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Length effect of methoxy poly(ethylene oxide)-*b*-[poly(ε-caprolactone)-*g*-poly(methacrylic acid)] copolymers on cisplatin delivery

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ABSTRACT

Novel comb-shaped amphiphilic copolymers based on methoxy poly(ethylene glycol)-*b*-[poly(ε-caprolactone)-*g*-poly(methacrylic acid)] (MPCL-*g*-pMAA), were synthesized via ring opening polymerization (ROP) and atom transfer radical polymerization (ATRP) for drug delivery systems. MPCL-*g*-pMAAs with various MAA repeating units self-assemble into a core-shell structure in an aqueous solution. Critical aggregation concentrations range within 5.6×10^{-3}–7.0×10^{-2} mg/mL in double deionized water and 8.9×10^{-3}–7.0×10^{-2} mg/mL in phosphate buffered saline of pH 7.4, decreasing with increase in pMAA length. The carboxylic groups of MPCL-*g*-pMAAs were utilized to coordinate cisplatin, forming polymer-metal complexes for chemotherapy. The average hydrodynamic diameters of particles are within 220–246 nm, slightly dependent on pMAA length. However, the cisplatin-loaded MPCL-*g*-pMAAs particles have average hydrodynamic diameters of 263–412 nm owing to increasing drug loading efficiency with increase in pMAA length. Nevertheless, the MPCL-*g*-pMAA with the least number of MAA repeating unit shows the fastest drug release rate as well as the highest cytotoxicity against CRL-5802 cells. The cellular uptake of MPCL-*g*-pMAA particles, involving mainly clathrin-mediated endocytosis, increases with incubation time. MPCL-*g*-pMAA particles are non-cytotoxic to CRL-5802 cells but the cisplatin-loaded MPCL-*g*-pMAA particles show profound cell-killing ability. The MPCL-*g*-pMAA is a potential carrier for drug delivery systems.

1. Introduction

Amphiphilic block copolymers, containing a hydrophilic shell and a hydrophobic core, possess very attractive properties for drug delivery systems (DDS) owing to their high stability, improved drug solubility, prolonged circulation time, and enhanced elimination half-life in the blood, thus affording a high drug efficacy with few side effects [1,2]. Amphiphilic block copolymers that use poly(ε-caprolactone) (PCL) as the hydrophobic segment and poly(ethylene glycol) (PEG) as the hydrophilic segment to form micelles have been extensively studied for DDS [3,4]. With intriguingly synthetic skills, different block structures such as PCL-PEG diblock [5,6], PCL-PEG-PCL [7,8] or PEG-PCL-PEG triblock [9], PCL/PEG star-shaped types [10,11] can be obtained. Owing to the lack of reactive functional groups on the side chain of PCL-PEG block copolymers, hydrophobic payloads used to be encapsulated in the PCL domain using a physical manner. Thus, a functional ε-caprolactone (CL) monomer with a reactive group is beneficial in performing a further chemical conjugation on the PCL side chain.

Methoxy poly(ethylene glycol)-*block*-[poly(ε-caprolactone)-*graft*-poly(methacrylic acid)] copolymers have been synthesized for oral delivery of ibuprofen [12]. γ-(2-Bromo-2-methylpropionate)-ε-caprolactone (BMPCL) was synthesized as a functional CL and the feed ratio of CL and BMPCL was adjusted to control the grafting ratio of pMAA. Nevertheless, the active agent, ibuprofen, was still encapsulated into the hydrophobic PCL domain using a physical method [12]. Another example of a graft polymer to the PCL-PEG block copolymer is glycopolymer-*grafted* PCL using 2-bromo-ε-caprolactone (2-BrCL) as a functional moiety of CL [13].

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Molecular architecture plays an important role in determining the properties and applications of copolymers. It is worthy to develop a simple method for preparing a functional CL that can be further utilized to link with reactive molecules. In this contribution, the functional 2-BrCL monomer was prepared in two steps with a higher yield. The synthesis of methoxy poly(ethylene glycol)-*b*-poly(2-bromo-ε-caprolactone) (MPCL-Br) was conducted using a ROP method with methoxy poly(ethylene glycol) as an initiator and zinc ethyl β-diiminate as a catalyst. Zinc ethyl β-diiminate has been reported to exhibit a highest activity and stereoselectivity for polymerization of rac- and *meso*-lactide to poly(lactic acid) (PLA) with a narrow polydispersity [14]. Here, the synthesized MPCL-Br block copolymer will be grafted with a hydrophilic side chain of poly(methacrylic acid) (PMAA) by atom transfer radical polymerization (ATRP) to yield methoxy poly(ethylene glycol)-*b*-[poly(ε-caprolactone)-*g*-poly(methacrylic acid)] (MPCL-*g*-pMAA) copolymers. In parallel, reversible addition-fragmentation chain transfer (RAFT) polymerization is also a good method to yield block copolymers like ATRP [15] or branched copolymers [16,17] for drug delivery or imaging systems.

PCL is a hydrophobic polymer approved by the US Food and Drug Administration (FDA) and PEG is a non-toxic polymer. Both are commonly employed to prepare amphiphilic copolymers for DDS [3,4]. The hydrophobic PCL can be utilized to encapsulate hydrophobic drugs and the hydrophilic PEG shows a prolonged plasma circulation due to the prevention of recognition by the reticuloendothelial system (RES) [18]. PMAA bears carboxylic groups with a pKa of about 5–6 [19], which is responsive to pH value and ionic strength of the physiological environment [20]. Thus, PMAA and its methacrylic ester copolymers (i.e., Eudragit®) have been developed for cutaneous, oral, and parenteral drug delivery systems in clinics [21].

