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Polymer micelles with cross-linked ionic cores for delivery of anticancer drugs

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Abstract

This work reports the design of polymer micelles with cross-linked ionic cores that display high stability. Block ionomer complexes of poly (ethylene oxide)-*b*-poly(methacrylic acid) copolymer and divalent metal cations were utilized as micellar templates for the synthesis of the cross-linked micelles. Such micelles represent hydrophilic nanospheres of core-shell morphology. The core comprises a network of the cross-linked polyanions, which is surrounded by the shell of hydrophilic PEO chains. The ionic character of the core provided for pH-dependent swelling/collapse behavior of the nanogels. Cisplatin, a potent chemotherapeutic agent, was incorporated into the ionic core of the micelles with remarkably high efficiency (22% w/w). The drug-loaded micelles were stable in aqueous dispersions exhibiting no aggregation or precipitation for a prolonged period of time. Slow release of platinum complexes was observed in sustained manner from the cisplatin-loaded cross-linked micelles in physiological saline. In vitro studies using human A2780 ovarian carcinoma cells demonstrated that the cross-linked micelles rapidly internalized and delivered cisplatin into cells. These results indicated that polymer micelles with cross-linked ionic cores are promising for further fundamental material studies and practical applications as drug delivery carriers.

Keywords: Block copolymer micelles; Nanogels; Template assembly; Core-shell morphology; Cisplatin; Cancer chemotherapy

1. Introduction

Polymer micelles have attracted significant attention as nanoscale carriers for delivery of low molecular mass drugs, proteins, genes [1–3], and imaging agents [4]. These micelles are supramolecular assemblies of nanoscale size (10 to 100 nm in diameter) from amphiphilic block or graft copolymers. They have a fairly narrow size distribution and are characterized by unique core-shell architecture, where hydrophobic blocks are segregated from the aqueous exterior to form an inner core surrounded by a shell of hydrophilic polymer chains. A micelle is thermodynamically stable, relative to disassembly to single chains, if the concentration of the block copolymer exceeds the critical micelle concentration. The core-shell architecture of the polymer micelles is essential for their utility as novel functional materials for pharmaceutical applications. The core of the micelles is a loading space that accommodates various therapeutic or diagnostic agents. The hydrophilic shell is a brush-like corona that stabilizes the micelles in aqueous dispersion. Poly(ethylene oxide), PEO, is frequently used as a hydrophilic block of micelle-forming copolymers, since this polymer is known to be highly hydrated, soluble, non-toxic, non-immunogenic, and able to serve as an efficient steric protector for various microparticulates (such as liposomes, nanoparticles, nanocapsules) in biological media [5–7]. In particular, PEO chains prevent interactions with serum proteins and cells, avoids particle opsonization and render them "unrecognizable" by the reticuloendothelial system (RES) in the liver and spleen. Since the molecular weight of polymer micelles is far above the renal threshold (~ 40 kDa for copolymers), they evade renal excretion and non-specific capture by the RES, and demonstrate prolonged circulation times in blood [8,9]. With this propensity, micelles eventually demonstrated their utility, especially in cancer therapy, because of their promoted accumulation in the solid tumors through the enhanced permeability and retention effect [10,11]. Recently, nanofabrication of polymer micelles was significantly advanced by using block copolymers containing ionic and nonionic blocks ("*block ionomers*"). Such

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block copolymers react with oppositely charged species forming block ionomer complexes, which self-assemble into core-shell micelles [12–17]. The latter enable, uniquely, encapsulation of charged therapeutic molecules into the micelle core. Since being proposed independently by Kabanov and Kataoka in 1995 [12,13] this approach is now widely used for the incorporation of various polynucleic acids into block ionomer complexes for developing non-viral gene delivery systems. Ionic block lengths, charge density, and ionic strength of the solution affect the formation of stable block ionomer complexes and, therefore, control the amount of the drug that can be incorporated within the micelles [18,19]. The pH- and salt-sensitivity of such block ionomer micelles provide a unique opportunity to control the triggered release of the active therapeutic agent [20–22]. Furthermore, block ionomer complexes can participate in the polyion interchange reactions that are believed to account for the release of the therapeutic agent and DNA in an active form inside the cells [23]. However, all polymer micelles have a drawback as a delivery system because they disintegrate after dilution in the body fluids, resulting in premature drug release. The multimolecular micelle structure can be reinforced by the formation of cross-links between the polymer chains. The resulting cross-linked micelles are, in essence, nanoscale single molecules that are stable upon dilution and can withstand environmental challenges such as changes in pH, ionic strength, solvent composition, and shear forces without structural deterioration. There are numeral reports on stabilization of the polymer micelles by cross-linking either within the core domain [24–28] or throughout the shell layer [29–32]. In these cases, the cross-linked micelles maintained the small size and core-shell morphology while their dissociation was permanently suppressed. For instance, stable nanospheres from PEO-*b*-polylactide micelles were prepared by using polymerizable methacryloyl group at the end of the core segment [24]. In addition to stabilization, the core of polymerized micelles solubilized hydrophobic molecules such as paclitaxel and retained high loading capacity even upon dilution [25]. More recently, Kataoka et al. prepared trypsin-loaded polymer micelles of PEO-*b*-poly(L-aspartic acid) cross-linked with glutaraldehyde via Schiff base formation between protein and polymer molecules in the core [33]. The cross-linked micelles showed remarkable stability against high salt concentrations while preserving the activity of the incorporated protein. Formation of the network of a temperature-sensitive polymer (poly-*N*,*N*-diethylacrylamide) within the core was also employed for stabilization of Pluronic micelles [34]. However, the increased micellar stability was not permanent and disappeared over a time period of days to weeks.

