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The effect of block copolymer structure on the internalization of polymeric micelles by human breast cancer cells

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Abstract

The objective of this study was to assess the effect of hydrophilic/hydrophobic block chain lengths on the internalization of poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles by cancer cells. PEO-*b*-PCL block copolymers with varied PEO and PCL chain lengths were synthesized, assembled to polymeric micelles and loaded with a hydrophobic fluorescent probe (DiI) through a co-solvent evaporation method of physical encapsulation. The slow release of the fluorescent probe from the micellar structure was evidenced following DiI transfer to lipid vesicles. The extent of micellar uptake by cancer cells was investigated through their incubation with MCF-7 cells followed by measurement of the fluorescent emission intensity of DiI ($\lambda = 550$ nm) in separated lysed cells. Cellular internalization of polymeric micelles was confirmed by laser scanning microscopy. The mechanism of micellar uptake was investigated by pretreatment of MCF-7 cells with chlorpromazine and cytochalasin B. Encapsulation of DiI in PEO-*b*-PCL micelles lowered the extent and rate of hydrophobic probe internalization by cancer cells. For polymeric micelles with 5000 g mol^{-1} of PCL and varied PEO molecular weights of 2000, 5000 and 13,000 g mol^{-1}, maximum uptake was observed at a PEO molecular weight of 5000 g mol^{-1}. For polymeric micelles with 5000 g mol^{-1} of PEO and varied PCL molecular weights of 5000, 13,000 and 24,000 g mol^{-1}, maximum uptake was observed at 13,000 g mol^{-1} of PCL. Chlorpromazine reduced the cellular uptake of PEO-*b*-PCL micelles independent from the block copolymer structure, pointing to the involvement of clathrin mediated endocytosis mechanisms in the uptake of polymeric micelles by cancer cells. Inhibition of cellular uptake of PEO-*b*-PCL micelles by cytochalasin B, on the other hand, was found to be dependent on the chemical structure of the core/shell forming blocks.

Keywords: Polymeric micelles; Drug delivery; Poly(ethylene oxide); Poly(ε-caprolactone); Endocytosis; Fluorescent microscopy

1. Introduction

Self-assembled block copolymer micelles have been the focus of much attention as potential carriers for the solubilization and controlled delivery of different therapeutic agents [1–4]. The unique feature that has driven this attention is the flexibility of chemical structure in polymeric micelles, which makes it possible to prepare "custom made" carriers for the delivery of individual therapeutic agents. Several systematic studies on the effect of different core and shell forming structures on relevant properties of polymeric micelles in drug delivery such as micellar size, thermodynamic and kinetic stability, drug solubilization, drug release as well as organ disposition of polymeric micelles have been conducted [5–11]. The result of such studies have led to the development of optimal polymeric micellar carriers that can incorporate therapeutic agents through physical or chemicals means efficiently, provide controlled release, prolonged plasma circulation properties and, eventually, passive tumour targeting for the incorporated moiety. Little is known on the effect of variations in the core/shell structure on the biological fate of polymeric micelles at the target site and its interaction with target cells, however.

Understanding of the effect of chemical manipulations on the extent and mechanism of micellar uptake by target cells is essential in the design and development of nano-engineered polymeric micelles for drug or gene delivery to subcellular

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targets. Few studies have assessed the rate and extent of polymeric micellar uptake by different cells and provided insight on the subcellular distribution of polymeric micelles [12–14]. Uptake of PEO-*b*-PCL micelles having average PEO and PCL molecular weights of 2000 and 2300 g mol^{-1}, respectively, by mouse embryonal carcinoma cells, rat adrenal pheochromocytoma cells (PC12 cells) and mixed neuron-glia cultures have been investigated by Maysinger and co-workers [15,16]. The same group has reported on the preferential subcellular distribution of PEO-*b*-PCL micelles in the cytoplasm and several cytoplasmic organelles of PC12 cells [17]. The aim of the present study was to evaluate the effect of block copolymer structure in micelles of PEO-*b*-PCL on their extent, rate and mechanism of internalization by human breast cancer cells and define optimal core/shell architectures that can achieve either intra or extracellular modes of drug delivery.

