Materials and methods

2.1. Instrumentation

Gel permeation chromatographic (GPC) analysis was performed by using Agilent 1200 GPC System equipped with a refractive index detector. A series of narrow MW distribution Pullulan samples were used as standards during the measurement. FTIR spectra were collected on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific). NMR spectra were taken on a Bruker AVANCE III 400 M Hz NMR spectrometer. Transmission electron micrographs were recorded JEM-1011 electron microscopy (JEOL, Japan). Scanning electron micrographs were collected on JSM-6360LV electron microscopy (JEOL, Japan). The samples were sputtered with gold metal during SEM measurement. The approximate thickness of the gold layers applied to SEM samples is 20 nm.

2.2. Materials

Glucono-δ-lactone (analytical grade) was available from Sinopharm Chemical Reagent Co., Ltd; tetrabutyl titanate (analytical grade) was purchased from Sigma Co.; ethylene glycol (analytical grade) was purchased from Shanghai Zhanyun Chemical Co., Ltd.

N,*N*-dimethyl formamide, tetrahydrofuran (THF), 5-flurouracil (5-FU), Span-80, isopropyl alcohol, Rhodamine 123, petroleum ether (b.p. 60–90 °C), and paraffin liquid, all in analytical grade, were purchased from Aladdin Reagent Co. (Shanghai).

Human ovarian cancer cell line (OVCAR-3) was purchased from BNCC (BeNa Culture Collection Institute, Beijing). Murine macrophage cell line (raw 264.7) was available from ATCC. Phosphate buffered saline (PBS, pH = 7.40 ± 0.10), 0.25% Trypsin (in 0.02% EDTA), penicillin-streptomycin solution (1×), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), were all purchased from Genom Biomedical Technology Co., Hangzhou. Fetal bovine serum (FBS) South America was purchased from MRC Biotechnology Co., Changzhou. Gibco™ DMEM (high glucose, 1×) was purchased from Thermo Fisher Scientific.

2.3. Synthesis of PGDL

10.0 g of glucono-δ-lactone (GDL), tetrabutyl titanate (0.4 ml), ethylene glycol (0.4 ml) were charged to a 100 ml three-neck round bottom flask which was equipped with a reflux condenser and a N_2 gas inlet. The flask was placed in a silicone oil bath, and the reaction mixture was stirred at 160 °C for 3 h. The product was cooled under the protection of the N_2, giving a red-brown waxy solid. 20 ml of DMF was added to the product and the solution was heated to 80 °C to completely dissolve the product. The solution was cooled to room temperature, poured into a 250 ml beaker, and precipitated with 80 ml of THF. The precipitated solid was repeatedly washed with THF for 3–4 times, dried in vacuum to remove the THF. The polymer obtained was characterized by ^{1}H NMR and FTIR spectroscopy.

2.4. GPC determination of the PGDL molecular weight (MW)

The MW measurement was undertaken at a flow rate of 1 ml/min and a column temperature of 35 °C with water as the mobile phase. A series of narrow MWD Pullulan with different molecular weights were used as the standard samples to obtain the calibration curve. PGDL samples were dissolved in pure water and filtered with a 0.22 μm microporous membrane before measurements.

2.5. Degradation of PGDL

100.0 mg of PGDL completely dissolved in 3.0 ml of PBS buffer were placed immediately in an ultra-filtration centrifuge tubes (NMCO 3000), centrifuged at a relative centrifugal force (RCF) of 11068 × *g* for 20 min, and dried in an oven to a constant weight. 9 PGDL samples each containing 100.0 mg were also dissolved in 3.0 ml of PBS buffer, incubated in a 37 °C shaker at 150 r/min, for 1, 3, 5, 7, 9, 11, 13, 14, 15 days respectively. The samples were placed in the ultra-filtration centrifuge tubes where the filter weights had been measured, centrifuged at 11068 × *g* for 15 min, and dried in an oven to a constant weight. The filters with the remaining polymer were weighed, and the net weight of the polymer was obtained by subtraction of the filter weight, giving the remaining PGDL weight. Three replicate tests were performed in the degradation studies.

2.6. Preparation of PGDL particles

To prepare drug-free PGDL particles, 100.0 mg of PGDL were dissolved in 5.0 ml of water to make a 2% (w/v) aqueous phase. 40.0 ml of paraffin liquid and 2.0 ml of 5% (v/v) emulsifier span-80 were homogenized to make an oil phase. 4.0 ml of the aqueous phase were added drop-wise through a 0.22 μm microporous filter to the oil phase at 50 °C while stirring at 600 r/min by a mechanical mixer under the reflux condition. The mixture was agitated for 4 h and poured into a 250 ml beaker in which an equal volume of petroleum ether was added and mixed with the emulsion. The mixture was set aside to settle for approximately 15 min to remove the upper layer, and the remaining emulsion was centrifuged at 1259 × *g* for 5 min to remove the supernatant. The polymer particles were then washed by petroleum ether and isopropyl alcohol repeatedly, centrifuged, dispersed in isopropyl alcohol, and vacuum dried at room temperature before they were characterized by SEM and TEM. Zeta potential (mV) of 1 mg/ml