Recently, we proposed to use block ionomer complexes as micellar templates to synthesize novel polymer micelles with cross-linked ionic cores that display high stability. Indeed, the cores of the block ionomer micelles formed between poly (ethylene oxide)-*b*-polymethacrylate anions (PEO-*b*-PMA) and divalent metal cations were utilized as nanoreactors for cross-linking reactions. Resulting particles were hydrophilic nanosphere, which combine several key structural features that make these systems very beneficial for effective drug delivery. These are: a cross-linked ionic core; a hydrophilic PEO shell; and nanoscale size. These favorable characteristics of the polymer micelles with cross-linked ionic cores motivated our ongoing efforts to elucidate their potential as efficient carriers for the delivery of anticancer drugs. The objective of the present work is to evaluate whether the polymer micelles with cross-linked ionic cores can be useful for immobilization of *cis*-dichlorodiamminoplatinum (II) (cisplatin), a potent chemotherapeutic agent. It reports the synthesis and characterization of the cross-linked polymer micelles, the preparation and physicochemical properties of cisplatin-loaded micelles and their interactions with cells.

2. Experimental section

2.1. Materials

Poly(ethylene oxide)-*b*-poly(methacrylic acid) (PEO-*b*-PMA) diblock copolymer ($M_w/M_n = 1.45$) was purchased from Polymer Source Inc., Canada. The block lengths were 170 and 180 repeating units for PEO and PMA respectively. The concentration of carboxylate groups in the copolymer samples was estimated by potentiometric titration. 1,2-Ethylenediamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, *cis*-dichlorodiamminoplatinum(II) (cisplatin), *o*-phenylenediamine, fluorescein isothiocyanate (FITC) and other chemicals were purchased from Sigma–Aldrich (St Louis, MO) and were used as received.

2.2. General procedure for the synthesis of cross-linked micelles

PEO-*b*-PMA/Ca^{2+} complexes were prepared by mixing an aqueous solution of PEO-*b*-PMA with a solution of $CaCl_2$ at a molar ratio of $[Ca^{2+}]/[COO^-] = 1.3$. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.9 mg, 0.01 mmol) and 1,2-ethylenediamine (0.3 mg, 0.005 mmol) were then added to the dispersion of PEO-*b*-PMA/Ca^{2+} micelles (20 ml, 0.05 mM of PMA units). The reaction mixture was allowed to stir overnight at room temperature. The extent of cross-linking (20%) was controlled by the ratio of amine functional groups to carboxylic acid groups. After completion of the reaction, an equimolar amount of EDTA was added followed by dialysis, first, against 0.5% aqueous ammonia, and then against distilled water to remove Ca^{2+} ions and byproducts of the cross-linking reaction.

2.3. Synthesis of fluorescein-labeled cross-linked micelles

In order to modify cross-linked micelles with fluorescent label, the cross-linking of the cores of PEO-*b*-PMA/Ca^{2+} micelles was performed in the presence of the excess of 1,2-ethylenediamine (20% molar excess relatively to activated carboxylic groups) to introduce free amino groups into the micelle structure. Thus prepared cross-linked micelles will be further conjugated with fluoresceine isothiocyanate (FITC) using standard procedure [35]. Briefly, 0.1 ml of 1% solution of FITC in dimethylformamide was added to 1 ml of the dispersion of polymer micelles (1 mg/ml) in 0.1 M sodium carbonate buffer, pH 9.0, and was stirred overnight at room