2. Materials and methods

2.1. Materials

Methoxy polyethylene oxide (average molecular weight of 2000 and 5000 g mol^{-1}), bovine serum albumin, Triton X-100 and *N*,*N*-dimethyl sulfoxide (DMSO) were purchased from Sigma chemicals (St. Louis, MO, USA). Methoxy polyethylene oxide (average molecular weight of 13,000 g mol^{-1}) was purchased from Polymer Source Inc. (Montréal, Canada), ε-caprolactone was purchased from Lancaster Synthesis, UK. Fluorescent probes DiI and Concavallin A Alexa fluor 488 conjugates were purchased from Molecular Probes, USA. Cell culture media RPMI 1640, penicillin–streptomycin, fetal bovine serum, L-glutamine, Hepes buffer solution (1 M) were purchased from Gibco, Invitrogen Corporation, USA. Culture flasks and plates were from Corning-Coaster (Cambridge, MA). All other chemicals were reagent grade.

2.2. Synthesis of block copolymers

Poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) block copolymers of different core and shell forming block lengths (Table 1) were synthesized by ring opening polymerization of ε-caprolactone using methoxy polyethylene oxide as initiator and stannous octoate as catalyst. Methoxy PEO (5 g), ε-caprolactone at different feed ratios and stannous octoate (0.002 equiv. of monomer) were added to a 10 mL previously flamed ampoule, nitrogen purged and sealed under vacuum [18]. The polymerization reaction was allowed to proceed for 4 h at 140 °C in oven. The reaction was terminated by cooling the product to room temperature. The reaction product was dissolved in chloroform, precipitated and washed with an excess of cold methanol and collected with centrifuge and dried under vacuum oven at 40 °C for 48 h.

2.3. Characterization of the block copolymers

^{1}H NMR spectrum of PEO-*b*-PCL in $CDCL_3$ at 300 MHz was used to assess the conversion of ε-caprolactone monomer to PCL, comparing peak intensity of –O–CH2– (δ = 4.223 ppm) for ε-caprolactone monomer to the intensity of the same peak for poly(ε-caprolactone) (δ = 4.075 ppm). The number average molecular weight of the block copolymers was also determined from ^{1}H NMR spectrum comparing peak intensity of PEO (–CH_2CH_2O–, δ = 3.65 ppm) to that of PCL (–O–CH2–, δ = 4.075 ppm). Gel permeation chromatography (GPC) using refractive index and light scattering detector characterized the number and weight average molecular weights and polydispersity of block copolymers. Samples of 20 μL from 10 mg/mL stock solutions of the polymers in THF were injected to 4.6 mm × 300 mm Waters Styragel® HT4 column (Waters Inc., Milford, MA). The mobile phase was THF with a flow rate of 1 mL/min. The elution pattern was detected at 35 °C by refractive index/light scattering detectors (Model 410, Walters Inc.). The calibration curve was prepared using polystyrene standards.

2.4. Assembly of PEO-b-PCL block copolymers and characterization of self-assembled structures

Micellization was achieved by dissolving PEO-*b*-PCL block copolymer (30 mg) in acetone (0.5 mL) and drop-wise addition (∼1 drop/15 s) of polymer solution to doubly distilled water (3 mL) under moderate stirring at 25 °C, followed by evaporation of acetone under vacuum. Average diameter and size distribution of prepared micelles were estimated by dynamic light scattering (DLS) using Malvern Zetasizer 3000 at a polymer concentration of 10 mg/mL. Critical micelle concentration (CMC) of block copolymers were determined following changes in the fluorescence excitation spectra of pyrene in the presence of varied concentrations of block copolymers [19].

2.5. Preparation and characterization of fluorescent labeled PEO-b-PCL micelles

Physical entrapment of hydrophobic fluorescent probe, DiI, was used to prepare fluorescent labeled polymeric micelles [21,22]. DiI (10 μg/mL) and copolymer (10 mg/mL) were dissolved in acetone (0.5 mL). This solution was added to 3 mL of water in a drop-wise manner and remaining of the organic solvent was removed by evaporation under vacuum. The micellar solution was then centrifuged at 11,600 × *g* for 5 min, to remove DiI precipitates. The hydrodynamic diameter of DiI loaded PEO-*b*-PCL micelles was measured by light scattering as described above. An aliquot of the micellar solution was diluted with an equal volume of DMSO and used to quantify the level of encapsulated DiI by UV–vis spectroscopy at 550 nm (Beckman coulter DU 530, USA).

The stability of DiI physical incorporation in polymeric micelles was assessed measuring the in vitro rate of DiI