Cisplatin is a commonly-used anti-cancer agent to treat a variety of cancers such as head and neck, ovarian, breast, bladder and lung cancer because of its potent activity to crosslink DNA upon entering the cells [22]. The clinical utility of cisplatin is limited both by intrinsic and acquired resistance and dose-limiting normal tissue toxicity [23–25]. That cisplatin shows little selectivity for tumor versus normal tissue may be a critical factor limiting its value. To overcome the low therapeutic ratio of the free drug, binding cisplatin to the polymers containing carboxylic groups like poly-(L-glutamic acid) [26,27] and hyaluronic acid [28,29], have been explored toward leveraging the enhanced permeability and retention (EPR) effect and promoting delivery of cisplatin to tumors.

PMAA also forms the complex with cisplatin via coordination of carboxylic groups and platinum (II) atoms [30]. Because the length of PMAA may decide the properties of formed particles and their responsiveness to pH and ionic strength; hence, this study synthesized MPCL-g-pMAA of three different pMAA lengths via ROP and ATRP to compare their critical aggregation concentrations (CAC), sizes, drug-loading efficiencies, drug release profiles under different pHs and ionic strengths. The cytotoxicities of cisplatin-loaded particles were tested in CRL-5802 cells using the MTT assay. The internalization pathways of particles were analyzed using flow cytometry and confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA), copper(I) bromide (CuBr), phosphate buffered saline (pH 7.4), methoxy poly(ethylene glycol) (mPEG, MW 2000), dimethyl sulfoxide-*d* (DMSO-d_6), and deuterium chloroform ($CDCl_3$) were purchased from Sigma-Aldrich (St. Louis, MO). ε-Caprolactone (CL), cisplatin, *N*-bromosuccinimide (NBS), cyclohexanone, *meta*-chloroperoxybenzoic acid (*m*CPBA), rhodamine 123, and pyrene were purchased from Acros (Hackensack, NJ). *tert*-butyl methacrylate (*t*BMA) was purchased from TCI (Tokyo, Japan); aluminum oxide neutral (Al_2O_3) from Seedchem Company PTY. LTD (Melbourne, Australia); 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide from MP Biomedicals (Eschwege, Germany) and fetal bovine serum (FBS) from Biological Industries (Beit Haemek, Israel), respectively. Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (Carlsbad, CA). All other unstated chemicals were from Sigma-Aldrich and used without further purification.

2.2. Synthesis of 2-Bromo-ε-Caprolactone (2-BrCL)

The synthesis of 2-bromo-ε-caprolactone (2-BrCL) monomer was referred to previous report with some modifications [13]. Briefly, the mixture of *N*-bromosuccinimide (NBS, 17.7 g, 0.1 mol) and cyclohexanone (9.8 g, 0.1 mol) was put in ethyl acetate (EA, 250 mL) and reacted at 50 °C in an acid condition overnight. The solution was washed with double deionized (DD) water (200 mL) three times to remove the side product (succinimide). After the evaporation of EA, the obtained dark liquid was redissolved in dichloromethane (CH_2Cl_2, 250 mL), followed by gradual addition of *meta*-chloroperoxybenzoic acid (*m*CPBA, 70–75%, 12.1 g) into the solution at 0 °C. The mixture was stirred at room temperature for 24 h and then filtered. The filtrate was washed in saturated $NaHCO_{3(aq)}$ (200 mL) three times and dried with $MgSO_4$. After the evaporation of CH_2Cl_2, the obtained brown liquid was redissolved in ethyl ether (50 mL) and refrigerated at −20 °C. A pure colorless crystal was collected and dried with a yield of 10.8 g (56%).

2.3. Synthesis of *mPEG*-b-*Poly(2-Bromo-ε-Caprolactone) (MPCL-Br)*

MPEG-2000 (0.20 g, 0.1 mmol) was dissolved in toluene (20 mL), followed by addition of zinc ethyl β-diiminate (0.05 g, 0.1 mmol) [14]. The mixture was stirred at room temperature for 12 h and 2-BrCL (0.50 g, 2.6 mmol) was added into the solution. After 12 h, polymerization was terminated by addition of *iso*propanol (20 mL). Finally, MPCL-Br was obtained from precipitation into hexane (80 mL).

2.4. Synthesis of methoxy poly(ethylene glycol)-b-*[poly(ε-caprolactone)-g-poly(methacrylic acid)] (MPCL-g-pMAA)*

To prepare MPCL-*g*-p*t*BMA, the MPCL-Br macroinitiator (100 mg, 0.01 mmol), *t*BMA (4, 7, 10 mmol), HMTETA (12 μL, 0.04 mmol) were added into a two-neck round-bottom flask containing toluene (2 mL). The reaction was degassed by six consecutive standard freeze-pump-thaw cycles. Next, CuBr (6 mg, 0.04 mmol) was quickly added to the mixture under an argon atmosphere. Polymerization was allowed to proceed under continuous stirring at 60 °C overnight. The reaction was terminated by addition of CH_2Cl_2 (10 mL) and the solution was passed through an alumina column and Amberlite® IR120 to remove the residual copper catalyst. Finally, the product was obtained from precipitation into excess *n*-hexane and dried under vacuum. The degree of tBMA repeating unit was analyzed by ^{1}H NMR using $CDCl_3$ as a solvent.

To prepare MPCL-*g*-pMAA, trifluoroacetic acid (TFA) was utilized to hydrolyze the *tert*-butyl esters of MPCL-*g*-p*t*BMA [12]. The typical procedure was as follows. MPCL-*g*-p*t*BMA copolymers (50 mg) were dissolved in CH_2Cl_2 (12.5 mL) at room temperature. The solution was cooled to 0 °C and TFA (5 mmol excess to *t*BMA